13.—THE EMBRYOLOGY OF THE SEA BASS (SERRANUS ATRARIUS).

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(Plates LXXXVIII to CVII and 12 figures in text.)

INTRODUCTION.

The following paper, dealing with the embryology, forms part of a monograph on the Sea Bass in course of preparation under the direction of the United States Commissioner of Fisheries, Hon. Marshall McDonald. The monograph will contain, besides the embryology, a description of the larval development and of the adult fish, an account of the habits and distribution of the fry and the adult, and an account of the Bass viewed as a food-fish from an economic standpoint.

As might be inferred from its wide distribution along the Atlantic coast, all the way from Cape Cod to Florida, the Sea Bass is known under a variety of popular names. South of Cape Hatteras it is called Blackfish, in the Middle States "Black Will," "Black Harry," and "Hannahills." Sea Bass is the common name along the New England coast.*

My work on the bass was carried on at the Wood's Holl Station of the Fish Commission. It was begun in May, 1889, and has been continued without interruption to the present time. The bass makes its appearance in the neighborhood of Wood's Holl (Buzzard's Bay and Vineyard Sound) about the middle of May, and the spawning season lasts from that time until near the first of July. The fish is one of several (mackerel, scup, tautog, etc.), which at this season are reared in the hatchery of the Wood's Holl Station, and I was therefore able to obtain, with the least amount of trouble, as complete a set of material as could be desired. The collector, Mr. Vinal Edwards, visits the fish "pounds" in the neighborhood every morning at the time when the fish are taken out. Any fish that are found to be ripe are at once "spawned," *i. e.*, the eggs are pressed out into jars of sea water, with which milt is then mixed. The eggs thus fertilized are brought as quickly as possible to the hatchery and are placed in "tidal" jars or boxes, in which the water alternately rises and falls. The eggs during most of the period of incubation float at the surface of the water, but, as in the case of some other species of fish, there is a short period of time, not long before hatching, when they sink as though they were dead. The specific gravity,

* The Fisheries and Fishery Industries of the United States, Section I, 1884, p. 407.

however, again becomes less, and the eggs once more rise to the surface. Not all of the eggs suffer this change in specific gravity. In the batch from which my material was taken not more than two-thirds sank, the time being about forty hours after fertilization.

My material was obtained in the first days of June, when the temperature of the water was 60° F. The time from fertilization to hatching was about seventy-five hours. Later in the month, of course, the time was considerably less. The percentage of fish hatched out was very large, and for three or four days after hatching the young fish kept in aquaria remained in good health. By that time the yolksac had almost entirely disappeared, and the now very active fry evidently needed more spacious and varied quarters, for they began to die at a great rate.

In sectioning I found that the eggs killed in Perenyi's fluid, both old and young stages, yielded the best results. The yolk never becomes hard, but is coagulated, and especially in the young stages (segmentation and formation of the periblast) contracts away from the blastoderm, which it is therefore easy to separate from the yolk by means of needles.

After the body of the fish is once well outlined, I find it is better not to section the whole egg, but with fine scissors to cut off the embryo, and if possible shake it free from the egg membrane. With a little care the whole body may be obtained unbroken and entirely free from yolk, and yet with the periblast layer attached.

For surface preparations of the blastoderm during the stages of segmentation and formation of the periblast, the Perenyi embryos mounted in balsam answered fairly well. But much better preparations were obtained by killing the eggs in acetic acid, or in osmic and acetic, and mounting the embryos directly in glycerine.

I take this opportunity of thanking the Boston Society of Natural History and its librarian, Dr. J. Walter Fewkes, for the extremely obliging manner in which they have sent to Wood's Holl whatever books and journals I have asked for.

There are already several monographs in which the growth of our knowledge of Teleost development has been traced with great care, and I have therefore not considered it necessary to give extensive historical reviews. Hoffman's long paper (17) especially contains a full account of past work, and Henneguy's "Embryogenie de la Truite" (1888) brings the record nearly up to the present date.

I. SEGMENTATION.

The egg of the Sea Bass is a small pelagic egg about 1 millimeter in diameter. The egg membrane is thin and horny, but even after months in alcohol does not grow very hard. The yolk forms a single translucent sphere which, after coagulation of the albumen, shows in sections a finely reticulated structure. Imbedded in the yolk but near the surface, is a single large oil globule, which is always uppermost in the floating egg. In the ripe unfertilized egg the yolk is covered by a thin layer of protoplasm, of about the same thickness at all points. Shortly after fertilization this diffused protoplasmic layer begins to concentrate towards a point just opposite the oil globule. The "streaming" of the protoplasm, which characterizes the concentration, has been well described and figured by Ryder for the Cod (34). A couple of hours after fertilization there is found at the lower pole of the floating egg a disk of protoplasm, lenticular in section. At its edge the disk thins away into an excessively thi

layer of protoplasm which is continued round the yolk. The yolk itself contains no protoplasm except in the immediate neighborhood of the oil drop. About a third of the surface of the latter is covered by a cap of coarsely reticulated protoplasm, lacking a nucleus. Fig. 1 shows a section through the oil globule, *o. g.*, and its protoplasmic cap, *o. g. p.*, which is entirely free from the superficial (almost imperceptible) layer of protoplasm. This cap of coarse protoplasm is easily seen in surface views, and I have observed it in Mackerel eggs as well as in those of the Bass.

The patch of protoplasm at the lower pole, or the blastodisc, is at first circular. Just before or during the first act of cleavage there arises an inequality of the axes, so that by the time the first two blastomeres are marked off, the germ is bilateral, or at least biradial (Fig. 2). In the Bass and Mackerel the first two blastomeres are of equal size. This is normally so with the Cod as well, but on one occasion I observed that in all of the eggs got from a single codfish, the first two blastomeres were unequal in size. The inequality was very marked, but the eggs were healthy and the average percentage of fish was hatched out.

The first plane of cleavage is claimed by Agassiz and Whitman (1) to represent the anteroposterior axis of the adult. I found that while I could follow with ease the succession of furrows until thirty-two cells were formed, and in some blastoderms could follow the process a step further until sixty-four cells were established, after that I could no longer trace the fate of the early cleavage planes.

The segmentation of the Bass is of the ordinary bilateral type characteristic of Teleostei. The first two planes of cleavage (Fig. 2) are meridional, and at right angles to each other. They cut very deeply into the blastodisc, but do not extend quite through to the yolk. The section (Pl. LXXXIX, Fig. 13) really belongs to a stage of four blastomeres, but does not differ in appearance from one through the two blastomere stage. As is seen in this section, the two segments and later the four are connected by a thin layer of protoplasm (*c. p.*, central periblast), in the center of the blastodisc. At the periphery the segments are continued into the superficial protoplasm clothing the yolk, which protoplasm is especially thickened round the immediate edge of the segments. The ridge thus formed (early periblastic ridge, *e. p. r.*, Figs. 2, 8, etc.) persists from the time when cleavage begins until the periblastic nuclei are established as such (Fig. 22). During all this time it constantly varies in distinctness, now being very obvious and again scarcely perceptible. It has long been known that the blastodisc increases in size during segmentation, and the fluctuation in the height of the ridge is undoubtedly due to some periodicity in the force, which effects the incorporation of the outlying protoplasm into the blastodisc. Agassiz and Whitman (*l. c.*, p. 49) discuss this point and conclude that the force is in some way associated with nuclear division. The ridge, which I have called the "early periblastic ridge," to distinguish it from the peripheral periblast wall which is formed in a much later stage by the fusion of certain of the blastoderm cells, is shown in section in Figs. 15, 16, Pl. LXXXIX, Fig. 17, Pl. XC. In Fig. 13, Pl. LXXXIX, and Fig. 14, Pl. LXXXIX, the ridge is at its lowest ebb, so to speak, and the blastomeres fade away gradually into the surrounding protoplasm.

The third cleavage plane is shown in Fig. 3. It is in most eggs nearly, and often quite, parallel to the first plane. In this stage the segmentation cavity, *s. c.*, which is perfectly distinct after another cell division, becomes recognizable. Figs. 14 and 15, Pl. LXXXIX, are two sections through the planes *a* and *b*, respectively, of Fig. 3. In the

outer section (Fig. 14) all the cells are fused at their bases. In the inner section (Fig. 15) one of the cells (1) is free from the periblast layer below *c. p.* Just a little nearer the middle line of the blastoderm the other cell (2) also becomes free of the periblast. The segmentation cavity, of which *s. c.* in Fig. 15 represents a part, is very obvious in surface views of this stage, when the lower surface of the blastoderm is brought into focus. In Fig. 3 the upper surfaces of the eight cells are closely joined, edge to edge, but at the lower level the central ends of the cells inclose a well-marked space, *s. c.*, the floor of which is formed by the periblastic layer. Occasionally there occurs at this stage a displacement of cells, or one of the blastomeres is retarded in its cleavage, so that there results an irregular blastoderm as shown in Fig. 4. In the Bass such irregularities are rare. I was surprised to find how comparatively common they were in mackerel eggs.

In Fig. 6 the fourth furrow is seen beginning. The sixteen cells formed by this furrow, after they have suffered another nuclear division, are shown in Fig. 8, Pl. LXXXVIII. A section through *a–b* of Fig. 8 is given in Fig. 16, Pl. LXXXIX. The segmentation cavity (*s. c.*) is now plainly established, the four central cells of Fig. 8 being entirely free from the periblast, and the cavity even extending well under the peripheral cells.

The fifth act of cleavage is indicated by the nuclear figures in Fig. 8. The cleavage plane of the corner cells (*x*) is meridional, but in the remaining peripheral cells the plane is equatorial. The four central cells on the other hand suffer a horizontal cleavage (plane parallel to the surface of the blastoderm), which can only be observed in sections, Fig. 16, Pl. LXXXIX. By no means do all of the eggs pass through this act of cleavage in such a strictly bilateral fashion. In a watch crystal of eggs, at least one-half of them will be found to deviate from the type. The most common variation concerns the terminal and lateral pairs of cells (*m, n; m′, n′; l, r; l′, r′*, in Fig. 8). In one or two of these cells the cleavage is often meridional instead of equatorial. Thus in Fig. 9, *l* and *r′* (both belonging to the lateral pairs) exhibit the variation, and in Fig. 10, *m′*, belonging to a terminal pair, likewise divides meridionally. The four central cells invariably divide in a horizontal plane, and it is rare to find a corner cell (*x*) which varies. Occasionally, however, one is found dividing equatorially.

Fig. 17, Pl. XC, lies in the plane *c–d* of Fig. 8, and is from a blastoderm, in which this stage of cleavage is near its close. The thirty-two cells are here nearly separate. When they are completely established and the resting stage comes on, there is invariably some rearrangement which partially destroys the bilaterality of the germ. Even, however, where the rearrangement is coupled with a previous variation, such as Figs. 9 and 10 exhibit, the origin of the thirty-two cells can often be made out. Thus Fig. 11 shows a resting blastoderm of this stage, in which I have given each cell the letter of its immediate parent, as used in Figs. 8, 9, and 10. In Fig. 12, Pl. LXXXIX, however, in which the thirty-two cells have suffered nuclear division, the displacement of cells has been so great that it is impossible to trace the original bilaterality, just as it was impossible to follow with certainty the movements of the cells in the living egg under the microscope. During the last minutes of the thirty-two cell stage, it may be said that all degrees in the loss of bilaterality are found, and consequently it is only in certain embryos that the cleavage from thirty-two into sixty-four can be accurately followed.

There are certain general features in this sixth act (32 into 64) of cleavage, which were found in all the eggs I studied, and from which, coupled with the behavior of

some unusually symmetrical embryos, it is possible approximately to deduce an ideal type, which, it may safely be said, is never exactly followed in actual segmentation. In Fig. 12 it is seen that the cells of the peripheral ring undergo either meridional or equatorial division, but it is impossible to decide from the relative frequency which should be regarded as the type.

Figs. 18 and 19 are two sections through unusually symmetrical germs, in which the thirty-two cells had nearly retained the positions indicated by the amphiasters of Fig. 8. They may be referred to the ideally symmetrical thirty-two cell stage, as represented by Fig. 1. Fig. 18 would lie in the plane *a–b*, and Fig. 19 in the plane *d–f*. In Fig. 18 the lower tier (four in all) of central cells, c_2, are in the first stages of nuclear division. The cleavage planes of these four cells, then, run in the direction x, Fig. 1. Fig. 19 shows that the four upper central cells (c_1) suffer cleavage in planes parallel to y. These planes, x and y, may be taken as typical for the central cells, though there is undoubtedly much variation, but never in the direction of horizontal cleavage. The horizontal cleavage is exclusively found in the cells which lie between the central cells and the peripheral ring, viz, in m_2, n_2, etc. In Fig. 19 two of these cells, m_2 and m_2', show this cleavage.

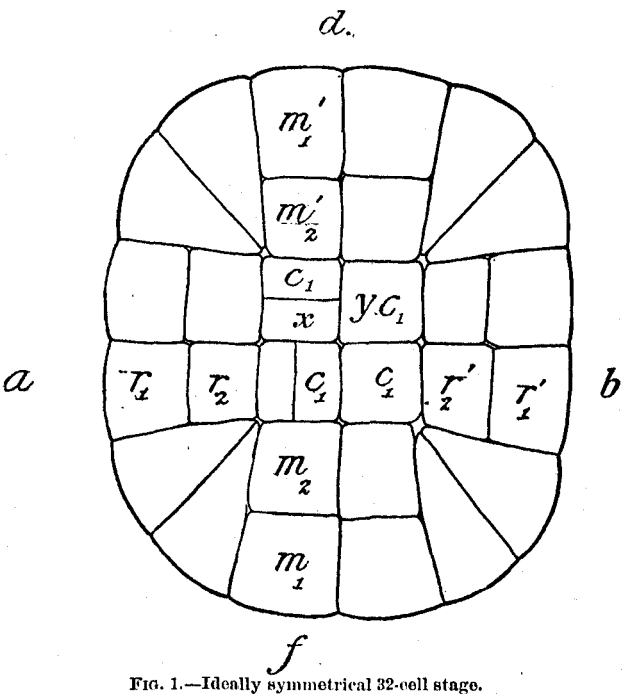

FIG. 1.—Ideally symmetrical 32-cell stage.

The typical cleavage from thirty-two cells into sixty-four may then be described as follows: The peripheral cells suffer meridional (equatorial) division, the intermediate cells, m_2, n_2, etc., horizontal division, the four upper central cells and the four lower central cells divide in planes parallel to y and x, respectively, these planes, being, in all probability, metamorphosed meridional planes. The further course of segmentation I found it impossible to follow. Fig. 20, Pl. XC, is through a blastoderm about an hour older than Fig. 19. The peripheral cells continue to divide meridionally or equatorially, the plane obviously being decided in many cases by the greater length of one of the cell axes.

During the segmentation the periods of rest are, as usual, long compared with those of activity. I give the following time record of the early divisions:

11:40 to 11:58 (18 minutes) period of rest; 4 blastomere stage.

11:58 to 12 (2 minutes) period of activity; 4 into 8 blastomeres.

12 to 12:27 (27 minutes) period of rest; 8 blastomere stage.

12:27 to 12:30 (3 minutes) period of activity; 8 into 16 blastomeres.

Bilaterality of the blastoderm.—Occasionally a blastoderm is met with which suggests that the bilaterality in at least the early stages is very deeply seated indeed.

Such a one is given in Fig. 7. Here both cells of one lateral pair (*l* and *r*) have deviated from the type and are undergoing meridional division, and both cells of the opposite pair are doing likewise. This state of affairs is, however, not the rule, and serves rather as a suggestion than an argument. But what is the rule, is the exact agreement between the corresponding cells of opposite halves as to the time when cellular fission begins. The furrows appear at precisely the same instant in *r* and *r'* and *l* and *l'*, and the same was true of the terminal cells *m*, *m'* and *n*, *n'*. In the ordinary blastoderm, as shown in Fig. 6, all the furrows start at exactly the same time. Such correspondence between the opposite halves plainly suggests that the bilaterality is not simply a morphological one, but that the symmetrical arrangement of cells is the mere outward expression of a physiological bilaterality which already exerts a control over the life of the organism.

Watase has shown in his careful study of the Squid segmentation (41 and 42) that the bilaterality is there even more prominent than it is in the Teleost. If, for instance, a cell on one side exhibits such a variation as multinuclear fission, the corresponding cell on the opposite side will do the same. The relation between the right and left halves of the blastoderm in regard to the time when activity begins is, however, of a very different character in the two animals. In the Squid there is an alternation in the activity of the two sides; one side undergoes nuclear division while in the cells of the opposite side the nuclei remain in the resting stage. In the Teleost, on the contrary, there is coincidence between the two halves of the blastoderm as regards activity and rest. This is certainly true of the cellular division, and without having studied the nuclear fission in anything like the detail which characterizes Watase's work on the Squid, I feel sure that the same coincidence is present in this form of activity as well. I never found resting nuclei on one side and amphiasters on the other, but the resting nuclei, sharply outlined and conspicuous, always made their appearance on the two sides at the same time, and as soon as amphiasters could be recognized on one side they could be found on the other.

Amphibian and Teleostean segmentation.—The teleostean segmentation has undoubtedly been derived from a total segmentation essentially like that of Amphibia, and, convinced of this, Rauber (36), Agassiz and Whitman (1), and Ziegler (47) have endeavored to homologize the early furrows in the two groups. In regard to the first two furrows there can be no difference of opinion. The homology of the third teleostean furrow is, however, by no means so clear. Ziegler, without entering into a detailed discussion of the matter, regards the first three furrows in the two groups as homologous. Agassiz and Whitman, after a critical examination of Rauber's views, also pronounce in favor of this homology, deciding that the third teleostean furrow represents the equatorial furrow of Amphibia. I do not find, however, their reasons sufficient for discarding the homology offered by Rauber, supported as it is by variations (atavistic) in the teleostean germ towards the Amphibian type, and by variations in the Amphibian segmentation which so exactly imitate the telpostean type.

In his extremely suggestive paper Rauber (36) shows that the generally accepted account of the frog's segmentation is erroneous. The furrows have not the ideally symmetrical arrangement given in the common figures. It is very rare even for the second meridional furrow to cross the first at the poles of the egg, and Rauber never observed an egg in which the first three meridional furrows so cross. Commonly the two furrows, which together compose the so-called second meridional furrow, meet the

first furrow at points on opposite sides and a short distance from the poles. For the sake of convenience the cut Fig. 2 may be used as an illustration, though it does not represent the most common type of the frog's segmentation. Supposing *p* to be the upper pole of the egg, the first furrow *a–a* and the second furrow *b–b* cross at points some distance from the pole. Whatever be the precise cause which makes the furrows in the frog thus avoid the pole, the avoidance is a fact, and to it Rauber has given the name of *Polflucht*. The third meridional cycle of furrows (the true third or equatorial is represented by the bounding line *c* in the figure) exhibits the same phenomenon. The individual furrows run from the equatorial furrow not to the pole, but into the several older furrows; for instance, the line *x* may represent the course of the furrow in this cycle. All degrees of *Polflucht* may be found, and when the degree is great enough, and is coupled with the occurrence of certain other features, the type of segmentation shown in Fig. 2 is produced.

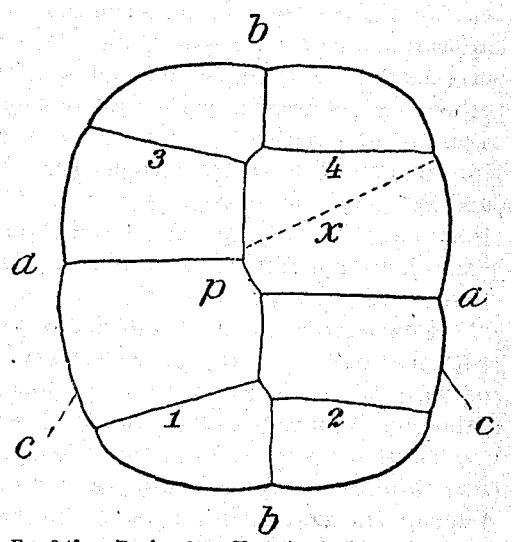

FIG. 2 (from Rauber, 36).—Variation in the frog's segmentation, imitating teleostean segmentation—*p.*, animal pole; *a.* to *a.*, first furrow; *b.* to *b.*, second furrow; *c.* to *c.*, equatorial furrow; 1, 2, 3, 4, fourth furrow.

This type while unusual is not very rare. Its similarity to, almost identity with, the 8-celled stage in the Teleost, can not be overlooked, and I think Rauber is justified in regarding the third teleostean furrow as homologous with the furrows 1, 2, 3, 4. This homology is further strengthened by the occurrence of a variation in the Teleost, which may be interpreted as a reversion, and as indicating that the third furrow in the fish egg was originally a true meridional furrow, or at least one with very little Polflucht. Such a variation is shown in Fig. 5, Pl. LXXXVIII (Mackerel). It is certainly very rare in the Bass and Mackerel, and the blastoderm figured was apparently pathological. Agassiz and Whitman record the occurrence of the same variation in *Ctenolabrus* (I, Pl. CVII, Fig. 35).

According to Rauber the first equatorial furrow of the frog has been lost in the Teleost. Agassiz and Whitman would seem to believe that the *a priori* improbability of such a loss taking place is so great that, in spite of the variations just described, it is preferable to regard the first three furrows as homologous in the two groups. I do not see the inherent improbability of the loss. On the contrary, the disappearance of segmentation in the ventral half of the egg, coupled with the early contraction of the protoplasm (belonging to this half) towards the upper pole, make it easy, I think, to understand how the loss was brought about.

II. FORMATION OF THE PERIBLAST.

The existence of yolk nuclei in the Teleost egg (parablast nuclei, His; periblast nuclei, Agassiz and Whitman) was discovered as long ago as 1854 (Lereboullet, 31), but their origin was first made out by Agassiz and Whitman in 1884 (1). These authors proved beyond a doubt that in *Ctenolabrus* the nuclei are derived from the marginal cells of the blastodisc, which, from the earliest stages of segmentation are

connected with the yolk (or periblastic) protoplasm. The marginal cells so connected eventually lose their cell outlines, and are drawn into the surrounding protoplasm. In *Ctenolabrus* there are two concentric rings of cells thus made use of. Before the appearance of their paper it had been held by Kupffer and others that the nuclei originate in the yolk independently of the blastodisc nuclei. Hoffmann (17), on the other hand, claimed that the first segmentation nucleus underwent a horizontal cleavage, the upper daughter nucleus giving rise to the nuclei of the blastodisc, while, from the lower daughter nucleus were derived the nuclei of the periblast. Agassiz and Whitman have sufficiently criticised Hoffmann's account and figures, and while there is good reason to believe that the periblast nuclei do not originate in all Teleosts from the peripheral cells of the blastodisc (von Kowalewski, 27) their criticism seems to me a very just one.

I have found that the nuclei develop in *Serranus* in a manner almost identical with that in *Ctenolabrus* There are minor differences concerning the number of rings of marginal cells drawn into the periblast, etc., but my account is essentially a confirmation of that of Agassiz and Whitman.

At the end of segmentation the marginal cells of the blastodisc are flattened and do not take the stain as readily as the other cells. In a surface view (Fig. 21, Pl. XC), they appear as a wreath of pale cells round the periphery of the blastoderm. This wreath of cells, often observed (Kupffer, 24; Van Beneden, 40; Ryder, 35; Cunningham, 8; Henneguy, 18), has been and is still the subject of great misconstruction. Kupffer and others believed that these cells were formed round nuclei, which had originated in the yolk, and that they then passed out of the yolk and were added to the blastodisc. It might be thought that the very lucid and exact account of Agassiz and Whitman would have cleared this part of teleostean embryology from any shade of uncertainty. But in his last paper (18) so old a student of the Teleosts as Henneguy concludes that the cells of the "wreath" are passing out of the periblast "pour s'ajouter au germe" (p. 461).

In Fig. 21 the marginal cells, though very different in appearance from the rest of the blastoderm, still retain their cell outlines. They are even marked off from the surrounding periblastic protoplasm, which continues to form round the edge of the blastoderm the "early periblastic ridge." A few minutes later there is no longer any line of separation to be seen between these cells and the outlying protoplasm, though they are still marked off from one another (Fig. 22, Pl. XC). Sections through this stage are the most important for the study of the formation of the periblast. Fig. 25, Pl. XCI, is such a section, in which the right half presents a slightly older condition than the left. On each side the marginal cells pass without the interruption of the ridge into the cortical layer of protoplasm (*cor. p.*). On the left, however, the marginal cell still preserves its earlier shape; the segmentation cavity cuts its way into it (compare earlier sections, Figs. 20, 19, 18, etc.). But on the right the cell is flatter, and the whole body passes uninterruptedly into the central periblast layer (*c. p.*). The section shows plainly enough that the marginal cells are being drawn into the periblastic protoplasm.

An hour later (Fig. 23, Pl. XC) the marginal cells have fused with one another, and the lost cell outlines are now only indicated by the accumulations of protoplasm round the nuclei. In sections through this stage it is seen how completely the peripheral cells have lost their identity in the process of fusion with each other and the periblast. In Fig. 26 the marginal cells of Fig. 25 have been metamorphosed into the

periblastic wall (*p. w.*), which as yet contains but a single circle of nuclei. There takes place at about this time a flow of protoplasm from the wall towards the center of the blastoderm, whereby the central periblast layer becomes thicker than in earlier stages.

During the next hour there is an active multiplication of nuclei in the periblast wall. The indirect method of division is followed, and the karyokinetic figures are very conspicuous, especially in surface views (Fig. 24). Succeeding the single ring of nuclei shown in Fig. 23 there is a stage with two concentric rings, though the two rings can not be distinguished at all parts of the periphery. After this the arrangement of the nuclei is no longer a regular one, as is proved both by surface views and series of sections. In Fig. 28 the periblastic wall contains four nuclei, but other parts of the wall show only two or three (Fig. 27). All the periblastic nuclei of a blastoderm do not suffer division at the same time, but it sometimes happens that the nuclei of a particular region all divide at once, as shown in Fig. 24, though on the opposite side of the blastoderm from which this view was taken the nuclei were in general at rest.

The multiplication of the nuclei in the wall is followed by a gradual migration towards the center of the blastodisc. In Figs. 27 and 28 the inner nuclei have begun to creep in this direction, and the migration continues until nuclei are scattered all through the periblastic layer. This migration from the edge of the blastodisc is illustrated by the series of sections, Figs. 29, 30, and 31, which are from blastoderms of 9½ hours, 14 hours, and 16 hours, respectively. The periblastic wall, which is so prominent at the beginning of migration (Fig. 29), grows less conspicuous towards its end (Fig. 31). This is due in part to a gradual flow of the periblastic protoplasm towards the center of the blastodisc, and in part to the increasing size of the blastodisc, the edge of which, as it grows round the yolk, carries with it the periblastic wall.

The histological change which occurs in the periblastic nuclei, and which appears to be universal in Teleosts and Selachians, comes on in the early stages of migration. After the stage shown in Figs. 28 and 29, of about the same age, there is no longer any indirect division of nuclei; and the nuclei themselves, which have hitherto not differed in appearance from those of the blastodermic cells, now become greatly vacuolated and also flattened. They gradually increase in size and their contour becomes very irregular, owing to the development of protuberances. The peculiar character which they retain throughout embryonic life is fully acquired before invagination begins (Fig. 32, Pl. XCI, and Fig. 40, Pl. XCIII).

The physiological use of the periblast nuclei and protoplasm is not known. The suggestion has, however, been advanced (Hoffmann, 17; Ziegler, 47) that the nuclei have the function of working over the yolk into some shape which is easily assimilated by the blastodisc cells. Their uniform histological character in the Teleosts and Selachians naturally leads one to believe they have some special physiological function, the more so because, though ancestrally a part of the entoderm, in the ontogeny (of the Teleosts, at least) they take no share in forming the embryo. Like Hoffmann (17), Wenckebach (43), Ziegler (47), Henneguy (18), and in opposition to Kupffer (26), and Gensch (13), I find that the nuclei do not give rise to blood cells, nor do they contribute to the formation of the alimentary canal. The ultimate fate of the nuclei is associated with the final disappearance of the yolk sac through the agency of the liver.

III. INVAGINATION.

During the formation of the periblast the superficial layer of the blastodisc cells becomes differentiated from the rest to form what German authors usually call the "Deckschicht," English writers the "epidermic stratum." The progressive flattening by which these cells are converted into a well-marked layer may be traced through Figs. 25, 26, 27, Pl. XCI, into Fig. 40, Pl. XCIII. The latter section is through the stage when invagination begins, and the epidermic stratum (*ep. s.*) is fully differentiated. The flattening continues through later stages until the layer is reduced to the condition of a very thin membrane, which stains deeply.

During the same period the blastodisc undergoes a change of shape, which is the preparatory step towards invagination and the differentiation of the embryo. During the last stages of segmentation, when the periblast wall is being formed, the under surface of the blastodisc is either plain or slightly convex (Figs. 26 and 29, Pl. XCI). Four hours later (Fig. 30, Pl. XCI) the under surface has become decidedly concave. As the hollowing out continues the concavity takes an eccentric position (Fig. 31, Pl. XCI), and thus before any invagination occurs one part of the peripheral region of the blastoderm is thicker than the rest. In a surface view of this stage the thin eccentric area appears as an ill defined clear circular space, surrounded by the more opaque periphery, which at one pole (*p. p.*, Fig. 32, Pl. XCI), the tail end of the future embryo, is considerably thicker than elsewhere. At this pole, in Fig. 32, the invagination has already begun, and hence the periphery is here separated by a sharp line from the central region. But before the invagination begins this part of the edge is noticeably thicker than the rest, though nowhere is the peripheral region sharply marked off from the central. As to the means by which the center is thinned out, and the periphery, especially the embryonic pole, left thicker, I can only say that in general I agree with Götte (14). In the absence of any absorption of cells or extensive migration, the cause would seem to lie in the direction of cell growth, which in the main determines the position of nuclear cleavage planes, and hence the direction in which new cells are pushed.

The change of shape which the blastoderm suffers during this period gives rise to what is commonly called the subgerminal cavity, *i. e.*, the cavity which may be supposed to exist between the blastoderm and the periblast layer in Figs. 30, 31, Pl. XCI, Figs. 40, 41, Pl. XCIII. This cavity was in almost all the embryos I examined a virtual one, except at certain points where the bounding layers separate slightly, as in Figs. 40, 41, etc. (*s. g. c.*) Occasionally, however, an embryo was found in which a comparatively spacious cavity separated blastoderm and periblast, as in Fig. 47, Pl. XCIV. It will be understood that the subgerminal cavity is merely a late phase of the segmentation cavity of the earlier stages, *s. c.*, Fig. 25, etc.

The next preliminary step in the process of invagination is illustrated by Fig. 32, Pl. XCI, and by an antero-posterior section through the posterior pole of a similar blastoderm, Fig. 40, Pl. XCIII. In the surface view the peripheral region at the embryonic pole is seen to be bounded internally by a sharp line, and in the section Fig. 40, the explanation of this line is found in the well-marked randwulst, the inner limit of which is indicated by the cells *m*, *m'*. The mode of formation of the randwulst may be inferred from the shape and arrangement of the cells and the position of the daughter nuclei. Cell-growth, following the arrow *a* (Fig. 40) first established in the blastoderm a

thinner center and a thicker periphery; then, curving round in the direction of arrows *b* and *c*, it gave rise to a marginal wulst (see cut, Fig. 3).

Still confining our attention to the embryonic pole of the blastoderm, we see, Fig. 41, Pl. XCIII (antero-posterior section through a stage slightly older than Fig. 40) that a tongue of cells grows out from the randwulst towards the center. The tongue (*pr. h.*), several cells deep, can be recognized with ease before there is any ingrowth at all round the rest of the blastoderm edge. (Compare opposite halves of Fig. 41.)

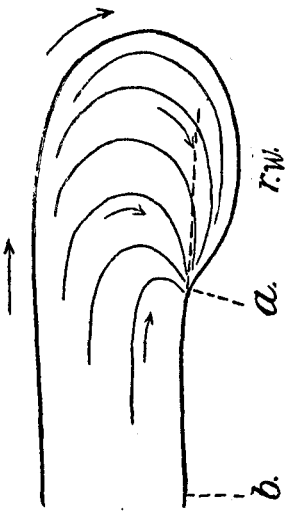

FIG. 3. Diagrammatic section to illustrate formation of randwulst—*r. w.*, randwulst; *a.*, apical line.

Round the rest of the edge the ingrowth is likewise, at least in most places, preceded by the formation of a randwulst, which, however, is inconspicuous. In Fig. 46, Pl. XCIV, a longitudinal section through the anterior pole of a blastoderm similar to Fig. 33, Pl. XCII, the periphery of the section is seen to be slightly thicker than the inner part. From the thickened periphery the ingrowth of cells (*v. mes.*) has already started, though only the apical cell is as yet clearly separated from the upper layer. In the transverse section, Fig. 47, Pl. XCIV (through *a–b* of Fig. 33), a randwulst can not be said to exist, the edge of the blastoderm from which the ingrowth (*v. mes.*) starts being actually thinner than the more central portion.

The early condition of the ingrowing layer at the cardinal points of the blastoderm, posterior pole, anterior pole, and lateral poles, is shown in the three sections, Fig. 41, Pl. XCIII (right half), Fig. 46, Pl. XCIV, and Fig. 47, Pl. XCIV, and a surface view of the blastoderm at this stage is given in Fig. 33. The ingrowing under layer is known as the germ ring. Before going further I must dissent from a point in the description which Agassiz and Whitman (*l. c.*) give of the formation of the ring. In their Fig. 6, corresponding with my Fig. 47, the cells of the ring are represented as forming a differentiated unicellular layer extending quite out to the epidermic stratum. In their paper, which is a preliminary one, the transverse section is the only one given, and they do not describe the condition at the embryonic pole. I have not found that the under layer cells are differentiated from the rest of the blastoderm at the extreme edge. The peripheral part of the blastoderm, both where there is a large randwulst, Figs. 41 and 46, and none at all, Fig. 47, is an undifferentiated area, and the germ ring consequently starts at some little distance from the extreme edge of the blastoderm.

I have not as yet mentioned the behavior of the epidermic stratum during the period of invagination. Like most authors I have found that this stratum does not share in the invagination. It is at no point continuous with the ingrowing layer, as all the figures show. The peripheral epidermic cells at the embryonic pole, however, act in a way which at least suggests the persistence of a tendency in them to take part in the invagination, though it is more probable that their behavior is due to some much less significant cause. The peripheral cells (*m. ep. c.* Figs. 40 and 41, Pl. XCIII, Fig. 45, Pl. XCIV), in this region during the early stages of invagination, are larger than elsewhere, and they project into the narrow ring-like space left between the periblast and the incurving surface of the randwulst. Fig. 41 shows the ordinary amount of this projection, but blastoderms are met with in which the epidermic layer follows the sur-

face of the randwulst in as marked a manner as is shown in Fig. 45. Round the rest of the blastoderm edge the marginal epidermic cells are often larger than the others, but there is nothing striking in their behavior, and after the first stages of invagination the peculiar character of the cells at the embryonic pole is lost (Figs. 43 and 44, Pl. XCIII).

The germ ring, the origin of which has now been described, continues its centripetal growth for a short while around the entire edge of the blastoderm, but especially in the region of the embryonic pole. In a surface view, Fig. 34, Pl. V, an hour later than Fig. 33, the inner outline of the germ ring (*g. r.*) is seen to have encroached upon the central clear space (the thin roof of the subgerminal cavity), and in a section, Fig. 43, Pl. XCIII, the short, thick tongue of cells shown in Fig. 41 is seen to have grown out into a thin sheet of uniform thickness, *pr. h.* (two layers of cells). Still an hour later this part of the germ ring has approached considerably nearer the center, Fig. 44, Pl. XCIII, preserving its characters of a simple two-layered sheet, which indeed it does not lose until the formation of the notochord begins. The growth of the germ ring round the rest of the blastoderm edge may be gathered from a comparison of Figs. 46 and 48, Pl. XCIV, both longitudinal sections through the anterior pole of the blastoderm (Figs. 48 and 44 are parts of same section). The ingrowth (*v. mes.*) is seen to be very slight compared with that at the embryonic pole, and moreover the cells of the under layer are not arranged in two strata.

The positive means by which the ingrowth is effected is undoubtedly cell division. (See the nuclear spindles in Figs. 46 and 48.) But as Agassiz and Whitman (*l. c.*) have pointed out, the width of the ring is probably also increased in a passive manner by the spreading of the blastoderm round the yolk.

There is one very striking and theoretically important feature which comes out in the comparison of the early and later stages of the germ ring. If Fig. 41, Pl. XCIII, be compared with Fig. 44, Pl. XCIII, it will be seen that the germ ring has not only grown in a centripetal direction, but that it has also been splitting off in a centrifugal direction from the randwulst. This point is brought out even better in a comparison between Figs. 46 and 48, Pl. XCIV. In other words, accompanying the ingrowth or invagination, there has been a backward (centrifugal) delamination, slight, indeed, but still a fact. Götte, the discoverer of invagination in the Teleosts, is the only writer who speaks of the centrifugal delamination in the randwulst, though, as I shall point out later, Götte's randwulst is really a stage of the germ ring in which the actual ingrowth has already begun.

The significance of the centrifugal delamination lies in the suggestion it gives of the way in which invagination may be converted into delamination. It is, to be sure, very doubtful whether there is any vertebrate in which the primitive hypoblast is really delaminated from the upper layer. But in the very similar case of the origin of the mesoblast from the primitive hypoblast, there is no doubt that in certain animals delamination has superseded the folding process (see section on mesoderm), and any occurrence which points to the way in which the one method may be converted into the other, seems worth recording.

In the cut, Fig. 3, the concentric lines of cell growth by which the randwulst is established, meet in what may be called the apical line *a*, which marks the inner edge of the randwulst. From this zone the ingrowth progresses centripetally, and a cen-

trifugal delamination also takes place in the direction of the dotted line. Now, by a slight alteration in the course of the lines of growth, the zone in which they all meet, or the apical line, may be made to move centrifugally or centripetally, and if, for instance, it take the position *b*, an extremely wide randwulst will be established. In this hypothetical randwulst, as in the real one, there would at first be no differentiation of layers, but by the quick and simple process of delamination the under cells of the wulst could be converted into a separate layer, as actually occurs in the real wulst. By moving the apical line far enough towards the center, the entire germ ring could be established by delamination, without there being any need of an actual ingrowth.

The germ ring as such reaches the height of its development about 20 hours after fertilization (Figs. 34 and 35, Pl. XCII, Fig. 44, Pl. XCIII, Fig. 48, Pl. XCIV, all of the same age). The thinning out of the central region to form the subgerminal cavity (*s. g. c.*), which was begun in earlier stages, has been continued. The thin region now, however, has very definitely circumscribed bounds. It is inclosed on all sides by the germ ring (Fig. 34) in the region of which, especially in the neighborhood of the embryonic pole, the upper layer of cells (which may now be called ectoderm) remains thick (Fig. 44). The thin region, or extra-embryonic part of the blastoderm, consists at this stage of three layers of cells—the epidermic stratum and two strata of nervous layer cells—which are still plump polygonal bodies. This region continues to grow thinner, but reaches its ultimate condition before the blastoderm has covered the yolk. It is then a thin membrane, made up of epidermic pavement cells and one or two layers of somewhat flattened cells.

Historical.—To Götte belongs the credit of the discovery that in the Teleost embryo there is a process of invagination leading to the formation of the primitive hypoblast, which in its turn splits up into entoderm and mesoderm. The account given in his first communication (1869, 15) was scarcely an exact description of the facts, but his second paper (14) contains a more accurate and detailed account than any I have found in subsequent papers.

In his first communication Götte described the edge of the blastodisc as suffering an actual involution to form the under layer, and this account was repeated by Haeckel in the description he gives of Teleost development in the "Gastræa Theorie" (19). In 1873 Oellacher published his well-known paper on the Trout (33), in which he claimed there was no invagination at all, but that the eccentric thinning out which led to the formation of the subgerminal cavity also left one portion of the peripheral region much thicker than the rest (embryonic anlage). This thicker portion of the periphery then split into the ectoderm, mesoderm, and entoderm of the embryo (delamination theory). Shortly after the appearance of Oellacher's paper Götte published his second account, with which my own description agrees in all essential particulars. The one point of difference concerns the randwulst. What Götte has described and figured under this name is really a stage of the germ ring, in which the free ingrowth has already begun. It corresponds with the ring shown in my Fig. 42, Pl. XCIII, or more highly magnified in Fig. 46, Pl. XCIV (anterior pole), and Fig. 41, Pl. XCIII, right half (posterior pole). As will be seen from the following brief abstract of Götte's description, the only improvement I have been able to make upon it lies in having defined the randwulst with somewhat greater precision. According to Götte the thinning out by which the subgerminal cavity is established is the result of the direction of cell growth, which

(see cut Fig. 4), starting from *a*, proceeds in the direction of the arrows. After the subgerminal cavity is formed the cell growth continues round the periphery in the same general direction (arrow) and a randwulst is established. In the region of the

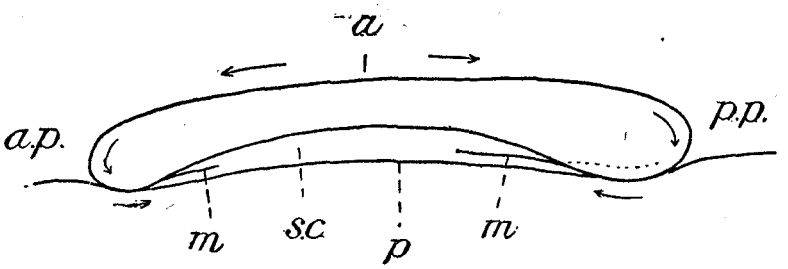

FIG. 4.—Diagrammatic section through a blastoderm to illustrate Götte's account of the development of the randwulst and germ ring; *p. p.*, post. pole; *a. p.*, ant. pole.; *m.* to *m.*, germ ring; *s. c.*, segmentation cavity; *p.*, periblast.

randwulst there is no differentiation of layers, but the cell growth continuing centripetally produces the layer *m–m*, sharply marked off from the layer above. In a somewhat later stage the differentiation of the under layer *m* extends back into the randwulst itself, in the direction of the dotted line.

Subsequent observers have either followed Oellacher's account (Hoffmann, 17) or have been content to describe the main fact of an ingrowth from the edge of the blastodisc, without entering into a discussion of the randwulst (Henneguy, 18; Agassiz and Whitman, 1; Cunningham, 8).

The consideration of the germ ring as a part of the gastrula will be found in a later section.

IV. NOTOCHORD, MESODERM, ENTODERM.

Formation of embryonic shield.—After the germ ring is completed the growth of the blastoderm round the yolk is continued with much greater rapidity than in earlier stages. In a stage 5 hours later than Fig. 35, Pl. XCII, two-thirds of the yolk is inclosed, Fig. 36, Pl. XCII. In the spreading of the blastoderm, the posterior pole or tail end of the embryo remains as a comparatively fixed point, while the anterior pole, *a. p.*, Fig. 35, travels rapidly in the direction of the arrow. The constant position of the oil globule with respect to the early blastodisc enables one to judge of this movement with certainty.

During the growth of the blastoderm the embryonic area in the region of the posterior pole becomes sharply marked off from the rest of the blastoderm. The area thus marked off is commonly known as the embryonic shield. The manner in which it develops is brought out in a comparison of Fig. 34, Pl. XCII (20 hours), with Fig. 37, Pl. XCII (25 hours). As the anterior pole of the blastoderm travels round the yolk, and the extra-embryonic region is thus increased, the head end (*h. e.*, Fig. 34) of the future embryo travels in the same direction, and in this way a roughly triangular area (embryonic shield) is established which projects into the surrounding extra-embryonic region. The outline of the embyronic shield in Fig. 37 is indicated by the letters *e. e. s.* In this figure the embryo itself is distinctly marked out as a median longitudinal thickening. A stage intermediate between Figs. 34 and 37 would show a clearly differentiated shield, though less extensive than that of Fig. 37, without the median thickening.

During the growth of the blastoderm the part of the germ ring not included in the embryonic shield, extra-embryonic part of ring, grows thin and its cells become flattened, as over the rest of the non-embryonic area. The change which takes place in this part of the ring between 20 and 25 hours is gathered from a comparison of Fig. 48 (F objective) with the older stage, Fig. 51 (D objective).

General structure of embryonic shield.—The ectoderm over the whole region forms a thickened plate, passing at the edge of the shield into the thin ectoderm of the non-embryonic area (Fig. 53, Pl. XCIV, transverse section through shield). The ectoderm plate is at first not thickened in the median line; on the contrary, just above the developing notochord it is somewhat thinner than elsewhere (Fig. 50, Pl. XCIV). The ectoderm cells are polygonal, except the epidermic stratum, and the layer is everywhere clearly marked off, often actually separated from the under layer or primitive hypoblast *pr. h.*, Fig. 50).

The primitive hypoblast over the greater part of the shield consists, as in earlier stages (Fig. 44, Pl. XCIII), of two strata of flattened cells. The strata are quite distinct, except in the middle line (Fig. 50, Pl. XCIV), where the cells are closely interlocked. The fusion which thus early appears in the primitive hypoblast along the median line is the first step in the formation of the notochord. At the lateral edge of the shield, where the primitive hypoblast ends abruptly, the two layers are not distinct (Fig. 53, Pl. XCIV), nor are they recognizable in the immediate neighborhood of the posterior edge, where the primitive hypoblast bends round into the ectoderm (Fig. 49, Pl. XCIV, a transverse section through the extreme posterior edge of the shield). As will be seen in Fig. 37, Pl. XCII, this edge of the embryonic shield exhibits a ring-like thickening continuous with the extra-embryonic germ ring, and the section given in Fig. 49, Pl. XCIV, shows that the thickening is located in the under layer.

In the anterior region of the shield the primitive hypoblast is not divisible into two layers. Fig. 52, Pl. XCIV, gives a longitudinal section, a little to one side of the median line, through a shield in which the embryo had just begun to be marked out as a linear thickening. Anteriorly the primitive hypoblast ends in a mass of polygonal cells, *a. m.*, which long occupy this position, just in front of and ventral to the future fore-brain. From this mass as far back as the point *x*, in other words, throughout the extent of the future brain area, the cells of the under layer are polygonal, and only indistinctly show two layers. Most, if not all, of the primitive hypoblast in the brain region becomes converted into mesoblast exclusively (head mesoblast; no somites).

Differentiation of the embryo and formation of notochord, etc.—The opaque linear streak which marks the body of the embryo, and which is shown in a somewhat advanced condition in Fig. 37, Pl. XCII, is in its first stage due almost exclusively to a thickening of the ectoderm. This thickening, by which the neural chord is formed, begins in the future head region of the embryo, which is thus marked off before the rest of the body. But in an hour's time the posterior region (trunk) of the shield has also acquired its median thickening (Fig. 53, Pl. XCIV, and Fig. 54, Pl. XCV, transverse sections through the trunk). The start thus obtained by the brain keeps it, however, from the beginning thicker than the spinal chord (Fig. 36, Pl. XCII, and longitudinal sections, Fig. 52, Pl. XCIV, and Fig. 55, Pl. XCV). For the present the neural chord may be passed over with this brief description of its origin.

By the time the neural chord has begun to form in the trunk region, the formation of the notochord and secondary layers has also commenced, by which the median

thickening is further increased. The development of these organs begins in the posterior region and travels anteriorly. Thus in the same embryo several stages in their differentiation may be observed. Fig. 53, Pl. XCIV, and Fig. 54, Pl. XCV, are two sections through the trunk of an embryo somewhat younger than that shown in Fig. 37, Pl. XCII. In the anterior section, Fig. 53, the condition is much the same as in the earlier embryo, Fig. 50, Pl. XCIV, but with this difference, the under, *en.*, of the two layers, found on each side of the incipient chorda, *nc.*, has definitely separated from the chorda and from the upper layer, *mes.* It is thus made up of separate halves which project slightly under the chorda cells. This layer constitutes the definitive entoderm. The intimate connection between the chorda cells and the layer marked *mes* (which develops into the mesoblast) is soon broken. The connection has no significance, for it sometimes happens that the chorda cells separate simultaneously from both entoderm and mesoderm. In the posterior section (Fig. 54) the differentiation has gone a step farther. The chorda has separated from both layers and has assumed a compact shape, though the cells have practically their former arrangement. The entoderm is in the same condition as farther forwards, but the unicellular mesoblast layer of Fig. 53 has begun on each side to thicken up at its inner angle. The thickening is as yet very slight, and extends but a short distance away from the notochord.

Let us trace the development of the notochord and secondary layers through a slightly older stage than that of Figs. 53 and 54. Of this stage a surface view from above is given in Fig. 37, Pl. XCII, from the side in Fig. 36, Pl. XCII, a median longitudinal section in Fig. 55, Pl. XCV, and a series of transverse sections (numbered from behind forwards) in Figs. 56, 57, and 58, Pl. XCV. From the surface views and the longitudinal section the relative extent of the head (brain) and trunk regions may be gathered. In the posterior trunk region (Figs. 55 and 56) the notochord is easily recognizable. The entoderm is in its former condition, or nearly so, still consisting of two lateral unicellular sheets which project under the notochord. The projection under the notochord (Fig. 56) is more marked than in earlier stages. The mesoderm plates have thickened considerably (compare Figs. 54 and 56). It is only near the edge of the shield that they now consist of a single layer of cells. On passing gradually into the anterior trunk region the notochord will be found to grow appreciably thinner (Fig. 55), likewise the mesoblast plates. Going still farther forwards into what may be called the neck region (*n*, Figs. 55 and 57), the notochord and entoderm fuse, and the mesoblast plates thin away into scattered cells (*mes.*, Fig. 57). In the head region (Figs. 55 and 58), in connection with the vertical development of the brain, the primitive hypoblast has thinned away into a unicellular layer, which in later stages is in part transformed into scattered mesoblast elements, and in part persists as the extreme anterior portion of the entoderm lamella.

Formation of the primitive streak and closure of the blastopore.—Before the early history of the notochord and layers can be concluded, it will be necessary to describe the course of development at the posterior end of the embryo. The condition at this end, after the completion of the germ ring, is shown in Fig. 44, Pl. XCIII, a median longitudinal section through a stage such as Fig. 35, Pl. XCII. When the blastoderm has grown round the yolk, as far as is shown in Fig. 36, Pl. XCII, the condition at this pole becomes a slightly different one. There is now at the posterior end of the embryo (Fig. 55, Pl. XCV, median longitudinal section) an undifferentiated mass of cells of considerable extent (*c. m.*). Let the blastoderm grow still farther, and the mass will be found

to increase in length (Fig. 59, Pl. XCV, median longitudinal section through a stage something younger than Fig. 38, Pl. XCII). In transverse sections the exact relations of this mass are made clear. It is then seen that in the posterior half of the apparently homogeneous mass (*c. m.*) there is no differentiation of layers at all, but in the anterior half the condition is such as is shown in Fig. 60, Pl. XCV. The posterior region, in which there is absolutely no differentiation, will be spoken of as the caudal mass (bourgeon caudale, schwanzknospe), while the anterior region, in which there is fusion between the neural chord and the entoderm, may conveniently be called the neurenteric streak. The whole tract from the termination of the notochord to the end of the embryo has been regarded by Henneguy (18) and Schwarz (39) as a primitive streak analogous to the streak of Amniota, and (Schwarz) homologous with that of Amphibia. I fully agree with these views and will use the term primitive streak in this meaning. The name neurenteric streak implies that it is in this region that the neurenteric canal belongs, or would belong if it ever came into existence. As to this location of the neurenteric canal most students are agreed. (See especially Henneguy, *l. c.*, and Schwarz, *l. c.*)

During the further growth of the blastoderm round the yolk the primitive streak may increase in length (*pr. str.* Fig. 65, Pl. XCVI, a median longitudinal section just before the closure of the blastopore), but the increase is scarcely measurable.

Before discussing the way in which the primitive streak is established, a word may be said on the condition in the *Salmonidæ*. There here appears to be from the start, *i. e.*, before the blastoderm has begun to encircle the yolk, a very considerable caudal mass (bourgeon caudale, schwanzknospe), and Henneguy, *l. c.*, p. 585, has satisfied himself that in the trout the region of the primitive streak does not increase appreciably in length during the closure of the blastopore. Now, in spite of the fact that embryos vary considerably in length, and exact measurements can not therefore be implicitly trusted, it is unmistakable that in the Bass the primitive streak suffers a considerable increase in length during the closure of the blastopore. When the rapid growth of the blastoderm begins there is really no bourgeon caudale (Fig. 44, Pl. XCIII), but by the time the yolk is half encircled there is a perceptible bourgeon (caudal mass), from which the fusion extends forwards in the median line (Fig. 49, Pl. XCIV, transverse section through the anterior part of the primitive streak). Still later (Figs. 36, Pl. XCII, and Fig. 55, Pl. XCV) the primitive streak has slightly increased in length, and in the yet later stages (Fig. 59, Pl. XCV, and Fig. 65, Pl. XCVI) its increase in length is unquestionable.

The apparent difference between the Trout and the Bass in this matter may not after all be so great as it would seem. Without going into a further comparison, however, I feel bound to regard the Bass development as the more typical, if only for the reason that it is as regards this point so easily harmonized with the Amphibian development.

The means by which the primitive streak is brought into existence is obvious. As the blastopore grows smaller the extra embryonic part of the germ ring is *pari passu* drawn into the tail end of the embryo, and there is thus built up in this region a constantly increasing mass of undifferentiated cells. As this mass is built up the mesoderm plates cut their way back, and thus give rise in the anterior part of the region to what I have called the neurenteric streak. In the Bass there is no actual concrescence in the middle line (see surface view of closing blastopore, Fig. 39, Pl. XCIII), but the terminal notch observed in some fish, as well as general considerations derived

from a comparison of Teleosts with Amphibia, warrant us in regarding the closure of the blastopore as a process of concrescence, the result of which is to establish the primitive streak. Schwarz has come to the same conclusion (29).

At the final moment of the blastopore closure there is added to the primitive streak the mass of cells, *sec. c. m.*, Fig. 65. This mass is of course the remnant of the extra-embryonic germ ring. Even after its fusion with the primitive streak, a dividing furrow makes it for some time recognizable. It may be called, for convenience of reference, the secondary caudal mass.

The entire mass of undifferentiated cells left at the tail of the embryo after the blastopore closes, serves as cellular material for the backward growth of the several organs. Thus, while the extra-embryonic germ ring, as has been insisted upon by Agassiz and Whitman (*l. c.*) and Cunningham (8), assuredly forms part of the embryo, it does not form any special part, but, on the contrary, its cells eventually find their way into ectodermic, mesodermic, and notochordal tissues.

The behavior of the periblast during the final moments of blastopore closure may be gathered from Fig. 65, Pl. XCVI. As will be seen, the periblast closes over the blastopore area before the blastoderm proper. After the closure the periblast plug (*p. pl.*) disappears, the layer forming an even and complete investment of the yolk.

Entoderm.—After this digression the early history of the secondary layers and the notochord may be resumed. Four hours later than the stage last described (Fig. 36, Pl. XCII, and Fig. 56, Pl. XCV) the entoderm is completely established as a connected unicellular layer. In sections through a small embryo like the Bass it is often impossible to decide whether the entoderm on each side grows under the notochord, as shown in Fig. 56, or whether it is split off from the base of the group of chorda cells. In order to reach a decision I was forced to cut a great number of sections, for it is only now and then that one is obtained in which all the lower cells are clearly defined. In the sections through the stage of 25 hours (Fig. 37, Pl. XCII, and Figs. 55 and 56, Pl. XCV) the extremely intimate connection between the entoderm and chorda was uniformly evident, and in some sections, for instance the one given in Fig. 56, it was incontestible that the entoderm was actually growing under the chorda cells. The exact state of affairs in the earlier stage (Fig. 53, Pl. XCIV, and Fig. 54, Plate XCV) was likewise often difficult to determine, but the best sections were such as I have drawn. After a careful study I feel safe in saying that the lateral sheets of entoderm grow under the chorda cells and meet in the middle line, thus completing the layer. Agassiz and Whitman state the same for *Ctenolabrus*. In the Trout, according to Henneguy (18), this method is not followed. The primitive hypoblast there breaks up into mesoblast, notochord, and definitive entoderm, the entoderm being from the first continuous across the median line. The condition in the Trout is of course a derived one, while the development of the Bass in this matter closely follows the ancestral lines: the chorda cells at first actually roof in the archenteron along the median dorsal line, as in *Amphioxus* and Amphibia.

The entoderm, after it has grown under the chorda, is shown in the longitudinal section, Fig. 59, Pl. XCV, and in the transverse sections, Figs. 60, 61, and 62, Pl. XCV, all from the same stage (29 hours). The cells of which the layer is composed are flattened except at the posterior end of the embryo, in the region of the neurentric streak (Fig. 60, Pl. XCV, and Fig. 59, Pl. XCV, *n–m*). Since the notochord does not extend into this region, it is evident that the entoderm cells here must have had a different mode of origin from that employed along the notochordal tract; and in fact they are merely

the lowest cells of the streak which have assumed a columnar shape, and have thereby differentiated themselves from the rest. The line of demarcation between them and the neurenteric streak is always more evident in transverse than in longitudinal sections.

Posteriorly they, together with the mesoderm and ectoderm, fade away into the caudal mass (Fig. 59, behind *n*). Anteriorly they are continuous with the flat entoderm. These columnar cells subsequently form the roof and walls of Kupffer's vesicle.

Brook (3) has tried to show that the entoderm lamella, instead of delaminating from the invaginate germ ring, is derived from the periblast. Kupffer has always maintained that the periblast is the source of the entoderm (24, 25, 26); but it seems impossible that this should be the case in any Teleost, when so many observers have failed to find that the periblast takes any share in forming the permanent layers.

Notochord.—By the time the entoderm layer is completely established the notochord has assumed its ultimate shape of a somewhat cylindrical rod. (Fig. 61, Pl. XCV, *n. c.*) Anteriorly it thins away (Fig. 62, Pl. XCV) and in the neck region disappears. The two layers of polygonal cells which primitively constituted the chord (Figs. 55 and 56, Pl. XCV) have in Fig. 59, Pl. XCV, begun to assume the well-known shape characteristic of chorda cells. They are already much interlocked, and a few hours later (Fig. 65) each cell, in a longitudinal section, extends the whole width of the chorda. In the early stages of the chorda the number of cells which together compose a cross section (Fig. 61) is much greater than in later stages. For, as the cells become flattened antero-posteriorly, they spread out in the transverse plane, and consequently two or three come to compose a cross section (Fig. 77, Pl. XCVII, 39 hours). When this stage is reached it is next to impossible to determine the cell outlines.

The next stage in the histological differentiation of the chorda is brought about by vacuolation. The beginning of vacuolation is shown in Fig. 110, Pl. CI (transverse section through an embryo of 53 hours). The vacuolation continues until, before the time of hatching, the protoplasm is reduced to a thin peripheral layer (Fig. 127, Pl. CIII), in which the nuclei are situated, and a few strands which cross the central cavity. By this time a well-defined sheath of high staining power is found round the chorda. After hatching the sheath becomes more conspicuous and the protoplasmic layer even thinner than before. (Compare Fig. 144, Pl. CV, part of a transverse section through a larva 3 days after hatching. The notochordal sheath is indicated by a heavy line, *n. c. s.*)

The vacuolation which probably puts an end to cell multiplication, does not extend into the posterior portion of the chorda. The chorda cells on the contrary retain in this region their embryonic, protoplasmic character. Compare Figs. 111 and 114, Pl. CI, transverse sections through the tail and head regions, respectively, of an embryo 59 hours old, and Fig. 119, Pl. CII, through the tail of a 65-hour embryo. At the tip of the tail there is found throughout embryonic life and for 3 days after hatching (possibly for a much longer time) a mass of undifferentiated cells (caudal mass) in which the chorda ends. At the time of hatching this mass of cells has become very small. The posterior growth of the chorda undoubtedly depends upon the presence of these undifferentiated cells, and possibly upon the embryonic character of its own cells in this region, though I have not observed nuclear figures.

If a comparison be made between Fig. 53, Pl. XCIV, and Fig. 83, Pl. XCVIII, it will be evident that a considerable forward extension of the chorda takes place after the

early stages in its formation. The amount of forward extension will, however, be overrated unless it be borne in mind how much greater the ratio of the brain region to the trunk is in the earlier stages than in the later.

Mesoderm.—The history of the mesoderm may here be conveniently carried up to the closure of the blastopore. The condition shown in Figs. 60, 61, 62, 63, 64, Pl. XCV and XCVI (successive sections from same embryo, 29 hours), is practically retained until the closure. The mesoderm now throughout the trunk consists of two thick lateral masses (Figs. 60 and 61, *mes.*), thinning away at the sides, but not exhibiting the two-layered arrangement which in the trout prefigures the differentiation of somatopleure and splanchnopleure. As will be seen in the sequel, the development of the body cavity in the Bass is secondarily modified in a high degree. The cells of the mesoderm plates are polygonal except at the surface, where they now begin to assume a columnar shape which later becomes more pronounced.

The mesoderm plates fade away as they reach the neck region, here giving place to scattered mesoblast cells (Fig. 62). In the posterior head region there are a few scattered cells (Fig. 63) and a rather well-marked layer (*en. mes.*) which posteriorly is continuous with the entoderm, and anteriorly breaks up into scattered mesoblast cells, which form a loose investment of the eye-balls and brain. The layer in question is no doubt in great part entodermic, but it is impossible at this stage to fix upon the precise spot at which the entoderm lamella ceases and the scattered mesoderm begins. All we can say is that in the anterior brain region (Fig. 64, Pl. XCVI) the primitive hypoblast becomes entirely converted into mesoblast. The anterior mass of mesoderm cells (*a. m.*, Fig. 55) disappears at about this time, its cells apparently migrating and sharing in the general investment of the brain.

Formation of the mesoderm in Teleosts and the Cœlom theory.—It has been universally recognized by students of the Teleosts that the mesoderm originates by delamination. It seems likewise certain that the primitive method of forming the mesoderm in the Chordata was by the outgrowth of paired sacs from the dorso-lateral walls of the archenteron. According to Balfour's idea of the cœlom theory there was no contradiction between the two facts. As is well known, he believed that in the Selachians the mesoblast was delaminated as two lateral masses (more recent investigations would seem to show that it arises as paired outgrowths, Rabl, 38), but that the delamination was the cœnogenetic representative of an original outgrowth. He was thus thoroughly convinced that evagination could be, and was, in certain vertebrates, replaced by delamination.

The cœlom theory of Hertwig as applied to the vertebrates is, however, of a different type. According to Hertwig not only has the method of forming the mesoderm in the various vertebrate groups been derived from the primitive method shown in *Amphioxus*, but in the ontogeny of all vertebrates it still follows the ancestral lines in the one essential point: the mesoderm arises as paired *outgrowths* from the walls of the archenteron. Hertwig has himself shown that the cavity of the sac may be obliterated and the outgrowth in consequence be solid, but he is convinced that the process is always one of outgrowth and never of delamination. His position is sharply defined in the following quotation:

Bei keinem der Wirbelthiere entsteht der Mesoblast durch Abspaltung, sei es vom äusseren, sei es vom inneren, Grenzblatt, da er von beiden mit Ausnahme eines sehr beschränkten Keimbezirkes, überall durch einem Spaltraum scharf abgegrenzt wird (20, p. 308).

In the Amniota there is such divergence of opinion as to the precise way in which the mesoderm originates that Hertwig is able to defend his position with some success from this quarter (20). But in the case of the Teleosts there is such remarkable agreement in the descriptions of the way in which the mesoderm is formed that Hertwig's theory must, I think, be regarded as in opposition to the facts. The conclusion is forced upon us that in certain vertebrates evagination has been replaced by delamination in the formation of the mesoderm.

Accepting this conclusion, we naturally look about for a type which suggests a transition from the one method to the other. In looking over the figures with which Hertwig illustrates the development of the mesoderm in the frog (20, 1882), the suggestion that there is here a partial delamination strikes one strongly, and is worth a word or two. In *Triton*, according to Hertwig, the invaginate entoderm or chorda-entoblast is wholly used in forming the chorda. The wall of the gut is formed exclusively by the darm-entoblast. In the frog, on the contrary, the invaginated layer is a wider as well as thicker one, and not only forms the chorda but also a part of the roof of the gut (Fig. 5). In the figure, a transverse vertical section, the invaginated

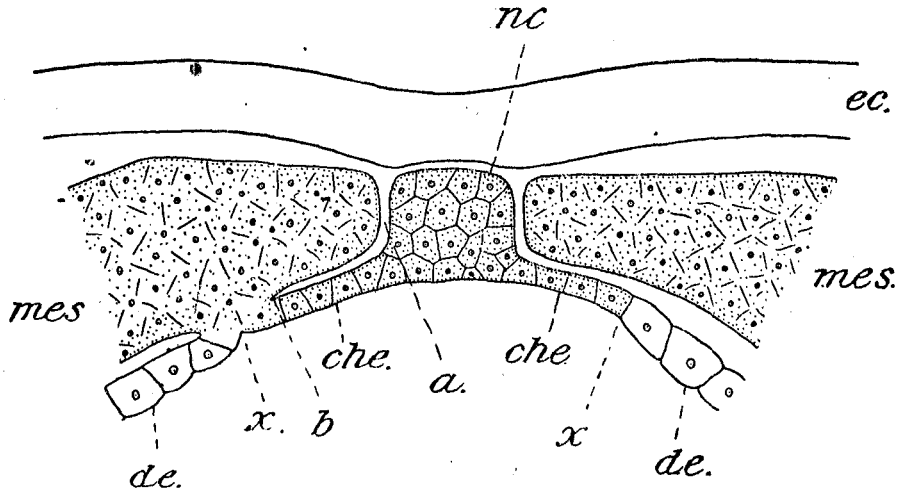

FIG. 5.—(after Hertwig, 20). Transverse vertical section through frog embryo to show partial delamination of mesoblast—*ec.*, ectoderm; *nc.*, notochord; *mes.*, mesoblast; *ch. e.*, chorda-entoblast; *d. e.*, darm-entoblast; *a.* to *b.*, delamination fissure; *x.* to *x.*, junction of chorda-entoblast and darm-entoblast.

layer is supposed to extend from x to x, at which points the chorda-entoblast, *ch. e.*, and the darm-entoblast, *d. e.*, pass into the mesoblast, *mes.* Now the figure, as far as it is possible to interpret it without having studied the previous stages, indicates that the invaginated layer in an earlier stage formed a continuous mass, stretching from x to x, and of about the thickness of the notochord. This mass appears to have broken up into notochord, chorda-entoblast, and the proximal parts of the mesoderm plates (from a to b). If this interpretation of the figure is a true one, and I am unable to understand the figure on any other supposition, then the mesoblast has delaminated from the notochord to x. The further growth of the mesoderm, peripherally from x, doubtless takes place as Hertwig describes.

It is very clear that in the frog the development of the derivatives of the primitive hypoblast does not take place in the simple ancestral manner shown in *Amphioxus* and *Triton*. The invaginate layer is made a thick one, so that the chorda is not forced

to come slowly into existence through a process of folding, but is at once cut out of a preformed mass of material, and, as I have said, I am inclined to believe the same is true of the proximal parts of the mesoblast plates. Indeed, Hertwig seems to entertain much the same idea when he speaks of the way in which the chorda and chorda-entoblast are separated from the mesoblast. Only he views the process from a different standpoint, and so compares the delamination fissure (*a* to *b*, Fig. 5) directly with the dorsal fold which lies between the outfolding notochord and mesoblast sheet of *Triton*.

If, now, the invaginate entoderm, which in the frog forms only a part of the roof of the alimentary canal, should take upon itself to form gradually more and more of the alimentary canal, it would probably from the very beginning become wider: the space from *x* to *x* would increase in width. The increase in width would naturally bring it about that a much larger part of the mesoderm plate would be delaminated than was formerly the case; there would constantly be less and less of the mesoderm which would have to arise as an outgrowth. Finally the condition in the Teleost would be reached, in which the mesoderm plate is split off from the primitive hypoblast along its whole width.

V. FORMATION OF THE ALIMENTARY CANAL.

The alimentary canal is formed from the simple entoderm lamella by a process of folding along the median line (Fig. 76, Pl. XCVII). The fold is converted into a tube by the meeting of its lower edges. There is a solid postanal gut formed as a thickening of the entoderm lamella, not as a fold. At the end of the postanal gut is Kupffer's vesicle, which is formed in essentially the same way as the permanent alim entary tube. It is scarcely necessary to say that Kupffer's vesicle and the entire postanal gut atrophy.

It seems worth while to give a detailed description of the formation of the alimentary canal, and this will best be done by going methodically through the several stages, taking up each series of sections at the posterior end of the embryo and passing forward.

The formation of the alimentary canal begins fir st in the region of Kupffer's vesicle and very shortly afterwards in the bra nchial region. Some hours before the closure of the blastopore the entoderm cells of the neurenteric streak (*n. str.*) become columnar (Fig. 60, Pl. XCV). These cells subsequently inclose Kupffer's vesicle. The rest of the entoderm lamella in this stage is made up of flattened cells.

Stage of 35 hours (Fig. 65, Pl. XCVI, and Figs. 66 to 72, Pls. XCVI and XCVII, series of sections from the same embryo). At the time when the blastopore closes sections through the neurenteric streak show an early stage in the formation of Kupffer's vesicle. The columnar entoderm cells have been pushed up in the middle line (Fig. 66), and arch over the cavity of the vesicle (*k. v.*), the floor of which is formed by the periblast. The vesicle is in this stage broad and shallow. Posteriorly the cavity fades away (longitudinal section, Fig. 65), and anteriorly likewise. The lining cells of the vesicle are directly continuous with the entoderm lamella. Passing forward (Figs. 67 and 69) the entoderm of the trunk is found to be thicker in the median line than laterally, the cells under the notochord approaching a columnar shape. Another feature will be noticed on comparing Fig. 67 with a section through an earlier stage (Fig. 61, Pl. XCV). The whole embryo is growing deeper and narrower, and coincidently

with this change in shape the entoderm is being drawn away from the sides towards the middle. This contraction of the entoderm towards the axis of the embryo becomes more apparent in later stages.

In this stage there is no trouble in discerning the precise anterior extent of the entoderm. In front of the medulla (*med.*, Fig. 147, surface view, of about same age as the sections we are now examining) there is scattered mesoderm but no entoderm. The transverse section, Fig. 71, through the middle of the branchial region, lies about in the plane *x* of Fig. 147. In this region the entoderm does not thicken in the middle line, but along two lateral lines (*br. f.*, Fig. 71), which in going back towards the posterior limit of the branchial region approach the middle. Along these lines the columnar cells have already begun to rise up in the shape of lateral folds (*br. f.*).

Stage of 39 hours (Figs. 73 to 79, Pl. XCVII, series of sections). In this stage Kupffer's vesicle is nearly as shallow as in the last, but its lateral limits (*m–m*, Fig. 73) are more sharply marked than was the case in Fig. 66. This is due, as the direction of the cells and subsequent stages show, to the fact that the entoderm cells at *m–m* have begun to grow towards the middle line. The formation of Kupffer's vesicle is a true process of folding quite like the formation of the alimentary tube in the trunk. There may be one point of difference which concerns the lateral entoderm (*l. en.*, Fig. 73). In the trunk the whole entoderm lamella is contracted towards the middle line and used up in forming the tube; but in the region of Kupffer's vesicle the lateral entoderm at least retains for a long time its original extent, while growing very thin. I am inclined to believe that the lateral entoderm is not drawn into the walls of the vesicle as it is into the walls of the gut, but that after becoming an excessively thin membrane it is absorbed in some other way. In a slightly later stage (41 hours) than the one just described the lateral entoderm has disappeared (Fig. 82, Pl. XCVIII). In this stage the increase in height and diminution in width of the vesicle, in consequence of the progress of folding, will be apparent.

Anterior to Kupffer's vesicle there is a median tract of thickened entoderm (transverse section, Fig. 74, *en.*) most of which will never be folded off, but will form the postanal gut. The anterior portion of the tract will, however, form a fold, and the axial cells in Fig. 74 have the typical arrangement and shape which precede its first appearance. Farther forwards there is a well-marked furrow (Fig. 75) which reaches its greatest height in about the middle of the trunk (Fig. 76). The distinctness of the cell outlines enables us to get at least a superficial insight into the process by which the furrow is produced. On comparing the three stages, Figs. 74, 75, and 76, there seems to be very little if any cell multiplication; no nuclear figures were observed. The cells on each side of the median line early exhibit a tendency to grow towards the axis; and in a general way, the process may, I think, be described as follows: In Fig. 74 the inner and lower ends of the cells 2, 2, grow towards the axis, and in so doing lift up the cells 1, 1, thus establishing an extremely minute cavity between them and the periblast. It is possible to follow every stage in the formation of the cavity from its first appearance as a minute, almost round aperture to the condition shown in Fig. 75. The cells 3, 3, follow the example of their predecessors, and thus the keystone cells 1, 1, are lifted still higher above the periblast. Let this course of action be followed by the cells 4, 5, etc., and it is easy to understand how the arch is increased in height, and at the same time how the lateral entoderm (*l. en.*, Fig. 74) disappears (as it has in Figs. 75 and 76); its cells, one after another, slip under their axial neighbors towards the median line.

The cell outlines in the several stages of the furrow, which I have tried to reproduce exactly in the figures, indicate that the method just described is the one followed; but complications undoubtedly supervene; for instance, the gut is often closed in ventrally before all the lateral entoderm has been drawn into its wall (Fig. 93, Pl. XCIX). Exactly how the cells thus left out disappear I do not know. In some cases, it would seem, they force their way between the cells which already line the tube, but again their position indicates that they are merely absorbed. In general, the method of forming the gut may be spoken of as a modification of the ordinary process of folding, such as is made use of in the Amniota, its peculiarity depending on the fact that the lateral entoderm is employed in helping to form the tube.

The condition in the branchial region is shown in the transverse section, Fig. 79, Pl. XCVII. If this figure be compared with the earlier stage, Fig. 71, Pl. XCVII, the course of development will be evident. The lateral folds (*br. f.*), barely begun in Fig. 71, are now well marked out. The further development of this tract of the enteron may be indicated in a few words. The apex of the fold, *a*, grows dorsally and ultimately fusing with the ectoderm, there is thus established the embryonic gill slit (only one in the embryo). The base, *b*, grows towards the median line and, meeting its fellow of the opposite side, closes in the foregut ventrally. As to the fate of the lateral entoderm of this region, I have not been able to come to a decision.

In passing backwards from Fig. 79 the lateral branchial folds gradually die out, Fig. 78, and in so doing approach the median line, where, in the œsophageal region (Fig. 77), they give place to a single broad low arch. The low œsophageal arch passes gradually into the deeper trunk furrow of Fig. 76.

Stage of 45 hours (Figs. 83 and 84, Pl. XCVIII, and Figs. 88 to 95, Pl. XCVIII and XCIX, series of sections). In this stage the tail begins to develop. The furrow, *t. f.*, which marks it off is shown in the longitudinal section, Fig. 84. Kupffer's vesicle in many individuals of this age is still bounded by the periblast, as in the longitudinal section, Fig. 84. In others, however (transverse section, Fig. 88), the process of folding has been completed, and the vesicle has an entire cellular wall; the lower edges of the fold, *m–m*, Fig. 82, have met in the middle line and closed in the vesicle ventrally.

In front of Kupffer's vesicle stretches the postanal gut (*p. a. g.*, Fig. 84), a mere thickened stripe of entoderm. At its posterior limit, just in front of the vesicle, at *r*, Fig. 84, it is fused with the notochord. A transverse section through this region of fusion is given in Fig. 90.

Directly in front of the postanal gut, at about *a*, Fig 84, a short tract of entoderm is found, in the condition shown in Fig. 91. This is the region of the anus, and the fold here appears last of all. In front of the anal region there is still for a short distance an open furrow, Fig. 92, but in the rest of the trunk and in the branchial region the enteron is now closed ventrally. The closure begins anteriorly and travels back. Figs. 93 and 94, Pl. XCIX, are through the trunk region. In the latter figure the remnant of lateral entoderm has finally disappeared. In the œsophageal and branchial regions the cavity of the enteron is almost obliterated. Fig. 105, Pl. C, is through the œsophagus (*oes.*) of a later stage than the one now under examination. Fig. 95, through the branchial region, is slightly oblique, and therefore it is only on one side that the gill slit is shown. In forming the gill slit the entoderm becomes continuous with the nervous layer of the ectoderm, as shown in the figure, while the epidermic stratum at first remains unbroken, dipping slightly into the branchial cavity.

Subsequently this stratum is perforated and a true opening is established. After the opening is once established there is formed round it a shallow depression of the general surface, so that in transverse sections the opening comes to be funnel-shaped (*g. s.*, Fig. 114, Pl. CI). The external appearance of the gill opening may be gathered from Figs. 149 and 150, Pl. CVII, *g. s.* As I have said, there is but this one opening into the branchial chamber during embryonic life. The remaining gill slits appear a day or two after hatching in front of the embryonic slit, but I have not studied their mode of formation.

Postanal gut.—In the stage which has just been under examination the tail had barely begun to develop. A few hours later (surface view from below, Fig. 98, Pl. XCIX), it becomes a prominent feature, and in the course of its development comes to contain the greater part of the postanal gut (*p. a. g.*). In this stage (Fig. 98) the cavity of Kupffer's vesicle has entirely disappeared. Indeed the cavity disappears in a much earlier stage of the development of the tail, as will be seen in Fig. 89, Pl. XCVIII, which is from a stage but slightly more advanced than Figs. 88 and 84, Pl. XCVIII. The obliteration of the cavity is brought about by the proliferation of the cells of its own wall. After the disappearance of its cavity Kupffer's vesicle is not distinguishable from the rest of the postanal gut, which at its posterior end gradually increases in size (Fig. 98) before vanishing in the caudal mass. The transverse sections, Figs. 99 and 100, Pl. XCIX, and Fig. 102, Pl. C, are all from an embryo nearly like Fig. 98. The two former sections show the condition of the gut within the tail, while Fig. 102 shows the condition of the tract *p. a. g.*, which the folding off of the tail has not yet reached. Referring them to Fig. 98, Fig. 99 lies in the Plane 1, Fig. 100 in Plane 2, and Fig. 102 in Plane 3. The position of the anus (not yet broken through) in the surface view is at *a*. Fig. 99 occupies the same relative position that Fig. 90 had in an earlier stage, is just in front of the neurenteric streak, and the entodermic mass, *p. a. g.*, is composed of the fused postanal gut and notochord. In Fig. 100 the postanal gut and notochord are separate. In front of the tail, Fig. 102, the postanal gut, *p. a. g.*, is less massive than farther back.

The postanal gut reaches the height of its development in the stage shown in Fig. 98. Almost immediately atrophy sets in. The atrophy begins at the anterior limit of the tail, and travels backwards and forwards. The sections, Figs. 99, 100, 101, and 102, are from an embryo in which the atrophy has just begun, and Fig. 101 is through the place where it starts. As will be seen in this figure, the atrophy of the gut leaves certain cells to mark its former position. These cells will again be touched on in dealing with the subnotochordal rod, the caudal extension of which they represent. The same cells in a later stage and farther back in the tail are again shown in Fig. 109, Pl. C, and Fig. 111, Pl. CI, *s. n. r.* The last part of the postanal gut to disappear is its swollen end.

Subsequent history of the alimentary canal.—The formation of the anus takes place before hatching, and there seems to be no ectodermal invagination to form a proctodæum. The mouth breaks through a couple of days after hatching. Shortly after the mouth appears, the cells which line the alimentary canal lose their embryonic appearance and come to look much like an adult mucous membrane; they secrete a cuticle and their limiting surface is no longer smooth (Fig. 144, Pl. CIV, *al. c.*). In the just-hatched larva, the condition of the alimentary canal in that part of the trunk which has been folded off from the yolk is shown in Fig. 126, Pl. CII.

Liver.—The formation of the liver begins about a day after hatching. It arises as a solid outgrowth from the dorsal wall of the enteron not far behind the limbs. Its condition in this stage is shown in Fig. 138, *l*, Pl. CIV (part of a transverse section through a larva 100 hours old). The cells of the solid outgrowth very soon arrange themselves so as to form the walls of tubes (Fig. 140, *l*, Pl. CV, larva of 112 hours). As the liver increases in size it grows down between the ectoderm and the yolk sac (Fig. 141, Pl. CV, 136 hours), to the posterior wall of which it henceforth clings.

A word may here be said as to the general characteristics of the larvæ in which the liver has become prominent. The yolk sac is now so small (Fig. 141) that it is limited to the anterior part of the trunk. The periblast layer still forms, as in earlier stages, a protoplasmic stratum clearly marked off from the yolk. The ventral surface of the yolk sac is closely pressed against the ectoderm, but elsewhere the ectoderm is separated from the rest of the embryo by a wide space (*b. si.*, Figs. 141 and 143). This space, which may be called the body sinus, is filled with a gelatinous fluid, which coagulates into a loose, stringy mass much like (only less dense than) the jelly of a medusa bell. After coagulation the jelly exhibits an irregularly radial arrangement. The body sinus is apparent in embryos just hatching, but the fluid which fills it does not develop its peculiar qualities until much later.

The further growth of the liver is connected with the final disappearance of the yolk and periblast. The three transverse sections, Figs. 143, 144, and 145, Pl. CV (from a larva 160 hours old), show how this disappearance is brought about. In the posterior section (Fig. 143) the connection of the liver with the alimentary canal is shown. The only part of the yolk which extends this far back is a vesicle, *o. g.*, which I take to be the oil globule. The oil globule has long before this (Fig. 151, stage of 65 hours), become intimately associated with the periblast, the protoplasm of which has grown entirely round it. In the two anterior sections (Figs. 144 and 145) the intimate connection between the liver (*l*) and the yolk (*y*) is obvious. The periblastic protoplasm, which in earlier stages formed a definite peripheral layer is now diffused through the yolk, which, in consequence, takes the stain. The yolk does not stain very deeply, but the contrast between its present and former condition is sufficiently striking. The periblastic nuclei have also undergone a change. They are no longer flattened and they stain much more uniformly than in earlier stages. Some of them have even a single nucleolus, and between such nuclei and the nuclei of the liver cells there is but very little difference. They are also no longer exclusively confined to the periphery of the yolk. The outlines of the liver cells adjacent to the yolk can not be made out. From these facts it is very evident that the liver is absorbing the yolk and periblastic protoplasm. The process is probably akin to ordinary intracellular digestion, but I could not discover the existence of any pseudopodia, nor do I believe that any such exist. As well as I could interpret the sections the process is something as follows: After the protoplasm has diffused through the yolk, the adjacent liver cells become actually continuous with this deutoplastic protoplasm. The cells which thus establish a connection with the yolk form a feeding or absorbing surface, which, as it incorporates new material on its yolk side, as constantly splits off new cells from its liver side. In this way the mass of yolk protoplasm shown in Fig. 145 is, bit by bit, entirely converted into liver cells. The precise fate of the periblastic nuclei I have not determined. That they, even in a slight measure, regain their early condition is

a matter for surprise. They probably in the end lose their identity and are absorbed as so much food material. Certainly there is no ground for believing (in the Bass) that they become blood corpuscles.

Historical.—In pelagic eggs the alimentary canal has usually been supposed to originate as a solid thickening of the entoderm lamella (Ryder 34, Kingsley and Conn 28, Agassiz and Whitman 1, Cunningham 8). Cunningham concluded that the ventral part of the thickening (floor of canal) was formed from periblastic elements, which migrated out of the yolk; but the grounds for this conclusion were very inadequate.

The formation of the canal in the trout has been studied in greater detail. From Oellacher's paper (33) it is difficult to arrive at a decision as to its exact mode of formation. Hoffmann (17), however, distinctly states that the process is one of folding, but aside from the branchial region his description is scarcely detailed enough to give one a satisfactory idea of the matter. Ziegler (48) and Henneguy (18) both describe the canal as arising in the main as a fold, and compare the process with the corresponding phenomenon in the Amniota. Henneguy's description is the more detailed of the two, and is no doubt in the main accurate for the trout. According to Henneguy, in the anterior part of the trunk behind the branchial region the canal is formed as a thickening which subsequently becomes hollow. In the rest of the trunk there is a median fold, the walls of which are so appressed that the cavity is a virtual one. Henneguy, however, does not speak of the postanal gut, and believes that the cavity of Kupffer's vesicle is continuous with that of the intestine: "La vesicule de Kupffer n'est donc que la premiere apparition de la cavité du tube digestif avec laquelle elle se confond plus tard" (*l. c.*, p. 563, Fig. 109, Pl. XXI). This can scarcely be so, for Schwarz's sections (39) prove the presence of a solid postanal gut in the trout, which subsequently atrophies together with Kupffer's vesicle.

Kupffer's vesicle.—The discovery of this vesicle was made by Kupffer in 1868 (24), and since then it has occupied a conspicuous place in the embryology of Teleosts. Its formation has never been satisfactorily worked out, and it has hence given rise to more discussion than could justly be claimed by it. The discoverer of the vesicle believed that it arose as an ectodermic invagination from the dorsal surface (25, 26), and in his figures the vesicle is shown as such a sac, closed below and opening on the dorsal surface. In his general scheme of vertebrate gastrulation Kupffer makes this sac play an important part. He regards it as homologous with the dorsal invagination of Reptilia, which he believes (26) constitutes the allantois (and part of rectum). The vesicle is then, for Kupffer, a structure which in higher groups becomes the allantois. From this standpoint it is a little misleading to speak of it as a rudimentary allantois, as is commonly done. It is rather a "prophetic" allantois. However, no one has ever confirmed Kupffer's account of the way in which the vesicle is formed, and until that is done it would scarcely seem possible to entertain any homology between the vesicle and the invaginated pit of reptiles.

Other investigators who have discussed this point of Teleost development depart widely from Kupffer's account, but differ among themselves. On the one hand Kingsley and Conn (28), Agassiz and Whitman (1), Cunningham (9), state that the vesicle arises as a space between the entoderm proper and the periblast. Henneguy (18) and Schwarz (39), on the other hand, contend that from the start the vesicle has a cellular floor, and arises as a closed cavity amongst the cells in front of the caudal mass. It will be noticed that the former group of investigators all worked on pelagic

eggs, while the latter two arrived at their conclusions from a study of the *Salmonidæ* (Schwarz also worked on the Pike). I have found that in regard to the point mentioned the former investigators were right—the cavity of the vesicle originally lies between the entoderm and the periblast.

The essential agreement, however, between the accounts of Henneguy and Schwarz leads one to believe that in the *Salmonidæ* the development of the vesicle may have suffered secondary modification. Whereas in the Bass the vesicle arises by a process of folding, in the *Salmonidæ* its development may be construed as the hollowing out of a solid thickening. After the vesicle has become a closed sac its further development and relations to the postanal gut have been correctly described by Schwarz.

Significance of the vesicle.—The significance of the vesicle is linked with the interpretation of the gastrula, which the vesicle on its side elucidates. In regard to gastrulation I am in thorough accord with Ziegler (48), and therefore regard the space (in great part virtual) between the entoderm and the periblast as the archenteron. The entoderm represents the dorsal hypoblast of the gastrula, the yolk and periblast the ventral hypoblast, and it is from the dorsal hypoblast alone that the alimentary canal is formed. The alimentary canal is formed by a process of folding, and Kupffer's vesicle, as the terminal part of the (postanal) gut, follows the same method. After the gut has been once folded off, the homology of the vesicle with the postanal vesicle of Selachians (instituted by Balfour in his text-book) is obvious. In each group the vesicle forms the dilated extremity of the postanal gut, and receives, or would receive if it existed, the neurenteric canal. But I think the homology is just as evident in the early stages of Kupffer's vesicle, as soon as it is recognized that the space between the entoderm and the periblast is the archenteron. In Fig. 65, Pl. XCVI, Kupffer's vesicle, *k. v.*, represents the dilated terminal portion of the archenteron, while in Fig. 88, Pl. XCVIII, it is the posterior end of a gut which has been folded off from the archenteron. The postanal vesicle of Selachians represents both. It forms the end of a gut produced in great part by folding, and it unquestionably represents the terminal portion of the archenteron.

But if Kupffer's vesicle in its early stages (Fig. 65) indicates that the terminal portion of the archenteron was primitively dilated, we naturally inquire both for the cause and for a corresponding phenomenon in the ontogeny of those animals in which the archenteron is bodily transformed into the permanent gut. As to the latter point, it would seem very common in the Amphibia for the archenteron to be thus dilated (see Morgan's figures, 32, and Götte's figure, Balfour, T. B., vol. 2, p. 105). The existence of such a dilatation in the enteron of primitive Chordata is further made probable by, and receives an explanation from, the relation of the neurenteric canal to the blastopore.

Morgan (32) has shown that in *Amblystoma*, after the neurenteric canal has been established by the closure of the upper part of the blastopore, the lower part of the blastopore remains as a common opening for the rectum and neural tube (persisting as the permanent anus). This condition he believes existed in adult early Chordata. Cunningham (8) advances a similar suggestion, and I see no reason for rejecting the idea. In the hypothetical animals which once existed with this arrangement of parts it is highly probable that the extreme end of the rectum into which the neurenteric canal opened was dilated into a kind of cloaca. And it is this cloaca which is represented in the ontogeny of vertebrates by the terminal dilatation of the postanal gut.

Balfour (T. B., vol. 11, p. 268) was the first to make this suggestion, though in a slightly different form; for being at that time unaware of the persistence of the blastopore in certain animals as the anus, he concluded that the common cavity (cloaca) was only established after the entire closure of the blastopore.

It will be seen that in the interpretation of Kupffer's vesicle I substantially agree with Cunningham (9)—it is the terminal part of the archenteron. Its significance is indeed a mere corollary of Ziegler's general interpretation of the Teleost gastrula, but on its side its existence strengthens this interpretation; for the presence of such a conspicuous cavity between entoderm and periblast can only be explained on the supposition that the space (virtual or real) between entoderm and periblast represents the archenteron.

It only remains to speak of the neurenteric canal. Various solid cords of cells have been spoken of by writers, which have been construed as representing this canal. These cords, however, have all been very ill defined and have not been represented in figures. Moreover, they have been found in entirely different places. In the Bass I have not succeeded in finding anything which could be interpreted as a neurenteric canal, nor do I believe that any representative of it exists. It is generally recognized that if the canal were present, it would open into Kupffer's vesicle. This is evident from the existence of the neurenteric streak (Fig. 82, Pl. XCVIII, *n. str.*). The canal could not, of course, open in front of the streak, and behind it the neural chord is not distinguishable from the mesoblast; or, in other words, just behind the neurenteric streak and Kupffer's vesicle lies the caudal mass of undifferentiated cells. It sometimes happens that the roof of Kupffer's vesicle is not vaulted, but forms a sharply indented arch (Fig. 82). Whether the indentation seen in such individuals has any significance may be doubted.

VI. NEURAL CHORD; SURFACE ECTODERM; EYE.

Neural chord.—In the early stages the ectoderm over the entire embryonic shield is greatly thickened (Fig. 53, Pl. XCIV) in comparison with the non-embryonic ectoderm. This wide plate of thickened ectoderm has been called by Götte the "axenplatte." Along the middle line a thickening forms the well-known "keel," and as this grows deeper the lateral parts of the plate grow thinner (the cells being used up in forming the keel), until there is finally a narrow deep keel passing at the sides directly into thin ectoderm (Figs. 57 and 58, Pl. XCV). This diminution in width of the embryonic ectoderm and formation of a neural keel is associated with a general diminution in the width of the embryonic area, as may be seen in the surface views and on comparing Fig. 53 with Fig. 57. In the Bass, in the posterior region of the embryo, there is no neural furrow (Fig. 53, Pl. XCIV; Figs. 54 and 56, Pl. XCV). In the brain and anterior trunk regions, however, there is a well-marked furrow, into which the epidermic stratum sometimes dips (Fig. 58), or as often passes over like a bridge (Fig. 57), as has been remarked by Hoffmann (17). (Henneguy is entirely mistaken when he explains the separation of the epidermic stratum from the bottom of the furrow, observed by Hoffmann, by supposing that the cells just beneath the epidermis were badly preserved). The neural furrow is a transitory feature, disappearing a short time after the stage shown in Figs. 57 and 58, Pl. XCV.

As to the manner in which the keel is formed, Götte's description (16) covers the ground pretty thoroughly. The lateral parts of the axenplatte, or the "medullar platten," as Götte calls them, crowd toward the median line. Here the "Zellenmassen nach unten ausweichen, und die Axenplatte so gewissermassen in derselben Richtung eine geschlossene Falte schlägt, was auch durch die vergängliche, oberflächliche Furche angedeutet wird." (Götte, 16, quoted from Hoffmann, 17, p. 21.) In the Figs. 53, 54, 57, and 58 the direction of cell movement in the region of the keel is easily discerned from the cell outlines, and is indicated by the arrows. Cell multiplication appears to play no part in forming the keel, which is produced from a mass of preformed material by "Zellenverschiebung." As may be gathered from the quotation, Götte believes that the "Zellenverschiebung," by which the keel (which equals a "geschlossene Falte") is built up, represents the invagination by which in most vertebrates the medullary groove is produced. I fully agree with him in this view of the process. The peculiarity of the Teleost in this respect may perhaps be expressed as follows: In the Teleost the cells which are destined to form the medullary cord are precociously developed in the requisite numbers (axenplatte). When the time comes to form the cord, the preformed cells move into their destined places, following in the main lines of movement (see arrows in Figs. 53, 54, 57) which, in the ancestor indicated the path along which the floor of the medullary groove traveled in the course of its deepening. In the ancestor, these lines of movement (see Fig. 6, arrows *b*) were (accepting for the moment the view of His and Hertwig) the resultants of the combined forces produced by cell multiplication taking place in direction of arrows *a*. In the Teleost, the causal connection between cell multiplication and direction of movement is not present, but the direction of movement itself has been retained.

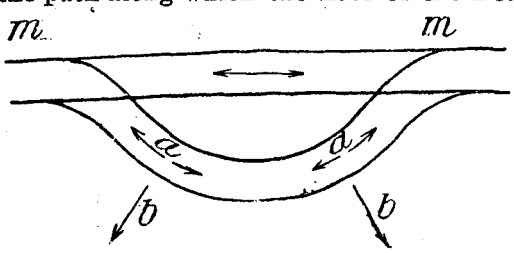

FIG. 6.—Diagram to illustrate formation of invaginate groove, as it is supposed to take place according to the "principle of unequal growth."

It is impossible here not to be tempted into carrying the comparison a little further, in the hope of getting some light thrown on the underlying principle of certain embryonic processes. One of the apparently simplest of such processes is seen when a unicellular membrane develops, by the method of invagination, a pit or a groove—as, for instance, in the development of an invaginate gastrula, or in the formation of an ideally simple neural groove. This phenomenon has received a well-known explanation, the strongest exponents of which are His and Hertwig. The principle involved in this explanation scarcely needs a description after Hertwig's name for it has been given. It is called by Hertwig (Lehrbuch, p. 58) "das Princip des ungleichen Wachsthums," and is briefly this: Suppose the membrane in the diagram (Fig. 6) to be composed of a single layer of cells. Now let rapid cell multiplication at right angles to the surface be set up over the area included between *m* and *m*. Pressure is exerted in the direction of the arrows, *a*, but meeting the lateral parts of the membrane which are not suffering cell multiplication, and hence act as rigid barriers, the effect of the pressure is first to form a groove, and then as the number of cells, and consequently the extent of surface, continues to increase, to drive the groove in the direction of arrows *b*. The mechanical simplicity of the explanation readily explains its popularity, and if true, its importance can scarcely

be overrated, for it would then give us a clear insight into that most interesting problem: what is the mechanism by which heredity works in the ontogenetic formation of organs? Accepting the explanation, the formation of a groove could be conceived of as taking place, because a certain hypertrophic stimulus had been applied to a long narrow area of the membrane. To give the stimulus was the duty of heredity, but after that heredity had no need to concern itself, for the mechanical pressure set up by the growth of the cells effected the rest.

In analyzing the "principle of unequal growth," we must give as great a precision as possible to the facts of the case. What we really see is (1) that cell multiplication takes place over the area *m* to *m*, and (2) that the area invaginates to form a groove. While it is possible that the second fact may be the mechanical necessity of of the first, it is also possible there may be no causal connection between the two. In such a problem as this, in which experimentation is out of the question, our only hope of a solution lies in a comparison of several cases where the result is the same, but where the two factors bear different relations to each other. Now, in the Teleost the factors, which in the invaginate ancestor were supposed to be causally connected, are wide apart in time. The cell multiplication takes place very early, and long after that is over the cells begin to move along lines, in general identical with those which in the ancestor marked out their path of progression. It would thus seem probable that in this case (that of the Teleost) heredity deals directly with the individual cells, though by what means a cell is induced to travel from the periphery of the axenplatte towards the axis, and is then sent out along the old lines, is assuredly beyond our present comprehension.

Returning to the ancestral type, it is clear there is not only no necessity of applying the "principle of unequal growth," but the probability is that the same relation between heredity and the individual cells which was deduced in the case of the Teleost exists here also; and that, as the new cells are brought into existence by fission, they stick together and move along their paths not because of a mechanical pressure but in obedience to what for want of a better term might be called the instinct of heredity. To sum up the case: When an area invaginates we get the impression that it does so because pressure is applied in a particular way. But the impression is due to the facts (1) that the cells migrate altogether and (2) that cell migration and fission take place coincidently. And this view of the case is borne out by the observation that cells follow the ancestral lines of migration when they are clearly not under the influence of a common pressure.

In this connection the peculiar modification of the folding process by which the alimentary canal of the teleost is produced, deserves a word. The manner in which the cells slip one under the other to take their place in the wall of the canal suggests strongly the comparative independence of the individual cells, and yet the result is the same as if a membrane had folded in the ordinary way.

Returning to the actual development of the neural cord, it is difficult to assign the proper significance to the neural furrow, *n.f.* It is clearly not homologous in the ordinary sense with the medullary groove of other vertebrates, for it disappears while the groove becomes the central canal. A careful examination of the surrounding cells (Fig. 57, Pl. XCV) leads to the conclusion that the furrow owes its existence to the fact that these cells share in the general movement indicated by the arrows. This movement we have seen represents the ancestral invagination, and thus, though the

furrow can not be said to be homologous with the medullary groove, the causes which produce the two grooves are the same. This consideration shows us, I think, that it is permissible to regard the furrow as representing the extreme upper part of the medullary groove, the chief part of which is ideally present in the middle line of the keel.

Minot has recently in a brief communication to the American Naturalist (November, 1889) urged that no good reason exists for believing the solid keel of Teleostei to be a derived structure. He regards the keel as representing an ancestral nerve cord, which arose as a thickening of the ectoderm, and hence as directly akin to the nerve cord of annelids. The formation of a canal within the keel is, for Minot, the primitive method. The open medullary groove is a secondary feature. This view does not, I think, find support in the ontogeny of the teleostean nerve cord, for all the details here point to the derivation of the keel from an open groove.

Like Götte and subsequent investigators I have not found anything which could confirm Calberla's account of the development of the keel in *Syngnathus* (10). In the Bass there is no special sheet of cells running down from the neural furrow into the keel mass.

The strange interpretation which Kupffer (26) has applied to the neural furrow in the Trout, regarding it as a primitive groove homologous with that of Amniota, has been sufficiently criticised by Henneguy (18, p. 531). The account which Miss Johnson (23) has given of a fusion of layers in the Newt, along the median dorsal line, does not find support in the Teleost, and its presence in the Newt must, I think, still be regarded as problematical.

Further development of the keel.—In the series of transverse sections (Figs. 60–64, Pls. XCV and XCVI) it is seen that the wide medullary plate disappears last in the posterior region. The neurenteric streak need not detain us. It is composed of the fused neural cord, notochord, and hypoblast. The notochordal cells are sometimes distinguishable from the rest (Fig. 66). The constriction of the keel from the surface ectoderm has begun in the neck region (Fig. 62). Fig. 63, through the brain behind the eyes, and Fig. 64, through the eyes themselves, call for no explanation. The constriction of the entire neural cord from the ectoderm is finished by the time the embryo is 45 hours old (Figs. 88 and 90, Pl. XCVIII, and Fig. 94, Pl. XCIX, through the trunk, and Figs. 95, 96, and 97, Pl. XCIX, through the head).

The formation of the central canal, cavities of the brain, and optic sacs, is accomplished in the manner described by Henneguy (18) by the simple separation along the middle line of the constituent cells. Oellacher (33) and Hoffman (17) state that the central cells disintegrate, and that thus the cavities are established. This is not so in the Bass.

In the stage when the optic sacs begin to form (Figs. 62, 63, and 64) the cells composing the brain and spinal cord are elongated at right angles to the median plane of the embryo, but they are interlocked and disposed in an irregular fashion. The beginning of this arrangement may be seen in Fig. 57, Pl. XCV. The cells thus arranged begin, as seen in transverse section, to dispose themselves in two rows (Fig. 72, Pl. XCVII) which, at first interlocked at their inner ends, gradually acquire a more even dividing surface (Figs. 75 and 78, Pl. XCVII). The process continues until the inner ends of the cells form an approximate plane (Fig. 97, Pl. XCIX). The separation of the opposing surfaces takes place first, at least in the spinal cord, at the upper and lower edges of the canal (Fig. 90, Pl. XCVIII, Fig. 94, Pl. XCIX). The characteristic shape of the

embryonic *canalis centralis* is then acquired (Fig. 102, Pl. C; Fig. 127, Pl. CIII). When the central canal is first established I am disposed to think the wall of the canal is everywhere but one cell thick (Fig. 90, Pl. XCVIII). It is difficult, though, to make sure of this in sections (see Fig. 94, Pl. XCIX). After the canal is once established the wall increases in thickness (Fig. 110, Pl. CI), but even at the time of hatching the posterior end of the cord has a unicellular wall (Figs. 119 and 126, Pl. CII). Likewise in the brain when the cavity is first established, the wall is but one or at most two cells thick (Figs. 95, 96, and 97, Pl. XCIX).

General development of the embryonic brain.—At the time of hatching, the brain is not only histologically undifferentiated but is morphologically exceedingly simple. From the surface views alone (Figs. 146–150, Pls. CVI and CVII) almost the whole development may be gathered. The cerebral vesicles of the higher vertebrates, which make their appearance in the Trout, are not developed in the Bass; at least I have never found a stage in which they were present. In Fig. 146, Pl. CVI, the optic sacs and that part of the brain with which they communicate have been hollowed out. Elsewhere the neural cord is solid. In Fig. 147, Pl. CVI, the central canal and its cerebral continuation are established, and there is a constriction (*m. con.*) marking off the anterior from the posterior part of the brain. The swollen portion of the brain directly in front of the constriction develops into the mid-brain while the portion behind the constriction becomes the medulla. It will be seen that at first the medulla is narrower than the mid-brain. In Fig. 148 the fourth ventricle is developed (section given in Fig. 96, Pl. XCIX), and in connection with the appearance of this cavity, aided also by the increase in thickness of its own walls, the medulla has become much wider than in preceding stages, and considerably exceeds the mid brain in width. A longitudinal section of this stage is given in Fig. 83, Pl. XCVIII. Figs. 149 and 150, Pl. CVII, are views of the brain from below and above, respectively, not long before hatching. The stage drawn in Fig. 150 is somewhat the older, but the difference scarcely concerns the brain. The constriction between the medulla and mid-brain is a deep one, but the increase in width of these two parts of the brain involves especially the dorsal surface, as may be seen on comparing the two surface views, or in the sections Figs. 131–133, Pl. CIV, and consequently both the eye and the ear are overarched by the medulla. Two folds, the subsequent history of which I have not followed out but which would seem to form the cerebellum, appear in this stage (*cer. f.*, Fig. 150). The optic nerves come off from the ventral surface of the brain and run into the anterior parts of the optic cups. The part of the forebrain in front of the optic nerves which will develop into the hemispheres is small and undifferentiated.

An examination of a series of transverse sections through the brain at the time of hatching will supplement this description of the embryonic brain. Figs. 128–131, Pl. CIII and CIV, are successive sections from behind forwards through the medulla. The increase in thickness of the walls and general change of shape in comparison with earlier stages is seen on referring to Fig. 96, Pl. XCIX. Fig. 123, Pl. CII, is through the cerebellar (?) folds (*cer. f.*). Fig. 133, Pl. CIV, is just in front of the cerebellar folds; the thin roof of the fourth ventricle here would seem to correspond to the valve of Vieussens. Fig. 134, Pl. CIV, is through the mid-brain and shows the iter and developing optic lobes. The infundibulum (*Inf.*) in its course backwards is met with in this section. Fig. 124 is a little in front of the last section, and from a slightly younger stage. The part of the brain here met with is the third ventricle, as

is shown by the presence of the infundibulum. The latter in this stage has barely begun to grow backwards. Its composition of long columnar cells needs no description. Fig. 135, Pl. CIV, is through the anterior part of the eyes. The third ventricle here sends up a dorsal process, presumably towards what will become the pineal gland. Ventrally the optic nerves (*op. n.*) are met with. The most peculiar feature in the embryonic brain is clearly the great forward extension (Fig. 134) of the thin roof, characteristic of the fourth ventricle. The condition of the spinal cord at the time of hatching is sufficiently indicated in the Figs. 119 and 126, Pl. CII, Fig. 127, Pl. CIII, representing transverse sections from the extreme posterior end forwards.

The histological differentiation into nerve cells and fibers begins both in the spinal cord and brain shortly (about 12 hours) after hatching. A peripheral accumulation of fibrous matter is formed, and some of the peripheral cells abandon their simple elongated embryonic shape, and assume the appearance of rounded nerve cells, having in general a single process (Fig. 136, Pl. CIV, spinal cord). The transformation of the elongated embryonic cells into rounded nerve elements proceeds rapidly, and on the second day after hatching there are no embryonic cells to be seen (Fig. 139, Plate CV, larva of 112 hours). During the histological transformation of the cells the canal of the spinal cord also loses its embryonic shape. The successive stages in the metamorphosis of the canal are shown in Fig. 136, Pl. CIV, and Figs. 139, 141, and 143, Pl. CV. The closure progresses gradually from the edges towards the center. The distribution of the fibrous matter on the fourth day after hatching (larva 160 hours) is shown in Fig. 143, Pl. CV. It is only on the third or fourth day after hatching that the spinal nerve roots can be made out (Figs. 141 and 143, *d. n. r.*, *v. n. r.*), and then they are so very small that their presence alone can be demonstrated. So with most of the cranial nerves, their minute size renders it impossible to follow their development.

Histological differentiation of the surface ectoderm.—The thin membranous ectoderm of the non-embryonic area has already been mentioned. It is composed of the epidermic stratum, and one or two strata of flattened cells. At the end of embryonic life, when the yolk sac begins to disappear, the ectoderm covering it gradually becomes thickened while the rest of the ectoderm grows thin (Figs. 139–141, Pl. CV). It is thus brought about that the yolk ectoderm in the larval stages is thicker than the rest of the layer.

After the wide neural plate (axenplatte) has been transformed into a deep keel, the ectoderm of the embryo, except in the immediate neighborhood of sense organs, is made up of the epidermic stratum and two strata of "nervous layer" cells. Its condition just before the separation of the neural cord is shown in Fig. 81, Pl. XCVII, part of a section, such as Fig. 75, Pl. XCVII. The further development of the general ectoderm during embryonic life consists in the vacuolation of the outer stratum of the nervous layer (1, Fig. 81), and the flattening of the inner stratum (2, Fig. 81). The vacuolation has begun in Fig. 81. Almost all of the cells of the outer layer become vacuolated, and usually there is in each cell a single large vacuole, as is shown in Fig. 112, Pl. CI, a more highly magnified view of the ventral portion of a section such as Fig. 111. Inside the vacuolated layer is the second stratum made up now of flattened cells. This condition of the ectoderm is indicated in the figures of the later embryonic stages, such as Fig. 126, Pl. CII.

The continuous dorso-ventral fin of the larva is indicated at a comparatively early stage in embryonic life by a groove in the nervous layer of the ectoderm (*f. gr.*, Figs. 88, Pl. XCVIII; 99, Pl. XCIX). The groove first appears just before the tail begins to fold off, at the posterior end of the body and on the dorsal side (Fig. 88, *f. gr.*). As the tail is folded off the groove extends round its tip to the ventral side (Figs. 99, 100, Pl. XCIX), and as the folding off of the tail continues, the formation of the groove progresses in an anterior direction on both dorsal and ventral surfaces. In the Bass, as in other Teleosts, after the tail has grown out a short distance it becomes laterally compressed, the dorso-ventral fin fold (*d. f.*, *v. f.*, Figs. 111, 112, Pl. CI; 119, Pl. CII) developing at the same time. By this means the tail end of the embryo is made to lie against the yolk on its side. From the start the cells which line the fin groove (*f. gr.*, Figs. 88, 99), and which belong to the second stratum of the nervous layer, are larger and not so flattened as the surrounding cells. When the fin fold begins to develop (Fig. 112) the groove extends into it, the cells of the second nervous stratum retaining an approximately cubical shape. The cavity of the fin fold (Fig. 112) disappears before the close of embryonic life, but the double sheet of nonvacuolated cells persists.

Eye.—The development of the eye does not differ from the generally accepted account for the Teleosts, except in regard to the lens, and it may therefore be run over very briefly. The solid optic sacs (Fig. 64, Pl. XCVI) are separated from the brain by a fissure which extends from above downwards and, as in other Teleosts, from behind forwards, and hence the position of the optic nerves in later stages (Figs. 135, Pl. CIV, and 149, Pl. CVII). The cavity of the optic sac is established in the way already described for the neural cord, and the wall of the sac is at first made up of a single layer of cells (compare Fig. 72, Pl. XCVII, through the still solid optic sac, with Fig. 80, Pl. XCVII, after the cavity is formed). In the folding of the optic sac to form the optic cup there is nothing which calls for special attention. The inner layer of the cup thickens and forms the retina, the outer layer becomes transformed into the ordinary stratum of flat cells (pigmented epithelium of the choroid, Figs. 80, Pl. XCVII, 97, Pl. XCIX, 108, Pl. C). The choroidal fissure is still present at the time of hatching (Fig. 151, Pl. CVII).

The development of the lens in the Bass affords an interesting illustration of how one modification in the embryo leads to another. In the trout the head region of the embryo is early lifted up above the yolk, so that it is possible for the lens to develop, as it does in the ordinary position. It is developed as a solid thickening in which the epidermic stratum takes no part (Henneguy). In the Bass, however, the whole embryo, head as well as trunk, is buried in the yolk (Fig. 97, Pl. XCIX), and it is only late in embryonic life, long after the lens has formed, that the head begins to be folded off (Figs. 130, Pl. CIII, and 135, Pl. CIV). On glancing at Figs. 72, Pl. XCVII, 80, Pl. XCVII, and 97, Pl. XCIX, it is seen that it is impossible, because of the reason just mentioned, for the lens to develop on the side of the head, as in other vertebrates—the layer here consisting of periblast (*p*) and not ectoderm.

The lens makes its first appearance while the optic sacs are still solid, as a thickening of the nervous layer of the ectoderm. Its position is on the dorsal surface in the angle between the ectoderm and periblast (*ln.* Fig. 72, Pl. XCVII). The thickening becomes transformed into an open invagination which grows down between the optic cup and the periblast (*ln.*, Fig. 80, Pl. XCVII). The inner wall of the invagination is made up of columnar cells (*c. ep.*), and is therefore conspicuous, but the outer wall is

composed of small flattened cells and is apt to escape observation, especially as its continuity is often broken. In stages subsequent to that shown in Fig. 80 the outer wall is not found (Fig. 97, Pl. XCIX), the lens being derived exclusively from the inner wall of the columnar cells. When the optic cup begins to form, the layer of the columnar cells (*c. ep.*) also suffers invagination, and the concavity thus established begins to be filled with cells (*ln.*, Fig. 97, on right side).

In this condition the connection of the layer of columnar lens cells with the surface ectoderm is maintained by a few flattened and rather irregularly shaped cells. The connection is, however, broken (left side of Fig. 97) before the lens becomes transformed into a solid mass, which is brought about by the increasing accumulation of cells in the above-mentioned concavity. After the invagination of the original columnar layer and the subsequent cell proliferation have converted the lens into a solid spherical mass, the superficial cells take on a columnar shape, while the inner cells become irregularly polygonal (Fig. 108, Pl. C). The further development of the lens was not traced with any exactness, though its condition some 15 hours before hatching is shown in Fig. 118, Pl. CII, where the distinction between the lens epithelium (*l. ep.*) and the lens fibers (*l. f.*) is obvious. The late development has probably nothing of especial interest, but the early development is a clear case of adaptation to the great delay in the folding off of the head.

VII. EAR; BRANCHIAL SENSE ORGAN; LATERAL LINE.

It has been noticed in the trout that the anlage, which was supposed to develop into the ear, is remarkably long (Oellacher). I have found that this anlage not only gives rise to the ear but to a functional branchial sense organ and to the organs of the lateral line as well. Before the blastopore closes there is found behind the eye a long shallow furrow (the sensory furrow, Fig. 62, Pl. XCV, *s. f.*) in the nervous layer of the ectoderm, the epidermic stratum sometimes passing over as a bridge, and sometimes filling the concavity with a few more or less detached cells. The transverse section (Fig. 62) shows that the groove is lined by nearly columnar cells. By the time the blastopore closes, the furrow is more marked, and presents in surface view the appearance shown in Fig. 146, Pl. CVI. The nervous and epidermic layers are henceforth distinctly separated, the latter always bridging over the groove. Partly owing to the convexity of the dorsal surface of the embryo, and partly to the greater thickness of its proximal than its distal wall (see Figs. 68–71, Pls. XCVI and XCVII), the sensory furrow has, when viewed from above, the aspect shown in Figs. 146 and 147, Pl. CVI; it is only the proximal wall that is obvious. At two points the furrow begins to deepen (Fig. 146, *a. s.*, and *B. s. o.*), the deepening taking place downwards and inwards. At these two points the auditory sac and the branchial sense organ will respectively be formed. Anteriorly and posteriorly the furrow with its thickened wall dies away in the general ectoderm. Its extent from behind the eyes nearly to the somites is indicated in the figure.

In Fig. 147, Pl. CVI, a further stage in the development of the sensory furrow is shown. The deepening of the furrow in the auditory and branchial sense organ regions has continued until there are now two well-marked sacs, the anterior of which is the branchial sense organ (*b. s. o.*), the posterior the auditory sac (*a. s.*). Between

the two sacs persists the connecting portion of the sensory furrow, and behind the auditory sacs the furrow is continued for some distance. The posterior portion (*l. l.*) of the furrow constitutes the anlage of the lateral line.

In the next stage (Fig. 148, Pl. CVI) the sensory furrow has definitely separated into its three derivatives. The auditory sac has closed, and with this closure was naturally brought about the division of the furrow. The three derivatives at this stage have not yet begun to move away from each other. In a subsequent stage (Fig. 149, Pl. CVII) they are, however, wide apart. In this stage the gill slit (*g. s.*) has broken through, and just in front of it is the branchial sense organ. The auditory sac is already overgrown (see dorsal view) by the medulla, and the anlage of the lateral line (*l. l.*) has moved some distance backwards from its original position in front of the somites. In a still later stage (Fig. 150) the lateral line anlage has grown still farther back, and is incompletely divided into three "sense organs of the lateral line."

After this brief survey we may return for a more detailed description of these parts. The series of transverse sections (Figs. 68–71, Pls. XCVI and XCVII, numbered from behind forwards) is from a stage slightly less advanced than Fig. 147, Pl. CVI. The anterior section (Fig. 71) is through that portion of the furrow which has already begun to differentiate itself into the branchial sense organ (*b. s. o.*). The greater thickness of the proximal wall of the groove, and the cavity of invagination need no description. In going backwards from this section an unbroken though comparatively shallow furrow leads to the auditory sac (Fig. 70). Here the invagination has a greater width and depth than at any other spot. In passing towards the posterior limit of the auditory sac the invagination draws away from the brain (Figs. 68 and 69), as is indicated in the surface view, Fig. 147. In Fig. 70, through the middle of the ear sac, where the invagination is nearest the brain, it is impossible to speak of an auditory nerve, for here there is direct continuity between the brain cells and the lining cells of the invagination. The auditory nerve becomes recognizable as a proliferation of cells only where the sac is separated from the brain by some of the surface ectoderm (Fig. 69, *a. n.*). Behind Fig. 68 the invagination becomes shallower and narrower, constituting the anlage of the lateral line.

Figs. 78 and 79, Pl. XCVII, are from a stage intermediate between Figs. 147 and 148, but in which the auditory sac had closed. Fig. 79 is through the branchial sense organ (*b. s. o.*), which is deeper and of a more compact shape than in early stages. Fig. 78 is through the posterior part of the closed auditory sac (*a. s.*) and the anterior part of the lateral line (*l. l.*). As the section indicates, though the sensory furrow has broken up into its several parts, these parts still closely adjoin one another.

Now that the history of the sensory furrow has been carried to this point, it will be more convenient to treat each derivative separately.

Ear.—The ear, after its constriction from the surface and the rest of the sensory furrow, forms a closed sac, the wall of which is made up of columnar cells (Fig. 95, Pl. XCIX). One side of the sac applies itself pretty closely to the medulla, and during later stages the cells of this wall become greatly flattened. Two stages are given in Figs. 114, Pl. CI, 130, Pl. CIII, the latter showing the condition of the ear at the time of hatching. At this time the columnar sensory elements are restricted to a rather closely circumscribed area. The overarching of the ear by the medulla has already been mentioned.

Branchial sense organ.—After its separation from the ear the branchial sense organ has the character of a sac, one wall of which is much thicker than the other, and over the cavity of which passes the epidermic stratum. Its appearance in surface view is given in Fig. 148, *B. s. o.*, and a transverse section through a corresponding stage in Fig. 96, Pl. XCIX. Anteriorly and posteriorly, as may be seen in the surface view, the sac is not sharply delimited. The further development of the organ consists in the loss of its cavity, in histological differentiation, and in the transformation of its ill-defined anterior extremity into two cellular cords which doubtless serve as a source for the production of new organs.

On comparing Fig. 96, Pl. XCIX, with a slightly older stage, Fig. 106, Pl. C, it is plain that the cavity of the sac has grown shallower, and with this change the epidermic stratum has dipped into the cavity, which it now lines (Fig. 106). The effacement of the cavity, begun in this way, is continued until the originally conspicuous cavity is reduced to an extremely shallow indentation (Fig. 115, Pl. CI), and at the time of hatching (Fig. 131, Pl. CIV, *B. s. o.*) even this indentation seems to have disappeared.

Coincidently with the loss of its cavity the sense organ changes its shape, as may be seen by glancing through the sections, Figs. 96, Pl. XCIX, 106, Pl. C, 115, Pl. CI, 131, Pl. CIV, and the surface views, Figs. 148, 149 and 150. Whereas in the earlier stages it is a vaguely delimited sac, in the later stages it is a sharply defined superficial sense patch, of an oval shape, from which there runs forward a short sensory cord, *a. s. t.*, Figs. 149 and 150. Histological modification accompanies the change of shape. In Figs. 96 and 106 the cells are embryonic columnar cells, but in Figs. 115 and 131 the organ is largely composed of peculiar sense cells, the nuclei of which are basal. Some of these cells terminate in short stiff hairs which project from the surface. No macerations were made, and I am consequently not able to go any farther into details of the histology, which appears to be identical with that of the sense organs of the lateral line. It is plain that the organ is functional during the later stages of embryonic and during larval life. Its position directly in front of the embryonic gill slit is shown in the surface views.

When the sense organ assumes its definite shape, there is left in front of it a narrow tract of columnar cells in perfect continuity with the sense organ and obviously derived from the anterior part of the anlage shown in Fig. 148. This tract of cells is conspicuous in surface views of some stages (Fig. 149, *a. s. t.*), but towards the time of hatching it becomes difficult to see in such views, though sections show that it not only persists but continues to develop. The entire anterior extension of the sense organ may be spoken of as the forward sensory tract. This tract differentiates into two tracts, one of which runs directly forwards (anterior sensory tract) while the other runs somewhat dorsally (dorso-lateral tract).

Figs. 106 and 107, Pl. C, belong to the same series of sections and are from a stage intermediate between Figs. 148 and 149. Fig. 107 is through the anterior part of the forward sensory tract where it differentiates into the anterior tract (*a. s. t.*) and dorso-lateral tract (*d. l. s. t.*). Figs. 115 and 116, Pl. CI, are from another series and belong to a stage about like Fig. 149. Fig. 116 shows the connection of the two sensory tracts at the anterior end of the whole forward extension. Near the time of hatching the dorso-lateral tract extends a short distance in front of the anterior tract, occupying the position shown in Fig. 123, Pl. CII, *d. l. s. t.* During larval

life one or two sense organs are found in this region, and it is extremely probable that they arise from the dorso-lateral tract. Indeed this is made nearly certain by the condition of the anterior end of the tract at the time of hatching (Fig. 132, Pl. CIV). It is here larger than elsewhere, and the cells begin to assume the appearance of sense cells, as if a sense organ were going to form *in situ* as a modification of a particular part of the cord. This calls to mind Allis's paper on the lateral line of *Amia* (2), in which he describes sense organs originating at various spots along a growing cord by local cell proliferation. The significance of this method of multiplication of sense organs will be discussed after the formation of the lateral line has been described.

The anterior sensory tract is at the time of hatching very short, and just what becomes of it I do not know. Nor do I know whether the gill slits, which are subsequently formed, have branchial sense organs. If they have, the organs must be extremely inconspicuous compared with the single embryonic organ.

I think Hoffmann must have seen the branchial sense organ in the embryo of the trout, for on page 7 (17, 1883) he gives a wood cut (surface view) in which the organ is shown fairly well, though he calls it an embryonic "Spritzloch." Hoffmann must surely be wrong in his statement that there is an embryonic spiracle in the teleost. What he figures as such in his sections is the embryonic gill slit, which does not disappear at all, as he states is the case.

Lateral line.—As has been said, it is the posterior end of the common sensory furrow which is transformed into the anlage of the lateral line. In Fig. 147 the general character of this end is indicated. The furrow is here long, narrow, and (compared with the auditory invagination) shallow. Becoming independent on the closure of the auditory sac, the lateral line anlage forms an elongated narrow sac, the opening of which is bridged over by the epidermic stratum. Its appearance and position, just behind the ear, is shown in Fig. 148, *l. l.* A section through the anterior end of the lateral line at this stage is given in Fig. 78, Pl. XCVII, *l. l.*

Once independent of the rest of the furrow, the cavity of the lateral line invagination begins to deepen, becoming at the same time narrrower, while the lining cells grow more columnar. Fig. 105, Pl. C, represents a section through a stage four hours older than Fig. 148 and shows these changes in the character of the lateral line. The cavity is sometimes a simple narrow slit as in the "line" on the right side, and sometimes it is dilated at the bottom as on the left. At the ends of the lateral line the cavity is considerably wider, especially in its upper portion where the lining cells become continuous with the surface ectoderm, than in the middle; on the left side of Fig. 105 the section cuts the anterior end of the line. When the section is unbroken the continuity of the lateral line cells with the nervous layer of surface ectoderm is perfect, as in the figures. But sometimes the "line" parts from the ectoderm and appears in sections as, at first sight, a closed tube lying on the mesoderm. Examination, however, always shows the incompleteness of the apparent tube. The attachment to the ectoderm is stronger at the ends than elsewhere, and occasionally the "line" will separate from the ectoderm along its length, remaining attached at one or both ends. I have mentioned these imperfect sections because it was probably such that led Brook (4) to the conclusion that the Wolffian duct splits off from the ectoderm in the Teleosts. As will be seen later, I agree with the majority of investigators

in deriving the Wolffian duct from the coelom. What Brook mistook for the duct was very probably the lateral line anlage.

While the anlage remains an undivided sac the surface view, Fig. 149, and the section, Fig. 105, fairly represent its character. During its existence as a simple sac it increases in length considerably and at the same time travels from its early position in front of the somites to a position some little distance behind the first or second somite (compare Figs. 149 and 150). As to the manner in which the sac travels backward, I can only say that it does not plow its way through the surrounding passive ectoderm, as Beard (5) states is the case with the lateral line anlage of Selachians. Its continuity with the surrounding ectoderm prohibits this idea.

The formation of the separate sense organs begins some 10 or 15 hours before hatching. The elongated sac suffers a constriction and gradually becomes divided into two parts, which are at first connected by a strand of cells. During the constriction the backward growth of the whole anlage continues, so that the final position of the first (anterior) lateral line organ is some distance behind the first somite (Fig. 150, *l. l. o*^{1}). Of the two parts into which the sac is divided, the anterior is the smaller and becomes the first (anterior) lateral line organ. The posterior portion by continued division gives rise to the remaining organs.

By the time the sac is divided into two distinct parts (Fig. 150, right side) a general change in the character of these parts, as contrasted with the early condition of the organ, is perceptible. The change especially affects the anterior part, as may be seen on comparing Fig. 113, Pl. CI, with Fig. 105. Fig. 113 is through the anterior half of the parent sac, which is connected with the posterior half by a short strand of cells. In the figure it is seen that the wide mouth of the parent sac no longer exists, and the connection of the sense-organ cells with the surface ectoderm is also of a different character The sense organ appears to have been constricted off from the general ectoderm, and in consequence the nervous layer as well as the epidermic stratum now passes over the mouth of the cavity. The cavity has also changed its character. It is much shallower and (compare Fig. 150, *l. l. o*^{1}.) is indeed almost spherical. It continues to grow shallower (Fig. 120, Pl. CII, *l. l. o*^{1}.) until it finally disappears (Fig. 127, Pl. CIII) or perhaps is represented by the slight superficial concavity which the sense organs of the lateral line possess at the time of hatching. During the later stages of its existence the cavity is so sharply outlined (Fig. 120, Pl. CII) as almost to suggest the presence of a cuticle. I have no stages in the development of the sense organ between that shown in Fig. 120 and the condition in Fig 127, but the path followed is probably the same as that pursued by the branchial sense organ: as the cavity of the organ continues to flatten out, the surface ectoderm dips into and lines it; only in this case the nervous layer must be pressed aside, for in the perfect organ (Fig. 127, Pl. CI) the sense cells are covered by the epidermic stratum alone. The histology of the sense organs of the lateral line is quite like that of the branchial sense organ already described.

The constriction by which the first lateral line organ is separated from the surface ectoderm affects all the rest of the original anlage. Both the connecting strand of sense cells and the posterior portion of the original sac are separated from the epidermic stratum by the nervous layer. Figs. 122 and 121, Pl. CII, are sections through different stages in the formation of the connecting strand (*con. st.*). In Fig. 122 the arrangement of the cells indicates the former presence of a cavity and suggests its

gradual obliteration by the elongation of the connecting strand. Fig. 121 shows the character of the strand commonly met with, in which the cells have no especial arrangement. The connecting strand continues to elongate, because of the backward growth of the posterior portion of the original anlage, until it becomes excessively thin and finally disappears. At the time of hatching I am unable to find any trace of it. However, my inability to trace the origin of nerves in the Bass shows that the apparent disappearance of the strand is not a conclusive argument against the supposition (Beard, 6) that it may persist as a fine nerve thread.

The posterior of the two parts into which the first constriction separates the original anlage is, as I have said, the larger of the two. In transverse section it presents about the same appearance as the anterior organ in Fig. 113, Pl. CI. Its cavity is, however, a much elongated one (Fig. 150, right side). This portion of the primitive sac suffers a constriction which begins before the first connecting strand has disappeared (Fig. 150, left side). Of the two parts into which it is separated the anterior (*l. l. o*^{2}., Fig. 150, left) becomes the second lateral line organ. The condition of this organ at the time of constriction is fairly represented by Fig. 120, Pl. CII. Its cavity subsequently disappears, and at the time of hatching it is a histologically differentiated organ (*l. l. o*^{2}., Fig. 126, Pl. CIII), lying just in front of the anus.

The posterior part of the original anlage, which the second constriction pinches off (Fig. 150, left), presents at first about the same appearance in section as the organ shown in Fig. 120, Pl. CII. It lies in front of the anus. At the time of hatching, some 10 hours after the constriction begins, this portion of the lateral line anlage forms a rod of cells lying behind the anus, on which sometimes one, sometimes two, sense organs are found in process of forming. The rod lies like the sense organs in front of it, along the middle line of the lateral surface, and I could not distinguish it in surface preparations. The series of sections, 126^{i}–126^{iv}, Pl. CIII, however, satisfactorily elucidates its nature. Each section represents the lateral line region of the opposite sides. Glancing through the series it will be seen that the rods do not lie exactly opposite each other, and that moreover on one side two sense organs are indicated, on the other but one. The section 126^{i} is the most anterior of the series and shows that on the left side the rod does not extend so far in front, while on the right there is a sense cord (*s. c.*) which, however, extends but a short distance in front of the sense organ (*l. l.* o_3, 126^{ii}) and must be regarded as the anterior end of that organ. Fig. 126^{ii} lies four sections farther back. On the left there is no sense cord; on the right there is a sense organ (*l. l.* o_3.) in process of forming. It is in about the same stage as that of Fig. 120. Going backwards from Fig. 126^{ii} to 126^{iii} the sense organ on the right gradually passes into the connecting strand (*con. st.*); on the left a short rod of cells is first met with which proves to be the anterior end of the sense organ, *l. l.* o_3^1. Continuing back from 126^{iii} to 126^{iv} on the right, the connecting strand passes into another incipient sense organ, *l. l.* o_4., but on the left there is behind the organ, *l. l.* o_3^1., nothing but a simple cord of cells, which comes to an end not far behind 126^{iv}. The organ *l. l.* o_4. is likewise prolonged backwards as a short cord of cells. In no part of the postanal sense tract on either side has histological modification set in.

From the series of sections it is plain that the cord of cells on the right is suffering constriction and is thus giving rise to two organs, *l. l.* o_3. and *l. l.* o_4. On the left there is no constriction and but one organ is forming. The cavity in each of these

postanal organs is probably a remnant of the cavity of the original lateral line anlage, and the manner in which the organs are formed appears to be essentially like that pursued in the case of the anterior organs; but henceforth whatever new organs are formed must follow a different course of development, for the cavity of the original anlage has, so to speak, been used up, and the posterior part of the sense tract is now a simple cellular cord without any cavity at all. It would seem probable that this terminal sense cord continues to grow backwards, developing sense organs along its course by local proliferation. The probability rests on the presence of a terminal simple cord in the tail, on the existence of sense cords in the head, and on the formation of sense organs along such cords in *Amia* (2).

The lateral branch of the vagus, which innervates the lateral line organs and which is so evident in Selachian and (Hoffman, 21) Trout embryos, can not be distinguished in the Bass during embryonic life, nor could I make it out in larvæ of 2 or 3 days.

COMPARATIVE.

Common sensory anlage.—As far as I know, the formation of the ear, lateral line anlage, and branchial sense organs, by the division of a common anlage, has never been recorded before. I was consequently pleased to find a couple of figures in one of Kupffer's papers (26, 1884) which strongly suggest that the organs are formed in the same way in the Trout; Kupffer's figures, which are surface views (Taf. 11), show that at first there is on each side of the neck one sac, then two, and then three. The structures appear in the figures to be hollow, but Kupffer says they contain a central mass of loosely connected cells. Kupffer describes the structures as branchial arches and says the ear arises independently of them. Unfortunately no sections are figured in the paper, and it is therefore impossible to decide whether Kupffer is right in his interpretation or not. The general resemblance of the "schlundbogen" in his Fig. 14, Taf 11, to the series of sense organs in my Fig. 148 is certainly very marked.

The fact that there is in the Bass a common anlage for the ear, branchial sense organ, and lateral line has certainly no phylogenetic significance. It can only be regarded as a convenient method of forming these organs, which the embryos of certain animals have adopted. It however serves to emphasize in a striking way the serial homology between the organs which previous work has already made so probable.

Branchial sense organ.—The existence of a histologically differentiated sense organ, which bears such obvious relations to the gill slit as does that of the Bass, makes one wish that the fate of the so-called primitive branchial sense organs of Selachian embryos were better known. In spite, however, of our ignorance regarding the precise fate of these organs, it seems impossible to avoid the homology between what I have called in the Teleost a branchial sense organ and the patch of thickened ectoderm which Beard (5) describes above each gill slit in the Selachian. There also seems good reason for accepting Beard's belief that these "primitive branchial sense organs" indicate the position of a series of sense organs of very ancient origin. We are thus led up to the rather surprising conclusion that while in the Selachians the position of these ancient organs is only indicated by thickened patches of undifferentiated ectoderm, in the highly modified group of Teleostei one at least of the organs has been retained in a functional condition, though probably very different in structure from the ancestral organ.

Beard (*l. c.*) supposes that the sense organs, primitively situated one above each gill, gradually increased in number and spread over the head. The phylogenetic method of increase he thinks was probably division, and in this I agree with him, basing my belief chiefly on the development of the lateral line organs in the Bass. In actual ontogeny the method generally employed would seem to be the formation of a sensory cord which grows out from a primitive center and along which sense organs develop by local proliferation (2).

The serial homology of the nose, ear, and branchial sense organs, so strongly supported by Beard, receives new confirmation in the development of the Teleost, though the original sac-like character of the branchial sense organ (lateral line organs also) in the ontogeny of the Bass makes it, I think, necessary to alter the details of the comparison instituted by Beard between these organs. Beard believed, and it is generally so believed, that the primitive condition of the segmental sense organ of vertebrates was that of a superficial sense patch, something like the lateral line organs of larval fishes. In the auditory and olfactory regions the originally simple sense patch gave rise by division to a number of such organs, which were confined to a small area. The whole area subsequently became invaginated to form respectively the auditory and olfactory organs, the sac-like structure of which is therefore highly secondary as compared with the superficial sense patch. Now, according to this view, it is difficult to account for the fact that the branchial sense organ and the (embryonic) organs of the lateral line originate in the Teleost as sacs, which subsequently flatten out into the well-known superficial sense organs. If the latter condition represents the primitive condition in the vertebrates, why need the organs go to the trouble of running through such a complicated metamorphosis? It is, of course, impossible to reach a decision in regard to this point when the known facts are so few, but for the present it must be borne in mind that there is at least a possibility that the sac-like condition of the organs in the embryo Bass represents a phylogenetic stage; and in view of this possibility, Beard's theory of the origin of auditory and nasal sacs from a collection of segmental sense patches can not be accepted, for there is at least as much to be said for the other theory, viz, that the ear and nasal sac represent single sense organs. According to the latter view the nasal sac (which arises in the Teleost as a simple invagination) has retained more closely than any of the other members of the series the structure of the ancestral segmental sense organ; the ear has been shut off from the surface and transformed into a closed vesicle, while the remaining organs have been flattened out into superficial sense patches.

Lateral line.—Beard has described the formation of the lateral line in the Salmon (6) as taking place in the following way: In the region of the neck just behind the ear a cord of cells is split off from the nervous layer of the ectoderm; the cord grows backwards along the whole length of the embryo; it then becomes thickened in each segment of the body, the intervening parts growing thin and finally passing out of sight, though the author thinks they may still persist as fine nerve strands. The thickened parts become the sense organs. The development of a sense bulb from one of the segmental thickenings takes place in the following manner: Certain of the cells which are next the outer surface lengthen until they reach the surface of the body, when they acquire terminal hairs; the remaining cells arrange themselves round the base of these cells as a center.

It will be seen that the chief points of difference between Beard's account and my own are two: (1) In the Bass the anlage of the lateral line is an elongated sac; in the Salmon it is said to be a cord of cells. (2) In the Bass the anlage divides into the separate sense organs during its growth backwards; in the Salmon there is first formed a continuous cord, and then special parts of it become thickened to form the sense organs, the intervening parts disappearing. The formation of a continuous cord may easily be looked on as a secondary modification of the method displayed in the Bass: the division into separate organs has merely been delayed until the anlage has grown all the way back.

Hoffmann's (21) account of the development of the lateral line is not so easily brought into harmony with my description. According to Hoffmann the lateral nerve develops some time before the sense organs. The former arises as a histologically modified cell string, which is at first a part of the nervous layer of the ectoderm. The string gradually moves out of the ectoderm, coming to lie at some distance internal to it, but at certain points connection is retained with the nervous stratum. The cells establishing this connection become the side twigs of the lateral nerve (each leading to a segmental sense organ). At the point of connection with the side twigs, the cells of the nervous layer become histologically modified and form a sense organ. Hoffmann points out the difference in the development of the two parts of the lateral line in the following words:

Zwischen den ersten Anlage des Ramus lat. nerv. Vagi und den der Sinneshügel besteht also nur dieser Unterschied, dass Erstgenannter in einem sehr frühen Entwicklungs-stadium auftritt und nicht segmentirt sich anlegt, während die Sinneshügel der Seitenorgane erst in einer viel späteren Periode der Entwicklung, zur Ausbildung kommen und *gleich vom Anfang an segmentirt sind.*

It seems impossible to reconcile Hoffmann's description with the development of the Bass. For in the latter the origin of all the lateral line organs from a single cervical sac is unmistakable, while according to Hoffmann they arise *in situ* by local modification of the ectoderm. The development of the Bass is so very clear in this respect that I do not think Hoffmann's account can be accepted without confirmation.

In the Selachians (Beard, *l. c.*) the lateral line anlage consists of a thickened stripe of ectoderm, made up of the "primitive branchial sense organs" of the last three or four gill slits. This anlage grows backward, plowing its way through the indifferent ectoderm and becoming transformed into the lateral nerve and the lateral line proper. The exact manner in which the lateral line itself develops has not been worked out very satisfactorily. In the main point, though, there is an agreement between the Selachians, Salmon (Beard, 6), and the Bass: the organs of the lateral line originate as an anlage confined to the cervical region. It is difficult to homologize precisely the lateral line anlage in the Bass and Selachian, for while in the latter the anlage is composed of the sense organs of the last four gill clefts, counting in the rudimentary cleft, in the former it lies behind the gill clefts and hence represents only those which have disappeared. It would thus seem that the anlage in the Teleost contains two less branchial sense organs than in the Selachian.

The agreement between the forms mentioned above, as to the origin of the lateral line anlage, is strong evidence that Beard's conception of the lateral line is the true one. The lateral line is, for Beard, comparable with a tract of head sense bulbs. The latter tract phylogenetically arose by the multiplication of a primitive branchial sense

organ, the multiplication taking place along a line which gradually extended farther and farther from its starting point. In the case of the lateral line the phylogenetic development has been the same; the sense organs of the last few gill slits instead of sending out tracts over the head proliferated in a backward direction along the middle line of the lateral surface of the body, and so gave rise to a row of organs stretching far beyond the region (branchial) to which they were originally confined. Balfour foreshadowed this theory when he suggested (T. B., vol. XI, p. 445) that the development of the anlage in the neck and the innervation of the line by the vagus indicated that "the lateral line was probably originally restricted to the anterior part of the body."

The homology instituted by Eisig (11) between the lateral line organs of fishes and the "Seiten organe" of certain annelids (*Capitellidæ*) is well known. Balfour in his text book declined to accept it, and though Beard favored the homology in his paper on the Teleostean lateral line (6), after studying the Selachians he gave it up. Now that the early development of the lateral line is approximately known in Teleosts and Selachians, there seems less than ever to be said for the homology. If it could be shown that the segmental sense organs of annelids, leeches, etc., arise from an anterior anlage, which grows back and, so to speak, distributes the sense organs along the trunk, the homology might well be supported. But, as far as I know, the invertebrate segmental sense organs arise *in situ*.

Professor Whitman, in a paper on the "Segmental Sense Organs of Leeches" (44), supported the homology in 1884, and has recently said (45) that he still regards the position as tenable, although aware of the difficulties. Professor Whitman's paper dealing with this point will be awaited with a good deal of interest. In the mean time one can not but think that the segmental arrangement of the lateral line organs in some fishes (Salmon) has been looked at too closely, for in Whitman's account of the leech segmental organs there is found the following passage: "The developmental history of these lateral organs in the fish, where they make their first appearance as *segmental papillæ in the strictest sense of the words*, can not at present be explained on a more satisfactory hypothesis" (*i. e.*, hypothesis of homology between leech organs and lateral line organs). But in the presence of so many fishes in which at the time of hatching there are only a few sense organs to the whole line (see Ryder, 34, p. 508), and in which there is consequently no segmental arrangement at all, it is obviously unwarranted to assume that fishes like the Salmon present the ancestral condition of the lateral line. Further, even if in the Salmon the lateral line organs do first make their appearance segmentally arranged, the Bass development and Beard's observations make it extremely probable that here also the lateral line anlage first forms in the neck and then grows back, producing a continuous long cord on which the organs subsequently develop, a method which it seems best to look on as a secondary modification of the simple increase by division, which the organs undergo in the embryo Bass.

VIII. ORGANS FORMED FROM THE MESODERM.

Cœlom.—The lateral mesoderm plates of the young embryo seen in transverse section in Fig. 61, Pl. XCV, *mes.*, have a forward extension such as is shown in Fig. 146, Pl. CVI, *som.* In front of the plate the mesoderm consists of scattered cells, which in

subsequent stages form the head mesoblast masses. The mesoderm plates give rise to somites and cœlom. The forward continuation of the cœlom (pericardial cavity) is derived from the head mesoblast, but does not come into existence until after hatching. The formation of the head mesoderm and pericardial cavity will be considered later, and for the present the cœlom, as contained in the trunk, will only be described.

The formation of the cœlom in the Bass takes place in a different way from that followed in the Trout. In the latter the embryo is not compressed laterally to such a great degree, so that the mesoderm plates lie in a more or less horizontal plane, and the cœlom mesoblast is divided from the somites in much the same way as in a bird. In the Bass the great lateral compression leads to a deviation from this mode of forming the cœlom, the deviation being probably a very common one amongst Teleosts.

Three stages in the formation of the cœlom are shown in Figs. 67, Pl. XCVI (35 hours), 75, Pl. XCVII (39 hours), 94, Pl. XCIX (45 hours). In Fig. 67 (compare the earlier stage, Fig. 61, Pl. XCV) the embryo has already undergone great lateral compression, and the mesoderm plates are now inclined at an angle of 45° to the surface. There is already present in this stage a shallow longitudinal furrow which tends to divide the plate into two parts, the somite mesoderm (*som.*) and the cœlom mesoderm (*coel.*). The furrow deepens as the next stage, Fig. 75, shows, and finally completely divides the plate into cœlom and somites, Fig. 94. (It may be said here that during the formation of the cœlom the somite mesoderm is at the same time dividing transversely into somites.) The cœlom as thus formed consists of a two-layered plate of flattened cells. The layers are not sharply separated, and during embryonic life it is only at the inner angle, where the Wolffian duct is constricted off, that a true cavity appears between them. The condition of the cœlom (*coel.*) at the time of hatching is seen in the successive sections, Figs. 126, 127, and 128, Plates CII and CIII. Fig. 128 is the most anterior, and on comparing it with Fig. 94 it is seen that the diminution in size of the yolk has brought the cœlom into nearly a horizontal plane. In the posterior part of the body, where the embryo has been entirely folded off from the yolk (Fig. 126), the lateral halves of the cœlom have met beneath the alimentary canal, and the somatopleure and splanchnopleure are sharply marked off from each other. The cœlom extends as far back as the anus, but at no time any further.

During the first three days of larval life the cœlom becomes transformed into something like its adult condition. A cavity appears between the somatopleure and splanchnopleure, in the immediate neighborhood of the alimentary canal (Fig. 136, Pl. CIV, 86 hours). The cœlom then extends beneath the canal (Fig. 138, Pl. CIV, 100 hours, and Fig. 139, Pl. CV, 112 hours) and the two halves ultimately meet, the ventral mesentery being absorbed. The gradual flattening which the cells composing the cœlom wall undergo may be traced in the figures 136, 138, Pl. CIV, and Fig. 141, Pl. CV. The next step taken by the cœlom is to grow down between the ectoderm and the yolk, on each side (Fig. 141, Pl. CV). By this time the liver has not only begun to develop (Fig. 138, 1) but has come to lie between the cœlom and the yolk, and hence the cœlom in its growth round the latter also envelops the liver. This is shown in Fig. 141, Pl. CV (136 hours) and in Fig. 145, Pl. CV (160 hours, ventral mesentery absorbed). When the two halves of the cœlom eventually meet in the median ventral line beneath the combined mass of liver and yolk (at *x* in Fig. 145) another ventral mesentery will be established. But it is plain that the new ventral mesentery will be only a continuation of the one which has been formed and absorbed

between the alimentary canal and yolk in Fig. 145. It therefore becomes evident that the growth of the cœlom round the yolk, begun in Fig. 141, is merely a part of the general growth of the cœlom round the ventral surface of the alimentary canal.

Near its ventral edge the somatopleure shows in the larval stages a band-like thickening (*m. b.*, Figs. 141 and 145) which looks in some respects like a muscle band.

Wolffian duct.—It is generally agreed that the Wolffian duct arises in the Teleostei as a fold of the cœlom. The formation of the duct begins anteriorly and travels back. Anteriorly the layers of the cœlom separate so as to inclose a true cavity, and there is formed a well-marked diverticulum (*w. d.*, Fig. 103, Pl. C). But there is no stage in which the duct exists along its whole length as a diverticulum. This may be due to the possible fact that as quickly as the duct is formed it is constricted off. The appearances indicate, however, that in its posterior half the duct is constricted off from the cœlom as a solid mass, the cells of which are radially disposed round an ideal lumen. The position and character of the duct, after separation from the cœlom, are shown in Fig. 110, Pl. CI, *w. d.*, at the time of hatching, in Figs. 126, Pl. CII, 127, Plate CIII. At the time of hatching the ducts extend as far back as the anus, but do not form a urinary bladder. The entire course of the duct is straight, and at its anterior end it opens into the cœlom.

The urinary bladder begins to form shortly after hatching by the fusion of the posterior ends of the ducts. It is very thin walled and opens just behind the anus. The only other step in the later development of the duct that I have observed is the formation of an anterior loop. On the second or third day after hatching, the anterior end of the duct bends round and runs posteriorly for a short distance. This is shown in the three successive sections, Figs. 143, 144, and 145, Pl. CV, of which Fig. 143 is the posterior. The opening of the duct into the cœlom (Fig. 144) at this stage could not be made out with certainty, but the part of the duct which I have represented as opening is morphologically the anterior end, and in an earlier stage this end very plainly opened into the cœlom.

Somites.—The marking off of the somites begins at about the same time as the separation of the cœlom. It begins anteriorly, but whether the first somite formed is the true anterior one I do not know. The somites are marked off by dorso-lateral constrictions (Fig. 98, Pl. CXIX, surface view, and Fig. 85, Pl. XCVIII, vertical longitudinal section to one side of median line) from which planes of division run into the substance of the mesoderm plates. The somites are, it is needless to say, solid. Hoffmann (17) describes them in the Trout as hollow, but he stands alone in this opinion.

The formation of somites travels antero-posteriorly, and during the greater part of embryonic life there is at the posterior end of the embryo, on each side, a quite long tract of undivided mesoderm (*un. mes.*, Fig. 85, Pl. XCVIII, and Fig. 98, Pl. XCIX) which at the tip of the tail ends in the caudal mass. New somites are constantly split off from the anterior end of the undivided mesoderm, and consequently the formation of somites and their gradual alteration may be studied in the posterior part of a single embryo quite as well as in corresponding parts of different stages. At the time of hatching there remains only a very little of the undivided mesoderm, the somites extending nearly to the tip of the tail.

In Fig. 98, Pl. XCIX, and Fig. 85, Pl. XCVIII, the somites next the undivided mesoderm are the younger, and on going forward it is seen that they gradually undergo certain changes of shape. When the somite is first formed, it is constricted off from the

mesoderm plate as a rectangular mass placed at right angles to the long axis of the body, Fig. 98. The vertical planes of division separating it from the mesoderm behind and the somites in front are simple planes (Fig. 85), and in transverse section the somite is undivided (Fig. 109, Pl. C). All that is changed as the somite grows older. Fig. 98 shows that the somites become inclined to the long axis of the body, the division planes running from without and posteriorly; Fig. 85 that they become bent forwards at about their middle; and Fig. 110, Pl. XCVII, that each somite becomes constricted into a dorsal and ventral portion (corresponding to the dorso-lateral and ventro-lateral trunk muscles).

While the somite is undergoing the changes of shape just described it also passes through a histological metamorphosis. The mesoderm plate, before the formation of somites begins, is made up of irregularly polygonal cells, which at the surface approach the cubical shape and give the plate a smooth bounding surface. The posterior remnant of the mesoderm plate (Figs. 85 and 98, *un. mes.*) preserves these characteristics, but the bounding cells become more decidedly columnar. A transverse section of the undivided mesoderm is shown in Fig. 90, Pl. XCVIII. When the somite is first constricted off, it is consequently made up of polygonal cells (Fig. 86, Pl. XCVIII, more highly magnified view of one of the posterior somites of Fig. 85, and Fig. 109, Pl. C, transverse section) and has on all sides a smooth bounding surface.

During the development of the somite the polygonal cells elongate in the direction of the chief body axis, and it finally is brought about that each cell stretches from the anterior to the posterior surface of the somite. In Fig. 87, Pl. XCVIII (more highly magnified view of one of the anterior somites of Fig. 85), the cells have undergone this elongation. Each of these long cells becomes transformed into a muscle fiber.

When the somite is first formed it is, as I have said, undivided in transverse section and has on all sides a smooth limiting surface. The smooth surface is lost on the proximal side of the somite (Fig. 94, Pl. XCIX), many of the cells coming to jut out in an irregular fashion. The next change to be described is the division into dorso-lateral and ventro-lateral muscle tracts. The somite constricts in a plane about opposite the notochord (Fig. 110, Pl. CI), and the tracts thus formed (*d. l. m.* and *v. l. m.*) are made more distinct by the existence of the cells *c. t.* While the remaining cells of the somite (exclusive of possible migratory cells, in regard to which I have no satisfactory observations) become transformed into elongated muscle cells, with large conspicuous nucleoli, the cells, *c. t.*, remain small, have inconspicuous nuclei and nucleoli, and form, as shown in the figure, a dividing wedge between the two muscle tracts. I really do not know what becomes of these cells, since the somite retains throughout embryonic life the character shown in Fig. 110. Sections through the larval stages suggest, however, that they become transformed into connective tissue. At least in the larva the muscle tracts are separated by a few scattered cells (Fig. 136, Pl. CIV).

The transformation of the elongated somite cells into muscle cells takes place after hatching, though, as is well known, the body is capable of strong muscular contractions before this time. Oscar Hertwig has described the metamorphosis which the homologous cells in *Triton* undergo (Lehrb., p. 270). In *Triton* distinct muscle fibrils appear in the cell protoplasm, first appearing in the peripheral part of the cell, but gradually forming in the inner portion also, until the whole cell is transformed into a bundle of fibrils. The development of the muscle cells in the Bass is of a

somewhat different character, in that the muscle substance is not formed as separate fibers, but as (apparently) homogeneous masses. During the first day of larval life, the peripheral part of the hitherto protoplasmic cell becomes transformed into three or four masses of muscle substance, separated by strands of protoplasm (Fig. 137, Pl. CIV, four muscle fibers from somites of Fig. 136). The protoplasmic strands meet in an axial remnant of protoplasm, in which is contained the nucleus. In a subsequent stage of development (Fig. 142, Pl. CV) the protoplasmic strands have disappeared and the amount of axial protoplasm at the same time has grown less, the great bulk of the cell having now become muscle substance. This is the condition of the fibers in the oldest larva I have studied.

The "intermediate cell mass" to which the somite is said to give rise in some fishes (Trout, Oellacher, Henneguy) does not exist in the Bass. Ziegler, who has carefully studied this structure (47), did not come to a positive conclusion in regard to its origin, but found that after giving off a number of blood cells it formed the "stamm vene" (fused cardinals) in the Salmon. It is undoubtedly absent in many fishes: *Serranus*, *Engraulis* (Wenckebach, 43), *Labrax* (Ziegler), and, as Ziegler says, has no homologue in other vertebrate groups. In those fishes in which it is absent, the vessels elsewhere formed by it are probably formed by scattered cells, as is the case with the aorta in the Bass. Ziegler's manner of looking at the structure commends itself; it is a part of the general Bildungs-gewebe (represented for the rest by wandering cells), which in some fishes early acquires an individuality as an anlage for certain great vessels (47).

In the Bass, as in other Teleosts, almost the entire somite goes to build up the great trunk muscles. What relation the cells, which subsequently form the skeleton, bear to the somites I am quite unable to say.

Mesoderm of the head.—Hoffmann (17, p. 26, 1883) says, without describing his observations, that the mesoderm of the head is undoubtedly segmented in the Teleosts. I have, however, not found any trace of segmentation in this part of the mesoderm, as the following description of its development will show. It is quite possible, however, that in this, as in some other respects, the small pelagic egg of the Bass is more secondarily modified than that of the Trout.

Before the closure of the blastopore the mesoderm in front of the paired plates (somite mesoderm) consists of scattered cells, and the total amount of it is small, as may be gathered from the transverse sections (Figs. 62, 63, and 64, Pls. XCV and XCVI.) The origin of this part of the mesoderm has already been described. The paired plates towards their anterior end dwindle in size, so that the transition from somite mesoblast to scattered mesoblast is not very distinct. When the somites begin to form, immediately after the closure of the blastopore, the scattered cells in the neck region (Fig. 62, Pl. XCV) increase in number and form a moderately compact mass (head mesoblast mass) in front of the somites, which extends forwards as far as the auditory invagination, and in front of that is continued as a collection of scattered cells. The condition of the head mesoblast at this stage is gathered from the series of sections, Fig. 68, Pl. XCVI (just in front of the somites), Figs. 69 and 70, Pl. XCVI (through auditory invagination), and Fig. 71, Pl. XCVII (through the branchial sense organ).

Forty-five hours, Figs. 95, 96, and 97, Pl. XCIX.—When the foregut closes in (compare Figs. 71, Pl. XCVII, and 95, Pl. XCIX) it is surrounded by scattered cells, but both

behind and in front of Fig. 95 the head mesoblast forms a compact mass. A section through the region of the branchial sense organ is shown in Fig. 96, Pl. XCIX, and on comparing this section with the earlier stage (Fig. 71, Pl. XCVII) it is seen that the head mesoblast masses have grown forwards. Still farther in front (Fig. 97, Pl. XCIX) the mesoblast consists of scattered cells.

The compact head mesoblast (head mesoblast masses) continues to increase in amount and grow forwards. At the time of hatching it extends up to the eyes. A series of sections (Figs. 127–134, numbered from behind forwards) through this stage will illustrate its condition. Fig. 128, Pl. CIII, is through the extreme anterior somite region. Compared with Fig. 127, it is seen that anteriorly the somites decrease greatly in size, and also that they come to lie beneath the medulla. Just in front of this section the somites come to an end and are followed by the head mesoblast masses, with which the body cavity is continuous (Fig. 129, Pl. CIII). Still further in front we come to the gill-slit region; Fig. 130, Pl. CIII, is, on the right side, through the slit, and on the left side just behind it. Comparing this figure with corresponding sections through earlier stages (Figs. 95, Pl. XCIX, 114, Pl. CI), the marked increase of mesoblast underneath the foregut is noticeable. The successive sections in front of the foregut, Figs. 131, 133, and 134, sufficiently indicate the condition of this part of the head mesoblast.

The pericardial cavity does not develop until after hatching. The cells which inclose it are split off from the under surface of the head mesoblast masses.

The development of the head mesoblast in the Bass, from a few scattered cells which proliferate and give rise to compact masses, which gradually acquire a greater forward extension, is evidently an extreme case of cœnogeny, and makes any attempt to study the early morphology of the head in such a fish as the Bass an almost hopeless task.

Mesoblast of the pectoral fins.—The pectoral fins do not form protuberances until a couple of days after hatching, but the mesoblast which gives rise to them begins to accumulate in the last few hours of embryonic life. Ziegler (47) has described the mesoblast of the fins as in direct continuity with, and as derived from, the head mesoblast. In the Bass there is no direct continuity between the two. On the contrary the accumulation of cells which gives rise to the fins (*pec. f.*, Fig. 136, Pl. CIV) is intimately associated with the body cavity. On passing forwards from the section represented in Fig. 136, the fin mesoblast comes to an end, the body cavity drawing away from the edge of the embryo and assuming an appearance about as shown in Fig. 127, Pl. CIII. Farther forward the head mesoblast begins. It is quite possible, however, that the lack of continuity with the head mesoblast is only apparent, and that cells may migrate (a few at a time so as to escape observation) from the head mesoblast to the position of the fins, though as to the probability of the supposition I have nothing to say. In a later stage (Fig. 139, Pl. CV, *pec. f.*) the intimate association of the fin mesoblast with the body cavity still exists, and as before there is no direct connection with the head mesoderm.

IX. HEART; AORTA; SUBNOTOCHORDAL ROD.

Heart.—During embryonic life the heart consists of a flattened sac lying to one side of the median line, in the space between the mesoderm and periblast (Figs. 133, Pl. CIV, and 151, Pl. CVII, *h*). The sac is composed of cubical cells, and is open

along one side over the greater part of its extent. Posteriorly, however, it forms a closed tube (Fig. 117, Pl. CII, *h*, arterial end of heart). The sac contains a plasma, which takes a light stain, and amœboid cells. The heart begins to beat while in this condition.

In regard to the origin of this simple embryonic heart and its further development I may say that my observations on the Bass differ in so many points from the careful accounts given by Henneguy (18), Ziegler (47), and Oellacher (33), of the formation of the trout's heart, that I prefer reserving my description until I have had an opportunity of studying the process in the *Salmonidæ*.

Subnotochordal rod and aorta.—Before the alimentary canal closes in ventrally there is found lying above it a single row of cells (*s. n. r.*, Fig. 76, Pl. XCVII). The cells are flattened dorso-ventrally, and their position with respect to the entoderm cells, from which it is sometimes difficult to distinguish them, makes it safe to conclude that they are the uppermost cells of the enteric fold, which have separated from the rest of the entoderm. There is thus in the Bass a homologue of the entodermic subnotochordal rod of Selachians, as there is in the Trout (Henneguy). The further relations of the rod in the Bass are extremely complicated. It becomes intimately associated with certain cells which form the aorta, though I do not believe that it enters into the composition of the vessel itself. I have thought it worth while to set down my observations on the development of the aorta, though they do not lead to a conclusion regarding the origin of the cells which form it.

To the subnotochordal rod are added other cells which form a string, some three or four cells in section, *a. an.*, Figs. 92 and 93, Pl. XCIX, and 103, Pl. C. The origin of these cells I do not know; sometimes I have thought them hypoblastic, and again mesoblastic. The probability is undoubtedly in favor of the latter origin. Occasionally the new cells added take a shape and position precisely like the subnotochordal cells (Fig. 104); usually, however, they are of an irregular shape. During the formation of this solid string of cells very few wandering cells are to be seen, though in a little later stage (Fig. 110, Pl. CI) they are conspicuous in the spaces between somites, chorda, etc. Whatever be the origin of this string of cells, it is a true "aorten strang" (Oellacher), in that it develops into the aorta. The cells, which compose it, separate so as to inclose, at first in an irregular fashion, a central space (*aor.*, Fig. 110), the aorta cavity. In subsequent stages the bounding cells become flattened and completely inclose the cavity. As I have said, I do not think the subnotochordal cells take part in forming the aorta, for even after it has become a perfectly closed tube they may sometimes be seen in their old position just above the vessel.

The atrophy of the postanal gut takes place from before backwards. As it progresses, there is left in the place of the gut a string of cells (one or two thick), which constitutes the caudal part of the subnotochordal rod (Figs. 101 and 109, Pl. C; Fig. 111, Pl. CI, *s. n. r.*). With this rod are associated some few amœboid cells, the origin of which is unknown (Figs. 101 and 109), but which are probably concerned in the formation of the aorta in this region. The formation of the aorta in the tail, like the atrophy of the postanal gut and formation of the subnotochordal rod, progresses from before backwards. There is no previous formation of a solid string of cells. In the section Fig. 112, Pl. CI, the subnotochordal cells, above the aorta, were unusually distinct.

X. GENERAL MORPHOLOGICAL QUESTIONS.

Concrescence.—The theory of His, that the vertebrate embryo is formed by the concrescence of two halves along the median dorsal line, has drawn many of the arguments used for its support from the development of the Teleosts; and in the study of any fish, the presence or absence of indications of concrescence must be looked on as one of the more important general questions involved. However attractive in the abstract the theory may be, I have failed to find in the Bass development any facts which should induce one to accept it, and the arguments commonly used in its favor seem to be very far from conclusive. Indeed, the only good argument I know of is Ryder's observation (35) that in *Elacate* the extra-embryonic germ ring gives indications of being divided up into somites. But I do not think this point can be made much of until Professor Ryder publishes a more detailed account of the embryos he observed, for the exact relations of the several parts of the embryo at the tail end can scarcely be ascertained from the existing account. Henneguy has in his last paper (18) reviewed the arguments for the concrescence theory, and as I agree in the main with his criticism it is unnecessary for me to recapitulate them. I will therefore simply describe the growth of the Bass embryo.

In the growth of the blastoderm round the yolk, the head end of the embryo does not remain a fixed point, the body lengthening in an antero-posterior direction, as His supposed. On the contrary, the tail end of the embryo (posterior pole of the blastoderm, *p. p.*, Fig. 35, 36, and 38, Pl. XCII) remains a comparatively fixed point, as Oellacher first showed, while the anterior pole of the blastoderm travels rapidly round the yolks (arrows, Figs. 35 and 36). The point where the blastopore closes is thus but a short distance from the original position occupied by the posterior pole of the blastoderm. Owing to the constant position of the single oil globule, these facts can easily be made out (compare Figs. 35, 36, and 38).

The growth of the embryo itself is more complicated, but still susceptible of what seems an accurate analysis. On comparing Figs. 35 and 36, it is seen that while the posterior pole of the blastoderm remains comparatively fixed, the head end (*h. e.*) of the embryo follows, though at a much slower rate, the anterior pole of the blastoderm in its growth round the yolk. The comparison of the two figures inevitably leads to the conclusion that the increase in length, which the embryo undergoes in passing from one stage to the other, is due to intussusception and not to concrescence. Extending the comparison to the later stage (Fig. 38, Pl. XCII, just before the blastopore closes) it is seen that the increase of length, which the embryo undergoes between the stages represented by Figs. 36 and 38, is brought about in a different way from that between Figs. 35 and 36. This is shown by the following examination: At the beginning of the older period (Fig. 36) the head end and tail end of the embryo are approximately equidistant from the oil globule, and at the end of the period (Fig. 38) the case is the same. The head end of the embryo has therefore continued to grow round the yolk, as in the period Figs. 35 to 36, and the body has also been lengthened at the opposite end in the opposite direction. The increase in length at the tail end of the embryo deserves especial attention. The great increase in length, which the body undergoes by the growth round the yolk of the head end of the embryo (Figs. 35 to 36, and also Figs. 36 to 38) can only be explained as ordinary growth by intussusception. If this is so, it is perfectly fair to assume, until the contrary is proved, that the comparatively

small increase in length, which the body receives at the tail end, is due to the same sort of growth. And I have no doubt that this is true of the greater part of the caudal increase. But the development, during the closure of the blastopore, of what has been called the primitive streak clearly leads to the conclusion that, as the blastopore closes, the germ ring is drawn into the tail end of the embryo, which is thus progressively lengthened, the final addition (of this sort) to its length being the incorporation of the secondary caudal mass, *sec. c. m.*, Fig. 65, Pl. XCVI. The formation of the teleostean primitive streak is obviously so similar to that of Amphibia (compare Schwarz, 39) that the two must be regarded as homologous. In *Triton* (Hertwig, 20) the blastopore closes in a slit-like fashion (see Fig. 7), leaving at its lower end a small opening, through which protrudes the *dotterpropf*. The line along which the blastopore closes is indicated after closure by a groove, *p. g.* Now compare with the diagram of the *Triton* blastopore the section given in Fig. 65, Pl. XCVI. The primitive streak in the Teleost represents the line of closure in *Triton;* at the posterior end of the streak there is the same opening; and the opening is plugged up by a *dotterpropf*. The formation of the primitive streak in the Amphibia, by a true concrescence of the blastopore lips, is undoubtedly the ancestral method, of which the process made use of in the Bass must be regarded as an embryonic modification.

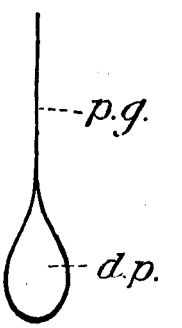

FIG. 7.—Diagram of closing blastopore of Triton.

To sum up, the growth of the embryo takes place, as I believe, in the following manner: The great increase in length is acquired by the head end of the embryo following the anterior pole of the blastoderm in its growth round the yolk. The embryo is also lengthened in a much less degree by the movement of the tail end in the opposite direction. The growth in each of these cases is brought about by intussusception. The blastopore closes in a manner which is clearly a modification of concrescence, and gives rise to the terminal portion of the embryo in which there is a median fusion of layers.

The concrescence theory of His certainly receives no confirmation in the development of the Bass. All the facts regarding the growth of the embryo that I have observed are incompatible with it, the only part of the embryo which is formed by concrescence being the posterior end, behind Kupffer's vesicle; and the increase in the length of the embryo, by the addition to it of the primitive streak, is obviously a secondary modification resulting from the transformation of a holoblastic egg (like the Amphibian) into a meroblastic egg. This is made plain by an examination of Fig. 65, Pl. XCVI. In the ancestral holoblastic embryo the primitive streak did not lie horizontally, but more or less vertically; *i. e.*, it represented the posterior end of an embryo, in which the yolk was comparatively small and went to form the ventral wall of the gut. With the formation of the large, purely nutritive, yolk, the posterior end of the embryo came to lie in a horizontal plane, and so added to the length of the embryonic body.

Among recent advocates of the concrescence theory (Ryder, 34, 35; Cunningham, 8) the closure of the blastopore in Teleosts has been regarded as affording strong evidence of the truth of the theory. But it seems to have been assumed, without any satisfactory grounds, that the tissue of the germ ring, as it is drawn into the embryo, comes to lie along the notochordal line. Cunningham's argument in brief is as follows: (1) the non embryonic part of the germ ring disappears; (2) it is not absorbed, and must consequently be drawn into the embryo; (3) the latter is hence formed by

concrescence. I fully admit 1 and 2, but the Bass development shows that the primitive streak is the only part of the embryo which it is fair to conclude is formed from the non-embryonic germ ring, and hence Cunningham's conclusion (3) is entirely inadmissible. Cunningham's whole conception of the non-embryonic germ ring is, as I shall try to show, an erroneous one.

Teleostean gastrulation and the significance of the germ ring.—The Teleostean gastrula is such a complicated embryonic form that it has given rise to many interpretations, and the disagreement as to the proper one still continues. So much is this the case that the light which the Teleostean development is capable of throwing on the embryology of the Amniotic vertebrates has been greatly obscured. I give a brief review of the several theories on this head, ending with Ziegler's, which, besides affording a satisfactory explanation of the Teleost embryo itself, makes practicable so many comparisons with both Ichthyopsidan and Amniotic embryos that it leaves little to be desired. The only obstacles in the way of the theory have been the lack of exact knowledge with regard to Kupffer's vesicle and the meaning of the extra embryonic germ ring.

Haeckel (19), who witnessed the inflection of the blastoderm edge to form the germ ring, regarded the Teleostean gastrula as a true discogastrula; that is, he believed, the inflected layer met in the center (as in Fig. 8), forming a complete layer beneath the ectoderm. The two-layered embryo thus formed he called a discogastrula, the yolk lying (morphologically) in the gastrula cavity. Haeckel's conception is based in the first place, as has long been recognized, on wrong observations; the invaginated tissue does not form a complete layer beneath the ectoderm, but remains incomplete in the center (Fig. 9). In the second place Haeckel's explanation, as Balfour pointed out (Elasmo. Fishes, p. 277), makes it impossible to regard the yolk as a part of the embryo, whereas the comparative embryology of vertebrates makes it absolutely certain that the yolk of meroblastic eggs is a part of the embryo and has been derived from the yolk cells of some such form as the Amphibian blastula. While Haeckel's theory, as he presented it, no longer receives any support, it has obviously influenced the views of more recent writers, such as Ryder and Henneguy. According to Ryder the Teleost gastrula has been derived in the following manner:

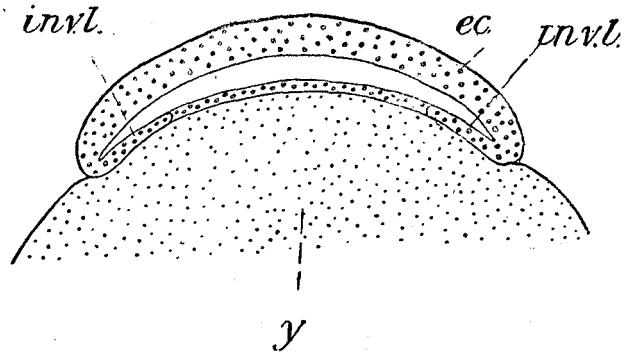

Fig. 8. Diagram to illustrate Haeckel's idea of teleostean gastrulation—*y.*, yolk; *inv. l.*, invaginated layer; *ec.*, ectoderm.

> A gradual loading of the entoblastic pole of the blastula (*Amphioxus* blastula) with yolk causes the latter to be constricted around its equator in the course of development, thus leading to the formation of a blastodisc with an inflected two-layered margin. (35, p. 493.)

The incomplete center of the inflected under layer, to which Ryder gives the convenient name of discopore, is "homologous with a circular opening which might be produced by a rupture near the center of the inflected entoblast of the gastrula of *Branchiostoma.*" It will be seen that while Ryder agrees with Haeckel in believing

that the yolk fills the archenteron, he on the other hand regards the yolk as representing a part of the entoderm of the *Amphioxus* gastrula.

Henneguy's theory is practically the same:

La gastrula des poissons osseux est, comme l'a bien vue Haeckel, une véritable discogastrula qui par son mode de formation et par sa constitution, se rapproche beaucoup plus de la gastrula type de l'Amphioxus que celle des autres Poissons (18, p. 596). Si l'on suppose, en effet, la blastula de l'Amphioxus ouverte à sa partie inférieure et s'invaginant autour d'une sphère (vitellus) on aura une image exact de la gastrula des Téleostéens. L'intestin primordial, le protogaster, est rempli par la masse vitelline (p. 597).

It will be seen that both of these writers refer the Teleost gastrula directly to that of *Amphioxus*, and accordingly regard the ingrowth of cells round the entire edge of the fish blastoderm as representing the invagination of *Amphioxus*, the cavity of the fish gastrula being filled with yolk, which has been derived from the bottom cells of the *Amphioxus* archenteron. It can not be denied that this theory offers an explanation of the early Teleost gastrula (diagram, Fig. 9), but it becomes utterly unsatis-

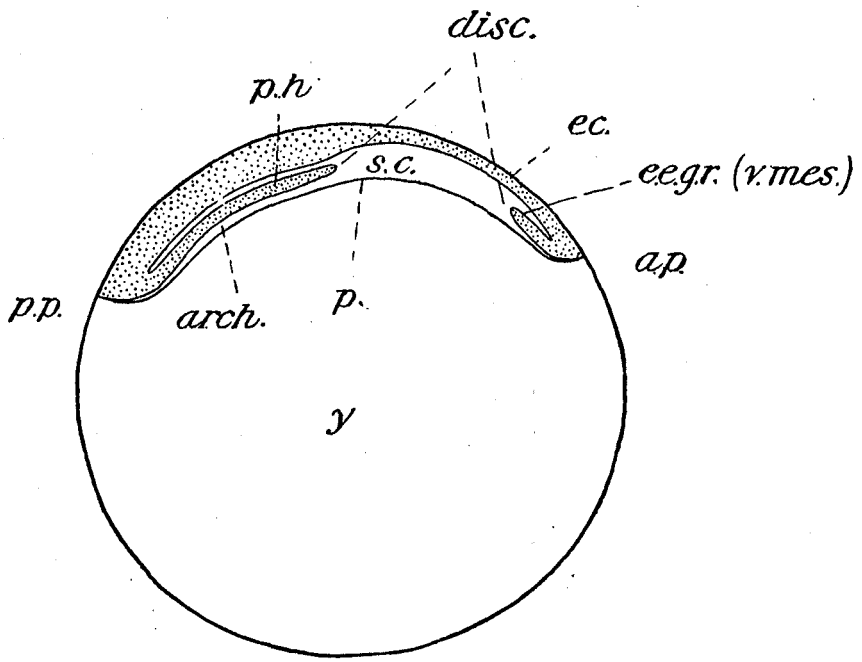

FIG. 9.—Diagram of early Teleost gastrula—*p. p.*, posterior pole; *a. p.*, anterior pole; *p. h.*, prim. hypoblast; *arch.*, archenteron; *p.*, periblast; *s. c.*, segmentation cavity; *disc.*, discopore; *ec.* ectoderm; *e. e. g. r.* (*v. mes.*), extra-embryonic germ ring (ventral mesoblast).

factory as soon as what Balfour has called (7) "the asymmetry of the vertebrate gastrula" begins to appear in the fish embryo. For the Teleost gastrula of Ryder and Henneguy is a symmetrical gastrula, and they are consequently unable to explain why it is that (continued) invagination takes place at one pole of the blastoderm (*p. p.*), while the other pole (*s. p.*) grows epibolically round the yolk. There are numerous other difficulties in the way of the theory, which become apparent as soon as the attempt is made to derive in detail the older Teleost embryo (Fig. 10, p. 264, and Fig. 65, Pl. XCVI) from a gastrula such as the theory assumes. But the greatest objections are, first,

the total absence of intermediate forms between the gastrula and that of *Amphioxus*, and second that the theory leads us nowhere; it does not admit of any exact comparison between the teleostean embryo and those of other vertebrates.

Kupffer's theory of gastrulation (25, 26) is very different, but stands in complete opposition to the facts. According to Kupffer the vesicle, which bears his name, arises by an invagination from the ectodermal surface, and alone represents the *Amphioxus* invagination. The functional entoderm is derived from the yolk and is regarded as a structure which has gradually replaced the invaginate entoderm.

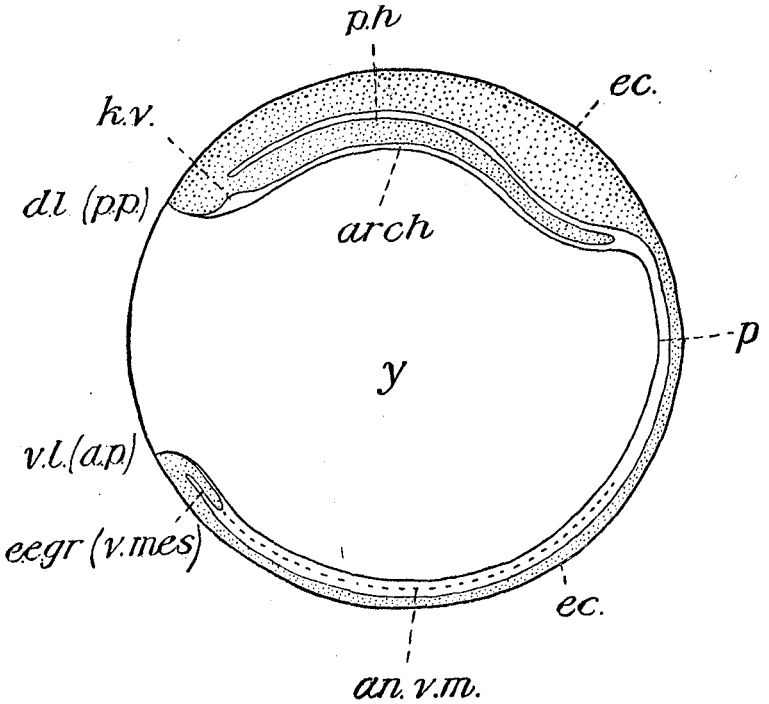

FIG. 10.—Diagram of Teleost gastrula, not long before blastopore closure—*d. l.* (*p. p.*), dorsal lips (post. pole) of blastopore; *v. l.* (*a. p.*), ventral lip (ant. pole) of blastopore; *k. v.*, Kupffer's vesicle; *arch.*, archenteron; *p. h.*, prim. hypoblast; *ec.*, ectoderm; *p.*, periblast; *e. e. g. r.* (*v. mes.*), extra-embryonic germ ring (ventral mesoderm); *y*, yolk; *an. v. m.*, extent of tract of ventral mesoderm in ancestor.

Kollmann (29) entertains the strange and difficult view that the discopore (*disc.*, Fig. 9) is the blastopore. His theory has already been criticised by Ryder (35, p. 493), with whose objections I agree. There is really nothing to support the theory; the generalized diagram of the meroblastic gastrula, which Kollmann gives, is a very different form from the Teleost embryo, and an acceptance of his homology of the discopore with the blastopore makes it impossible to understand any part of the further development. The closure of the blastoderm edge at the tail end of the embryo becomes incomprehensible, while the position of the "blastopore" (discopore) lip directly under the head of the embryo (when compared with its position in *Amphioxus*), and the fact that the "blastopore" (discopore) never closes, remain absolute mysteries.

To Ziegler (48) is due the credit of having first instituted a detailed comparison between the teleostean and Amphibian gastrulas, for it is only through such a comparison that the intricacies of the fish development become comprehensible. Ziegler's

homologies are as follows: Yolk together with the periblast represents the yolk cells of the Amphibian gastrula; the invagination at the posterior pole of the fish blastoderm represents the invagination round the dorsal lip of the Amphibian blastopore, which forms the so-called chorda-entoblast; the gastrula cavity in the fish is morphologically between the invaginated layer and the periblast. Ziegler's theory is concisely stated in the following quotations (48):

Bei der Unke und den Salmoniden wird die Bildung der unteren Schichte am ganzen Rande der Keim scheibe eingeleitet; sie beginnt aber an der dorsalen Seite früher als an der anderen, und schreitet nur auf dieser fort, während sie im übrigen Umfang bald wieder sistirt wird.

Phylogenetisch ist das Entoderm der Teleostier nur der dorsale Theil des Darmdrüsenblattes; es entsteht aber aus demselben das ganze Darmepithel, indem es medianwärts aufstülpt, und darauf die so entstandene Rinne vom Dotter abgeschnürt wird.

Der bei diesem Vorgang in der Kiemengegend entstehende freie Raum zwischen Entoderm und Dotter entspricht einem Theil der Gastrula und Darmhöhle der primitiveren Entwicklungstypen; dasselbe gilt vielleicht von der Kupffer'schen Blase.

Accepting Ziegler's homologies, it will be seen that the whole course of the fish development becomes easy to understand. Starting with the blastula (Fig. 25, Pl. XCI, *s. c.*, segmentation cavity) and disregarding for the present the non-embryonic part of the germ ring, the primitive hypoblast (*p. h.*) which invaginates at the posterior pole (*p. p.*) of the fish blastoderm (Fig. 9) corresponds to the primitive hypoblast (*p. h.*) which invaginates round the dorsal lip of the blastopore in the frog gastrula (Fig. 11). The chief point of difference is the lack of continuity in the fish embryo between the inner edge of the invaginated layer and the yolk, easily explained as an adaptation to the method of forming the alimentary canal from the invaginated layer exclusively. The archenteron (*arch.*) lies between the primitive hypoblast (*p. h.*) and the periblast (Fig. 9, *p.* 263). In consequence of the absence of continuity between the yolk and the invaginate layer, the archenteron at its edge is not separated from the segmentation cavity (*s.c.*). The growth of the anterior pole of the blastoderm round the yolk (compare Figs. 9, *p.* 263, and 10, *p.* 264) represents the growth of the small cells round the yolk cells in Amphibian gastrulation. The closure of the blastopore takes place in the same way as in the Amphibia; there is formed a short primitive streak behind the position of the neurenteric canal (Kupffer's vesicle in Teleost); at the posterior end of the primitive streak the final closure takes place (the comparison must be made with those

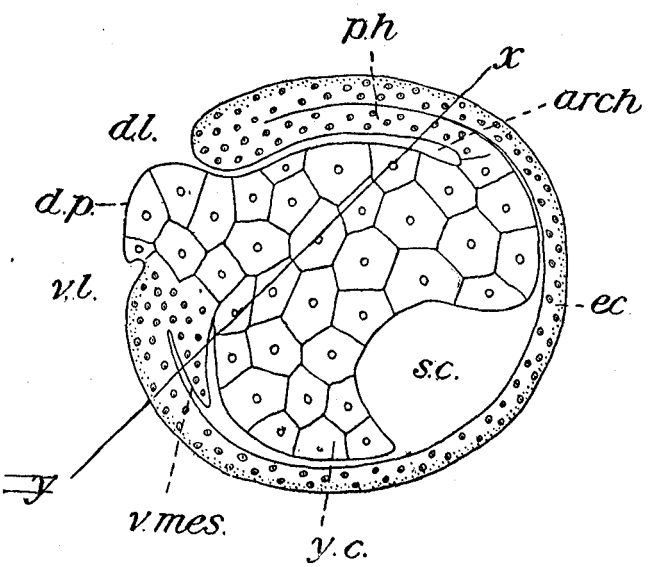

FIG. 11 (after Hertwig, 20).—Median longitudinal section through frog's gastrula—*d. l.*, dorsal lip of blastopore; *v. l.*, ventral lip of blastopore; *d. p.*, Dotterpropf; *s. c.*, remnant of segmentation cavity; *y. c.*, yolk cells; *ec.*, ectoderm; *p. h.*, prim. hypoblast; *arch.*, archenteron; *v. mes.*, ventral mesoblast.

Amphibia in which the posterior end of the blastopore does not persist as the anus, but closes), Fig. 65, Pl. XCVI, the blastopore remnant being plugged up in both types by the yolk.

The asymmetry which Balfour (7) showed to be a characteristic attribute of vertebrate gastrulation, is present in the highest degree in the Teleost gastrula. At the posterior pole of the blastoderm (dorsal lip of the blastopore) there is an extensive invagination, which gives rise to the roof of the archenteron. The opposite pole of the blastoderm (ventral lip of blastopore) incloses the yolk (=yolk cells=floor of archenteron) in an epibolic fashion. The cause of the asymmetry must be looked for in a peculiarly localized distribution of the yolk in the egg. The yolk not only lies in the hypoblastic part of the egg (so to speak), but in that part of it which corresponds to the *ventral* hypoblast of the gastrula. Consequently when the meroblastic blastula is perfected (Fig. 30, Pl. XCI) the so-called "blastoderm" contains not only the ectodermic half of the primitive simple blastula, but also contains one half of the hypoblast. This half of the hypoblast invaginates as it did in the yolkless ancestor, but the other half (yolk) must be inclosed epibolically.

The alimentary canal is formed from the roof of the archenteron exclusively. How this was effected is easy to see. The increase in size of the mass of yolk cells (of Amphibia) brought it about that the dorsal parts of the embryo were early folded off—some time before the alimentary canal was completed ventrally. The division of labor already far advanced between the dorsal and ventral hypoblast of the gastrula next took the final step: the dorsal hypoblast assumed the entire function of forming the gut, while the ventral hypoblast became transformed into pure food material. The yolk is consequently to be looked on as an organ of the gastrula which has lost its original function, but which in doing so became adapted to another function to which it owes its large size.

Up to this point the discussion of the extra-embryonic germ ring has been avoided. The interpretation of this part of the embryo is a mere corollary of Ziegler's conception of the gastrula, as originally stated (48). A comparison of the frog's gastrula (Fig. 11) with the fish gastrula (Fig. 10), after the preceding discussion, leads at once to the homology of the extra-embryonic germ ring, (*e. e. g. r.*) with the ventral mesoblast of the frog (*v. mes.*) In my preliminary communication (46) I made the following statement: "With reference to the meaning of what may be called the non-embryonic part of the germ ring, Ziegler is by no means clear, though the interpretation seems to me a mere corollary of the foregoing (Ziegler's) propositions." Since writing this I have received a letter from Professor Ziegler which, read in connection with his brief mention of the point in his second paper (47), satisfies me that he has held for some years the view of the extra-embryonic germ ring to which the present piece of work has led me. It gives me pleasure to find that as regards this point as well as in the general interpretation of the gastrula, I have been led to the same conclusions as Professor Ziegler. The point is one, however, which deserves a somewhat ampler notice than Ziegler gives it.

The adherents of the concrescence theory in vertebrates regard the extra-embryonic germ ring as hypoblastic. Thus Agassiz and Whitman (1) state their opinion as follows: "We think that what we have described as the entodermic ring (germ ring) corresponds to the chorda-entoblast of Rana; and it seems plausible that the periblast should correspond to the 'Darmentoblast.' On this view we should expect the periblast to take some share in forming the alimentary canal, which can not be admitted if

our observations are correct" (*p.* 79). With respect to concrescence this statement is made:

> It appears quite certain to us that the principle of concrescence underlies the formation of the embryo. The concrescence appears under the disguised form of a migratory movement of the cells, which accompanies the epibolic growth of the blastoderm. (P. 74.)

Now, before accepting the hypothesis that the extra-embryonic ring represents chorda-entoblast, we must be satisfied that the two halves of the ring meet along the chorda line. And I have tried to show that in the Bass there is no reason for believing this. Until the concrescence is actually proven, I do not think this manner of looking at the germ ring is admissible.

But, if the concrescence theory were established, even then it would scarcely be an explanation of the germ ring to call it chorda-entoblast. For at all events a part of the ring occupies a ventral position with respect to the blastopore (*e. e. g. r.*, Fig. 10, *sec. e. m.*, Fig. 65, Pl. XCVI), and it is obviously impossible to regard this part as chorda-entoblast. Evident as this would seem to be, Cunningham has been curiously misled into regarding the whole ring as equivalent to dorsal hypoblast, because it eventually comes to occupy a dorsal position with respect to the yolk. Cunningham's position is fairly given in the following quotations (8):

> The whole of the embryonic ring thus belongs to, and is formed into, the dorsal region of the embryo. (P. 17.)
>
> It is probable that the inflected ring in the Teleost is the dorsal hypoblast. It has already been pointed out that the whole of the inflected ring comes to lie beneath the axis of the embryonic rudiment, between that axis and the yolk. The invaginated layer thus ultimately occupies the same position as the layer in the blastoderm of the bird, to which the name hypoblast was first applied. (P. 20.)

With respect to the occurrence of concrescence, Cunningham gives no actual evidence, and there is nothing in his account which would cause me to believe that the growth of the embryo, in the fishes he studied, takes place in a different way from that of the Bass. The fact that the whole of the germ ring is absorbed into what Cunningham calls the dorsal region of the embryo (more properly terminal portion) is surely no argument that the germ ring is equivalent to dorsal hypoblast. The morphology of the ring must be determined before the blastopore closes, and an ingrowth from the ventral lip of the blastopore can scarcely be called dorsal hypoblast.

A satisfactory explanation of the extra-embryonic germ ring can only be obtained by regarding it as mesoblast. The ingrowth from the dorsal lip of the blastopore in the Teleost consists of primitive hypoblast (mesoblast plates, chorda, roof of archenteron). This ingrowth is continuous with that which grows in from the ventral lip, and which consists of mesoblast. Precisely the same state of affairs is found in the frog (Fig. 11, p. 265), as may be gathered from Oscar Hertwig's account (20, p. 273) of its development:

> Müssen wir schliessen, dass am Urmundrand der Ektoblast in das Innere der Embryonal form hinein wuchert, und hier einerseits in einen Streif ihrer dorsalen Wand übergeht, der den Darm nach oben als Chorda-Entoblast begrenzt, andererseits *sich in den Mesoblast continuirlich verfolgen lässt.*

In the phylogeny of the fish gastrula the entoblast and mesoblast have suffered a very similar fate. In the case of the former the ventral entoblast has lost its function, the dorsal entoblast assuming the entire duty of forming the alimentary canal. Likewise the ventral mesoblast, which in the Amphibian grows forwards underneath the yolk cells and forms the ventral mesodermic tissues of the adult, has in the fish lost

its function. The causes which led to the loss are plain enough. The yolk sac remains very large long after active muscular movements begin (movements begin before hatching) and a ventral musculature beneath the yolk (which would occupy the position indicated by dotted line in Fig. 10, p. 264, *an. v. m.*) would consequently be of no service until late in larval life, when the sac should have disappeared. This being the case, a much more economical method of forming the mesoderm was to put a stop to the subvitelline ingrowth (*an. v. m.*) and allow the lateral plates to form the ventral as well as dorsal mesoblastic tissues. The ventral (subvitelline) mesoderm, having in this way lost its function in the Teleost, must be regarded as a rudimentary organ of the gastrula. It always remains very small, and does not form any special organ or set of organs in the embryo. Being present it is however made use of, and goes to form a mass of indifferent material (caudal mass) at the expense of which the organs in the tail develop.

The germ ring, *in toto*, according to the view which has just been given, is not a peculiarity of the Teleost. It is a feature which the Teleost gastrula owes to an ancestor more or less like the gastrula of Amphibia, but which has gained in the Teleost a distinctive character, owing to its appearance all round the lip of the blastopore at a time when the latter is very large.

Significance of the germ ring with respect to the amniotic gastrula.—The fact that the ventral mesoderm, which in Amphibia is an important organ of the gastrula, is in Teleostei reduced to a rudimentary organ round the edge of the blastoderm, acquires a peculiar significance when the still more complicated gastrula of amniotes comes up for explanation. The fundamental features of this gastrula, it seemed, were satisfactorily explained by the Balfour-Rauber hypothesis, according to which the primitive streak plus the blastoderm edge represents the blastopore, the dorsal lip of which is indicated by the neurentric canal. The manner in which these writers believed the primitive streak to have been phylogenetically formed, was thought to receive a confirmation from the actual concrescence of the blastoderm edge, which takes place in the Selachian embryo behind the neurentric canal. During the last few years, however, the tide has set against this hypothesis, and in the direction of a new one, the chief exponents of which are Kupffer, Cunningham, Oscar Hertwig, and Rabl.

Kupffer's work on reptiles (25, 26), in the course of which he found that in this group there is no primitive streak, but in its place a definite invagination, led to the first step in the new direction. Kupffer came to regard the reptilian "invagination" (prostoma) and its homologue the Sauropsidan (and mammalian) primitive streak as alone representing the blastopore; the edge of the blastoderm was looked on as totally independent of the blastopore and was explained in a very unique fashion.

Cunningham (8), Oscar Hertwig (22), and Rabl (38) have all adopted this view of what constitutes the blastopore in the Amniotic gastrula. Hertwig says in his Lehrbuch (p. 104):

> Als Urmund schlage ich vor nur diejenige Stelle des Keims zu bezeichnen, an welcher wirklich wie bei der Gastrulabildung des Amphioxus und der Amphibien, eine Einstülpung von Zellen stattfindet, wodurch die Furchungshöhle verdrängt wird.

Such a process, according to Hertwig, does not occur round the edge of the blastoderm, and is only found in the region of the primitive streak and the "prostoma" of reptiles. The edge of the blastoderm is hence not a part of the blastopore; it is—

> eine Besonderheit der meroblastischen Eier, die mit der Entstehung der partiellen Furchung auf das innigste zusammen hängt.

Hertwig's explanation of the blastoderm edge is practically the same as Kupffer's.

Rabl (38) states his position, which is identical with the preceding, by means of a neat comparison between the bird and the frog gastrula. The line *x–y*, in Fig. 11, p. 265, cuts the frog's gastrula in two parts. The part to the left corresponds to the Amniotic embryo, the part to the right to the Amniotic yolk. The blastopore (*d. l.–v. l.*) equals the primitive streak, all round which there is an ingrowth of cells. The part to the right of *x–y* becomes transformed entirely into yolk. Rabl does not offer an explanation of how the transition from one gastrula to the other was accomplished. All he says is, that the effect is due to the great increase in size of the yolk (yolk cell mass). The inference, however, is that he adopts Cunningham's explanation, and supposes an actual hernia to take place.

The only argument for the view of the Amniotic gastrula entertained by the above-mentioned authors is that all round the lip of the blastopore in the Amphibia, etc., there is an ingrowth of cells, and that round the prostoma of reptiles and primitive streak of birds and mammals there is the same ingrowth. The two structures it is concluded must therefore be homologous. It then becomes a question of how to explain the blastoderm edge. Two explanations of the structure have been offered, one by Kupffer which is substantially the same as that of Oscar Hertwig, and one by Cunningham to which Rabl seems inclined. Kupffer's explanation is contained in the following passage (26):

Ich fasse also die Ausbreitung des Blastoderms über den Dotter als Blastulabildung auf. Der Abschluss dieser Bildung erfolgt um so später je grösser der zu umwachsende Dotter ist, und es tritt der Gastrulation vor Vollendung der Blastulabildung ein, *d. h.*, während ein Blastotrema [so-called Dotter-Blastoporus] noch vorhanden ist.

On analyzing Kupffer's view it will be seen that by "blastula" he means not a one-layered but a two-layered embryo. His theory is illustrated by the diagram, Fig. 12, in which *a–á* mark the blastoderm edge of an Amniote; *s. c.* is the segmentation cavity, and *y* is the yolk—the embryo being in what would commonly be called the blastula stage. Now the growth of the blastoderm over the yolk does not take place, according to Kupffer, in a true epibolic fashion, but is accomplished through the medium of a zone of tissue (Keimwall) in which the yolk cells (nuclei) become transformed into the cells of the two primary layers. Hertwig holds the same opinion (Lehrb., p. 105). If this be really the case in Amniota, two explanations of the process are possible: First, that it is a modification of the ancestral, epibolic growth (such as occurs in Teleosts), which view Kupffer and Hertwig would of

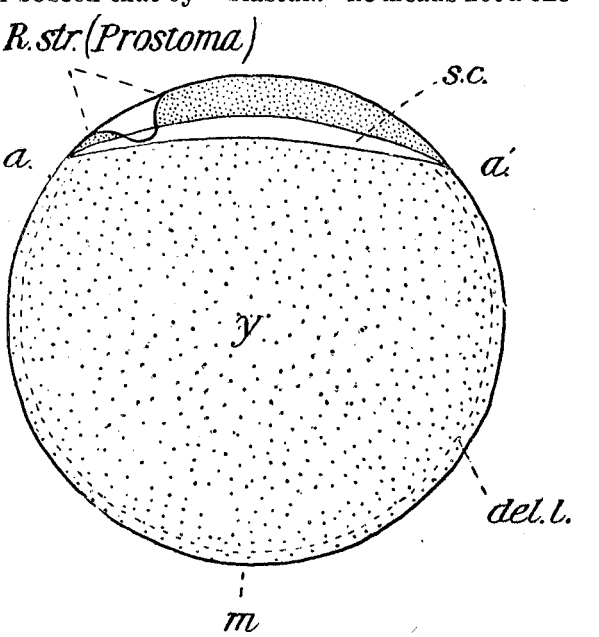

FIG. 12.—Diagram to illustrate Kupffer's theory of the Amniotic gastrula—*s. c.*, segmentation cavity; *y*, yolk; *a.* to *a.*, edge of blastoderm; *P. str.*, primitive streak (Prostoma of reptiles); *del. l.*, line along which, according to Kupffer, the yolk splits off ectoderm.

course reject, because it is equivalent to admitting the homology of the blastoderm edge in Teleosts and Amniotes, and consequently the correctness of the Balfour-Rauber hypothesis; secondly, that the process is, to refer it to simple embryonic forms, one of progressive delamination. It will be seen that Kupffer's hypothesis really implies the occurrence of the latter process, for when he explains the spreading of the blastoderm as the completion of the blastula stage, he really means that the yolk splits off ectoderm progressively from *a* and *a'* towards *m*. Thus, again to reduce the processes to their simplest forms, over one-half (yolk-half) of the blastula (Fig. 12, p. 269) delamination occurs; but in the other half there is a true invagination (region of prostoma and primitive streak). Neither Kupffer nor Hertwig illustrates his theory with diagrams, and since the embryonic processes dealt with are extremely complicated, it is a difficult matter to form a precise conception of their meaning. However, I think the analysis I have given is a perfectly fair one, and the result is evidently prejudicial to their theory. For the conclusion is that the Amniotic vertebrates have a blastula, which invaginates over one half and delaminates over the other. Such an embryonic form is nowhere known to occur, and the theory which is forced to assume its existence is in so far a weak theory and must give place to any other which can explain the facts by making use only of known processes.

The explanation which Cunningham gives to the blastoderm edge, and to which Rabl is logically forced, is much simpler than the hypothesis just discussed. It is equally objectionable, however, for it invokes a process never observed, and which is moreover *a priori* extremely improbable. Adopting as an ancestral form a gastrula like that of the frog, Cunningham supposes the Amniotic gastrula to have been derived as follows: The increase in size of the mass of yolk cells, instead of enlarging the blastopore (the result which one would suppose would naturally follow) produced a rupture in the ectoderm. In this way the lower surface of the yolk came to be exposed and the blastoderm edge was brought into existence, the latter being a purely secondary structure, dating no further back than the occurrence of the hernia.

The fact that, having once accepted Kupffer's homology between the primitive streak and the blastopore we are forced to assume the occurrence of such improbable embryonic forms and processes as have just been described, leads us to reconsider the older Balfour-Rauber hypothesis. The only objection to regarding the blastoderm edge as part of the original blastopore, is that round the latter there is an ingrowth of cells, round the former there is none. But the study of the meroblastic Teleost egg has already shown us that the part of the ingrowth which, according to the Balfour-Rauber theory, belongs to the blastoderm edge in the Amniota, became a rudimentary organ in the fishes. There is no more natural supposition than that it went a step farther and was altogether lost, or became still more reduced in size in the Amniota. The disappearance of a rudimentary organ is one of the commonest inferences in the study of the comparative anatomy of adults, and of course must occur in embryos as well. It is therefore a very different assumption from that of the occurrence of an embryonic hernia or a new and complicated embryonic form. Assuming that the mesodermic ingrowth has been lost round the blastoderm edge in Amniota, the only objection to the Balfour-Rauber theory falls to the ground, for of course this theory as well as the other is capable of explaining the existence of what Hertwig calls "unpaired mesoblast" behind the primitive streak.

It must be remembered, moreover, that the exact nature of the blastoderm edge in

Sauropsida is not well known, and that it is quite possible that further study may show the presence of a peripheral ingrowth in these embryos. Indeed Kollmann's figures (30) of sections through young chick blastoderms almost prove the existence of such an ingrowth. The later history of the blastoderm edge is extremely complicated, and the morphology of the region must be studied before the complications arise. If it should turn out that there is, as Kollmann believes, an ingrowth of cells from the blastoderm edge (which becomes a source of blood cells) the homology of the blastoderm edge in Ichthyopsida and Amniota would be proved. But Kollmann's conclusion that this peripheral ingrowth, or akroblast, is a special organ (homologous with the mesenchyme cells of certain invertebrate embryos) would not hold. For the akroblast would merely represent the last stage in the course of development, which the ventral mesoderm of simple vertebrate embryos (Amphibia, Petromyzon) has undergone.

WOOD'S HOLL, *July* 22, 1890.

LIST OF PAPERS REFERRED TO.

1. AGASSIZ and WHITMAN, C. O. On the development of some Pelagic Fish Eggs. Preliminary Notice. Proc. Amer. Acad. Arts and Sciences, XX, 1884.
2. ALLIS, E. P. Jr. Anatomy and Development of the Lateral Line System in Amia calva. Journal of Morphology, 1889.
3. BROOK, G. On the Origin of the Hypoblast in Pelagic Teleostean Ova. Quart. Journ. Micr. Sci., 1885.
4. BROOK, G. Note on the origin of the Wolffian Duct in Teleostei. Proc. Royal Soc., Edinburgh, Vol. XIV, 1887.
5. BEARD, JOHN. Branchial Sense Organs of Ichthyopsida. Quart. Journ. Micr. Sci., 1885.
6. BEARD, JOHN. On the Segmental Sense Organs of the Lateral Line, etc. Zool. Anzeiger, 1884.
7. BALFOUR, F. M. A Comparison of the Early Stages in the Development of Veretbrates. Quart. Journ. Micr. Sci., 1875.
8. CUNNINGHAM, J. T. On the Relations of the Yolk to the Gastrula in Teleosteans, and in other Vertebrate Types. Quart. Journ. Micr. Sci., 1885.
9. CUNNINGHAM, J. T. The Significance of Kupffer's Vesicle, etc. Quart. Journ. Micr. Sci., 1885.
10. CALBERLA, E. Zur Entw. des Medullarrohres und der Chorda dorsalis d. Teleostier und d. Petromyzonten. Morph. Jahrbuch, 1877.
11. EISIG, H. Die Segmentalorgane der Capitelliden Mitt. a. d. Zool. Station zu Neapel, 1879.
12. G. BROWN GOODE and Associates. Fisheries and Fishery Industries of the United States. Sec. 1, History of Aquatic Animals, Washington, 1884.
13. GENSCH, HUGO. Das secundare Entoderm und die Blutbildung beim Ei der Knochenfische. Inaugural Dissertation, Konigsberg, 1882.
14. GÖTTE, A. Der Keim des Forelleneies. Archiv f. Mikr. Anat., 1873.
15. GÖTTE, A. Zur Entwickl. d. Wirbelthiere. Vorlaufige Mitt., in Centralblatt fur die Medicin. Wissenschaften No. 26, 1869.
16. GÖTTE, A. Ueber die Entwicklung d. Central Nerv. System d. Teleostier. Archiv f. Mikr. Anat., 1878.
17. HOFFMANN, C. K. Zur Ontogenie der Knochenfische. Verhandelingen d. Konink. Akad. d. Wetenschappen zu Amsterdam, 1881–1883.
18. HENNEGUY, FELIX. Récherches sur la developpement des Poissons Osseux. Embryogenie de la Truite. Journal de l'Anat. et de la Physiologie, 1888.
19. HAECKEL, E. Die Gastrula und die Eifurchung der Thiere. Jena Zeitschrift, 1875.
20. HERTWIG, O. Die Entwicklung des Mittleren Keim-blattes der Wirbelthiere. Jena Zeit., 1881, 1882.
21. HOFFMANN, C. K. Zur Ontogenie d. Knochenfische. Archiv f. Mikr. Anat., 1883.
22. HERTWIG, O. Lehrbuch der Entwicklungsgeschichte des Menschen und der Wirbelthiere. Zweite Auflage, Jena, 1888.

23. JOHNSON, ALICE. On the fate of the Blastopore and the Presence of a Primitive Streak in the Newt. Quart. Journ. Micr. Sci., 1884.
24. KUPFFER, C. Beobachtungen uber die Entwicklung d. Knochenfische. Archiv f. Mikr. Anat., 1868.
25. KUPFFER, C. Die Entstehung der Allantois und der Gastrula der Wirbelthiere. Zool. Anz., 1879.
26. KUPFFER, C. Die Gastrulation an den meroblastischen Eiern d. Wirbelth. und die Bedeutung des Primitiv-streifs. Archiv f. Anat. und Entwicklungsgeschichte, 1882, 1884.
27. KOWALEWSKI, MIECZ VON. Ueber die ersten Entwicklungsprocesse d. Knochenfische. Zeit. f. Wiss. Zool., 1886.
28. KINGSLEY, J. S., and CONN, H. W. Some observations on the Embryology of the Teleosts, Mem. Boston Society. Boston, 1883.
29. KOLLMANN, J. Gemeisame Entwicklungsbahne d. Wirbelthiere. Archiv f. Anat. und Entwickl., 1885.
30. KOLLMANN, J. Der Randwulst und der Ursprung der Stützsubstanz. Archiv f. Anat. und Entwick., 1884.
31. LEREBOULLET, M. Récherches sur la developpement du Brochet, de la Perche et de l'Écrevisse. Annales des Sciences Naturelles, 1854.
32. MORGAN, T. H. On the Amphibian Blastopore. Studies from the Biological Laboratory of the Johns Hopkins Univ., Vol. IV., No. 26, Baltimore, 1890.
33. OELLACHER, J. Beitrage zur Entwicklungsgeschichte der Knochenfische, nach Beobachtungen am Bachforellenei. Zeit. f. Wiss. Zool., 1872-'73.
34. RYDER, John A. A Contribution to the Embryography of Osseous Fishes. Report of U. S. Fish Commissioner for 1882, Washington, 1884.
35. RYDER, John A. On the Development of Osseous Fishes. Report of U. S. Fish Commissioner for 1885, Washington, 1887.
36. RAUBER. Neue Grundlegungen zur Kenntniss der Zelle. Morph. Jahrbuch, 1883.
37. RUECKERT, J. Zur Keimblattbildung bei Selachiern. München, 1885.
38. RAHL, CARL. Theorie des Mesoderms. Morph. Jahrb., 1889.
39. SCHWARZ, DANIEL. Untersuch. des Schwanzendes bei den Embryonen d. Wirbelth. Zeit. f. Wiss. Zool., 1889.
40. VAN BENEDEN, E. A Contribution to the History of the Embryonic Development of the Teleosteans. Quart. Journ. Micr. Sci., 1878.
41. WATASE, S. On a New Phenomenon of Cleavage in the Ovum of the Cephalopod. Circular of the Johns Hopkins Univ., Vol. VIII, No. 70, Baltimore, 1889.
42. WATASE, S. Karyokinesis and the Cleavage of the Ovum. Circular of Johns Hopkins Univ., Vol. IX, No. 80, Baltimore, 1890.
43. WENCKEBACH, K. F. Beitrage zur Entwicklungsgeschichte d. Knochenfische. Archiv. f. Mikr. Anat., 1886.
44. WHITMAN, C. O. Segmental Sense Organs of Leeches. American Naturalist, 1884.
45. WHITMAN, C. O. Some New Facts about the Hirudinea. Journal of Morphology, Vol. II, No. 3, 1889.
46. WILSON, H. V. On the Development of the Sea Bass. Circular of Johns Hopkins Univ., Vol. IX, No. 80, Baltimore, 1890.
47. ZIEGLER, E. Die Entstehung des Blutes bei Knochenfischembryonen. Archiv f. Mikr. Anat., 1887.
48. ZIEGLER, E. Die Embryonale Entwicklung von Salmo Salar. Dissertation. Freiburg, 1882.

COMMON REFERENCE LETTERS USED IN THE FIGURES.

a. s.	Auditory sac.	*br. f.*	Branchial fold.
a. n.	Auditory nerve.	*br.*	Brain.
a. an.	Aorta anlage.	*b. s. o.*	Branchial sense organ.
al. c.	Alimentary canal.	*b. f.*	Body fold (separating body from yolk).
aor.	Aorta.	*b. si.*	Body sinus.
a.	Anus.	*bl. ed.*	Blastoderm edge.
a. p.	Anterior pole of blastoderm.	*cœl.*	Cœlom.
a. s. t.	Anterior sensory tract.	*c. t.*	Connective tissue (?) cells.
a. m.	Anterior mass of prim. hypoblast.	*cer. f.*	Cerebellar (?) fold.
bl.	Blastopore.	*c. c.*	Canalis centralis.

COMMON REFERENCE LETTERS USED IN THE FIGURES—Continued.

c. ep.	Columnar epithelium of lens invagination.
cer.	Cerebellum.
con. st.	Connecting strand, bwt. organs of developing lateral line.
c. p.	Central periblast.
cor. p.	Cortical periblast.
c. m.	Caudal mass.
d. l.	Dorsal lip of blastopore.
d. l. s. t.	Dorso-lateral sense tract.
d. l. m.	Dorso-lateral muscle tract.
d. f.	Dorsal part of embryonic fin.
d. n. r.	Dorsal root of spinal nerve.
e. p. r.	Early periblastic ridge.
ep. s.	Epidermic stratum.
e. e. s.	Edge of embryonic shield.
ec.	Ectoderm.
e. m.	Egg membrane.
en. mes.	Layer of cells which posteriorly becomes entoderm, anteriorly mesoderm.
en.	Entoderm.
f. gr.	Fin groove.
g. s.	Gill slit.
g. r.	Germ ring.
h.	Heart.
h. e.	Head end of embryo.
Iter.	Iter a tertio ad quartum Ven.
k. v.	Kupffer's vesicle.
l. en.	Lateral entoderm.
ln.	Lens.
l. l.	Lateral line anlage.
l. l. o.[1]	1st organ of lateral line.
l. l. o.[2]	2d organ of lateral line.
l. l. o.[3]	3rd organ of lateral line.
l. l. o.[4]	4th organ of lateral line.
l.	Liver.
l. f.	Lens fibers.
l. ep.	Lens epithelium.
med.	Medulla.
m. con.	Constriction separating medulla from mid-brain.
m. br.	Mid-brain.
m. b.	Muscle band (†) of cœlom wall.
m. c.	Marginal blastoderm cell.
m. ep. c.	Marginal epidermic cell.
mes.	Mesoderm.
n. str.	Neurentric streak.
n. s.	Nasal sac.
nc. s.	Notochordal sheath.
nc.	Notochord.
nr. ch.	Neural chord.
n. f.	Neural furrow.
op. s.	Optic sac.
op. l.	Optic lobes.
oes.	Œsophagus.
o. g.	Oil globule.
o. g. p.	Protoplasmic cap of oil globule.
p.	Periblast.
p. pl.	Periblast plug—Dotterpropf.
p. a. g.	Postanal gut.
p. c.	Pigment cell.
pec. f.	Pectoral fin.
p. w.	Periblast wall.
p. p.	Posterior pole of blastoderm.
pr. h.	Primitive hypoblast.
pr. str.	Primitive streak.
ret. l.	Retinal layer of optic cup.
s. f.	Sensory furrow—Common anlage for ear, lateral line, and branchial sense organ.
sec. c. m.	Secondary caudal mass (remnant of extra-embryonic germ ring).
som.	Somites or somite mesoderm.
sp. c.	Spinal cord.
s. n. r.	Subnotochordal rod.
s. c.	Segmentation cavity.
s. g. c.	Subgerminal cavity (late stage of segmentation cavity).
t. f.	Tail furrow.
un. mes.	Undivided mesoderm in tail.
v. l.	Ventral lip of blastopore.
v. l. m.	Ventro-lateral muscle tract.
v. f.	Ventral part of embryonic fin.
v. n. r.	Ventral root of spinal nerve.
v. mes.	Ventral mesoderm (non-embryonic part of germ ring).
w. d.	Wolffian duct.
w. c.	Wandering cell.
y.	Yolk.
1 *d.*	1st cleavage plane.
2*d.*	2d cleavage plane.
3*d.*	3d cleavage plane.
4*d.*	4th cleavage plane.
3 *ven.*	Third ventricle.
4 *ven.*	Fourth veneticle.

The several series of sections are numbered from behind forwards. Objectives and oculars referred to are Zeiss's.

EXPLANATION OF PLATES.

PLATE LXXXVIII.

Fig. 1. Section through oil globule of segmenting egg.
Fig. 2. Surface view of a segmenting egg, two blastomeres in which nuclear division has already taken place.
Fig. 3. View from below of 8-blastomere stage.
Fig. 4. Irregular 8-blastomere stage.
Fig. 5. 8-blastomere stage of mackerel, radial division.
Fig. 6. Blastoderm showing cellular division, 8 into 16.
Fig. 7. 16 into 32 cells; opposite cells divide at same time.
Fig. 8. Blastoderm showing normal cleavage, 16 into 32.
Fig. 9. Stage of 16 into 32, variation.

PLATE LXXXIX.

Fig. 10. Stage of 16 into 32—variation.
Fig. 11. Resting blastoderm of 32 cells.
Fig. 12. Blastoderm—32 into 64.
Fig. 13. Section through center of 4-blastomere stage.
Fig. 14. Stage of 8 cells—section is through *a* of Fig. 3.
Fig. 15. Stage of 8 cells—section is through *b* of Fig. 3.
Fig. 16. Stage of 16 into 32 cells—section lies through *a* to *b* of Fig. 8.

PLATE XC.

Fig. 17. Stage of 16 into 32 cells—section is through *c* to *d* of Fig. 8.
Fig. 18. Stage of 32 cells—lower cells just beginning to divide—through *a–b* of woodcut, Fig. 1, Pl. 9.
Fig. 19. 32 into 64 cells—through *d–f* of woodcut, Fig. 1.
Fig. 20. Section through late segmentation stage.
Fig. 21. Surface view of edge of blastoderm of 4.40 hours—marginal cells still distinct.
Fig. 22. Surface view of blastoderm edge—7.30 hours—marginal cells not marked off from the periblast.
Fig. 23. Surface view of blastoderm edge—8.30 hours—outlines of marginal cells entirely lost.
Fig. 24. Surface view of blastoderm edge—9.30 hours—multiplication of periblastic nuclei.

PLATE XCI.

Fig. 25. Section through blastoderm of about same stage as Fig. 22. D. 4.
Fig. 26. Section through a blastoderm such as Fig. 23. D. 4.
Fig. 27. Section through periblastic wall (about same stage as Fig. 24). D. 4.
Fig. 28. Section through periblastic wall (about same stage as Fig. 24). D. 4.
Fig. 29. Section through blastoderm (9.30 hours) of about same stage as Figs. 24, 27, 28. A. 4.
Fig. 30. Section through blastoderm of 14 hours. A. 4.
Fig. 31. Section through blastoderm of 16 hours. A. 4.
Fig. 32. Surface view of blastoderm (16 hours) in which the "randwulst" is just marked out at embryonic pole—first stage in formation of germ ring. A. 4.

PLATE XCII.

Fig. 33. Surface view of blastoderm (17 hours)—germ ring formed all round blastoderm edge. A. 4.
Fig. 34. Surface view of blastoderm (20 hours)—h. e.= head end of embryonic anlage. A. 4.
Fig. 35. Side view of embryo (20 hours). A. 4.
Fig. 36. Side view of embryo (25 hours). A. 4.
Fig. 37. Embryo of 25 hours—from above. A. 4.
Fig. 38. Embryo of 31 hours—from the side. A. 4.

PLATE XCIII.

Fig. 39. Tail end and blastopore of embryo, 31 hours. A. 4.
Fig. 40. Antero-posterior section through the posterior pole of Fig. 32, $m-m'$ = apical cells of the randwulst. F. 4.
Fig. 41. Antero-posterior section through a blastoderm just a little older than Fig. 32—to show ingrowth of germ ring (*pr. h.*) at embryonic pole. D. 4.
Fig. 42. Antero-posterior section through a stage such as Fig. 33. A. 4.
Fig. 43. Longitudinal section through posterior pole of a blastoderm between Figs. 33 and 34. F. 4.
Fig. 44. Longitudinal section through posterior pole of a blastoderm of 20 hours. F. 4.

PLATE XCIV.

Fig. 45. Part of longitudinal section through posterior pole of blastoderm of about same stage as Fig. 41—unusual involution of epidermic cells. F. 4.
Fig. 46. Long section through anterior pole of same stage as Fig. 43. *v. mes*, extra-embryonic part of germ ring (homologous with ventral mesoblast of Amphibia). F. 4.
Fig. 47. Transverse section through *a–b* of Fig. 33.
Fig. 48. Longitudinal section through anterior pole of same blastoderm as Fig. 44.
Fig. 49. Transverse section through posterior pole of Mackerel blastoderm (27 hours)—intermediate between Bass blastoderms given in Figs. 35 and 36. D. 4.
Fig. 50. Part of transverse section through embryonic shield of Mackerel blastoderm, 27 hours.
Fig. 51. Section through extra-embryonic germ ring (*v. mes.*) of Mackerel, 27 hours—blastoderm half round yolk. D. 4.
Fig. 52. Longitudinal section to one side of median line of Mackerel blastoderm, 29 hours. C. 4.
Fig. 53. Trans. section through anterior part of embryonic shield of Mackerel blastoderm, 30 hours—just a little less advanced than Fig. 36.

PLATE XCV.

Fig. 54. Transverse section through posterior pole of embryonic shield of Mackerel, 30 hours. (Same embryo as Fig. 53.)
Fig. 55. Median longitudinal section of embryo, 25 hours. C. 4.
Figs. 56 (one-twelfth immersion), 57 (D. 4), 58 (D. 4). Transverse section through embryo, 25 hours. (Letters refer to objectives.)
Fig. 59. Median longitudinal section through embryo, 29 hours. D. 4.
Figs. 60, 61, 62. Transverse sections through embryo, 29 hours. D. 4.

PLATE XCVI.

Figs. 63, 64 (same series as 60–62). Transverse sections through embryo, 29 hours. D. 4.
Fig. 65. Median longitudinal section through posterior end of embryo, 33 hours. D. 4.
Figs. 66 (D) 67 (D), 68 (F), 69 (D), 70 (F). Series of transverse sections through embryo of 35 hours. (Letters refer to objectives.)

PLATE XCVII.

Figs. 71 (D), 72 (D) (same series as Figs. 66–70). Series of transverse sections through embryo of 35 hours.

Figs. 73 (D), 74 (F), 75 (D), 76 (F), 77 (F), 78 (D), 79 (D). Series of transverse sections through embryo of 39 hours.

Fig. 80. Transverse section through optic sac of embryo, 39 hours. D. 4.

Fig. 81. Ectoderm of embryo, 39 hours. F. 4.

PLATE XCVIII.

Fig. 82. Transverse section through embryo, 41 hours, in region of Kupffer's vesicle. D. 4.

Fig. 83. Median longitudinal section through head end of embryo, 45 hours. C. 4.

Fig. 84. Median longitudinal section through tail end of embryo, 45 hours. D. 4.

Fig. 85. Longitudinal section to one side of median line through embryo, 45 hours. C. 4.

Fig. 86. One of posterior somites of Fig. 85. F. 4.

Fig. 87. One of anterior somites of Fig. 85. F. 4.

Figs. 88 (D), 90 (D), 91 (F). Series of transverse sections through embryo of 45 hours.

Fig. 89. Transverse section through region of Kupffer's vesicle of embryo slightly more advanced than Fig. 88. D. 4.

PLATE XCIX.

Figs. 92 (F), 93 (F), 94 (D), 95 (D), 96 (D), 97 (D), from same series as Figs. 88–91. Transverse sections through embryo, 45 hours. D. 4.

Fig. 98. Surface view of tail end of embryo, from below, 49½ hours.—*t. f.* (tail fold), marks line along which the ectoderm covering the tail bends round over the yolk. D. 4.

Figs. 99 (D), 100 (D). Transverse sections through embryo of 49½ hours.

PLATE C.

Figs. 101 (D), 102 (D), 103 (F), 104 (F), 105 (D), 106 (D), 107 (F), 108 (D), from same series as Figs. 99 and 100. Series of transverse sections through embryo of 49½ hours.

Fig. 109. Transverse section through tail end of embryo, 53 hours. D. 4.

PLATE CI.

Fig. 110. Transverse section through trunk of embryo, 53 hours. F. 4.

Fig. 111. Transverse section through tail of embryo, 59 hours. D. 4.

Fig. 112. Ventral portion of a section similar to Fig. 111. F. 4.

Figs. 113 (D), 114 (D), 115 (D), 116 (F). Series of transverse sections through embryo of 59 hours.

PLATE CII.

Fig. 117 (D), belongs to same series as Figs. 113—118, through embryo of 59 hours.

Fig. 118. Transverse section through eye of embryo, 59 hours. D. 4.

Fig. 119. Transverse section through tail of embryo, 65 hours. D. 4.

Fig. 120. Transverse section through 1st organ of lateral line, 65 hours. F. 4.

Figs. 121, 122. Transverse sections through connecting strand of lateral line of 65-hour stage. F. 4.

Fig. 123. Transverse section through cerebellar (?) folds and dorso-lateral sense tract of embryo, 65 hours. D. 4.

Fig. 124. Transverse section through eyes and infundibulum of 65-hour stage. D. 4.

Fig. 126. Transverse section through posterior trunk of embryo, 75 hours. D. 4.

PLATE CIII.

Figs. 126^{I}, 126^{II}, 126^{III}, 126^{IV}. Series of sections through postanal lateral line of embryo, 75 hours. F. 4.
Figs. 127 (D), 128 (D), 129 (D), 130 (D)—Fig. 126 belongs to this series. Series of transverse sections through embryo, 75 hours.

PLATE CIV.

Figs. 131 (D), 132 (F), 133 (D), 134 (D), 135 (C) from same series as Figs. 126–130. Series of transverse sections through embryo, 75 hours.
Fig. 136. Transverse section through region of pectoral fins of larva, 86 hours. D. 4.
Fig. 137. Four muscle fibers of Fig. 136.
Fig. 138. Part of transverse section through larva, 100 hours, to show origin of liver, *l*. D. 4.

PLATE CV.

Fig. 139. Transverse section through pectoral fins of larva, 112 hours. C. 4.
Fig. 140. Part of transverse section through region of liver in larva of 112 hours. D. 4.
Fig. 141. Transverse section through larva of 136 hours—region of liver. C. 4.
Fig. 142. Three muscle fibers from Fig. 141.
Figs. 143 (C), 144 (D), 145 (D). Transverse sections through larva of 160 hours—region of yolk and liver.

PLATE CVI.

Fig. 146. Surface view from above of embryo 33 hours. C. 4.
Fig. 147. Surface view from above of part of embryo 37 hours. D. 4.
Fig. 148. Surface view from above of embryo 45 hours. C. 4.

PLATE CVII.

Fig. 149. Surface view from below of embryo, 62 hours. C. 4.
Fig. 150. Surface view from above of embryo, 65 hours. C. 4. b–f = fold which constricts body from yolk.
Fig. 151. Side view of embryo, 65 hours. A. 4.

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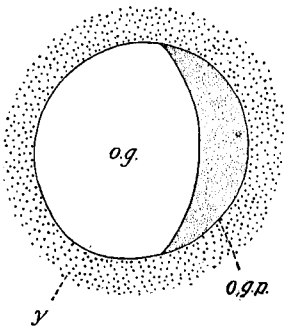


Fig. 1

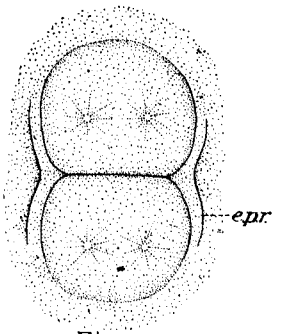
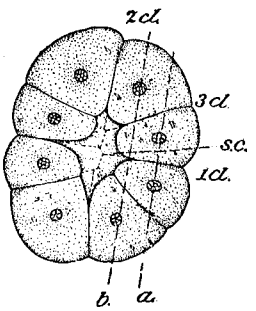


Fig. 2



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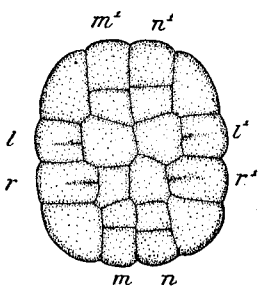

Fig. 7

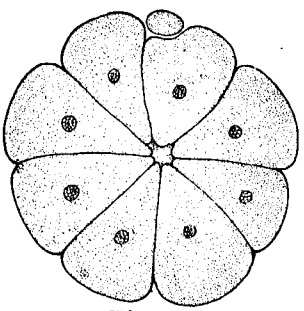

Fig. 5

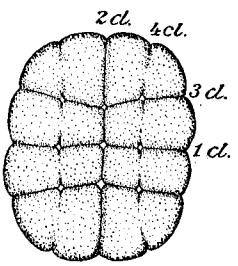

Fig. 6

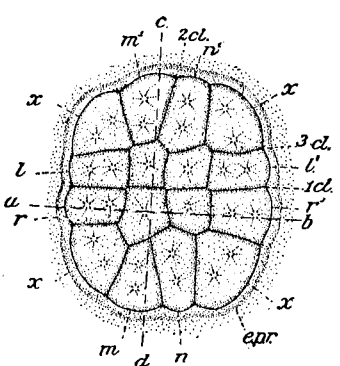

Fig. 8

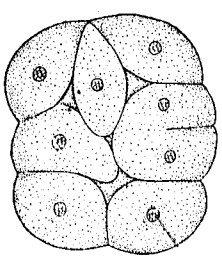

Fig. 4

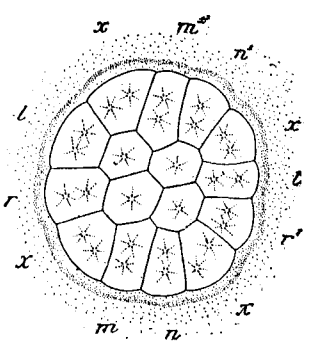

Fig. 9

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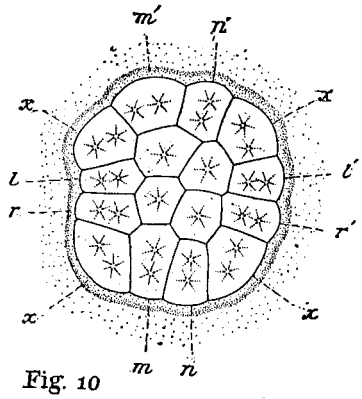

Fig. 10

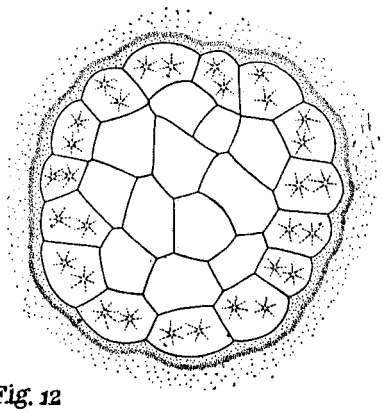

Fig. 12

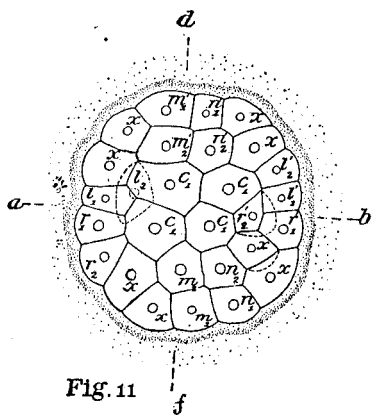

Fig. 11

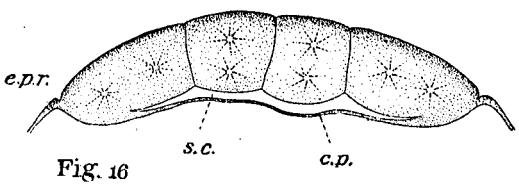

Fig. 16

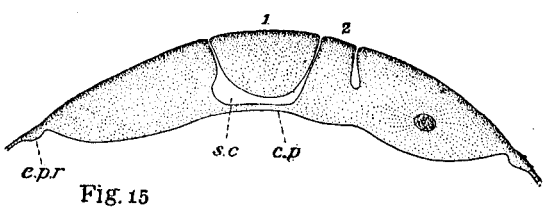

Fig. 15

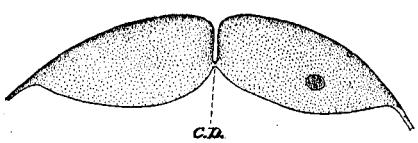

Fig. 13

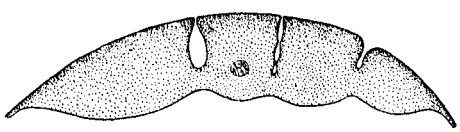

Fig. 14

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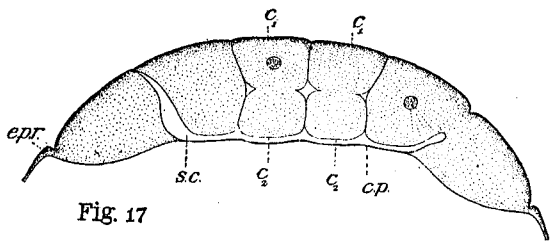

Fig. 17

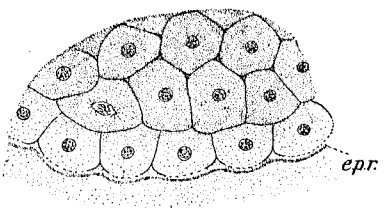

Fig. 21

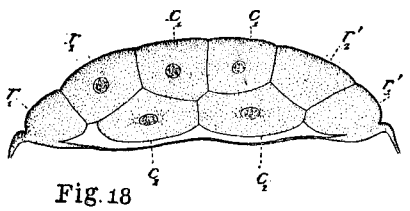

Fig. 18

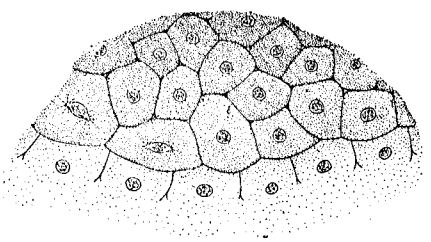

Fig. 22

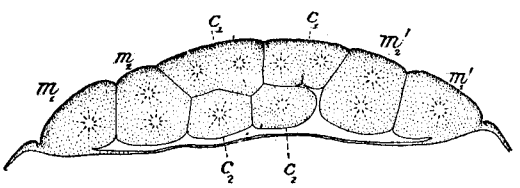

Fig. 19

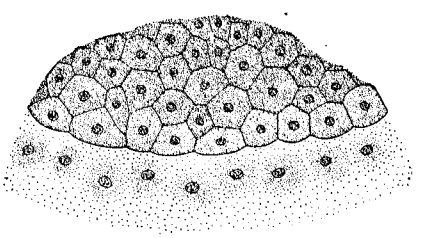

Fig. 23

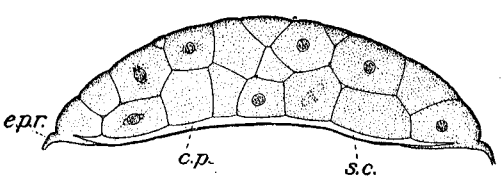

Fig. 20

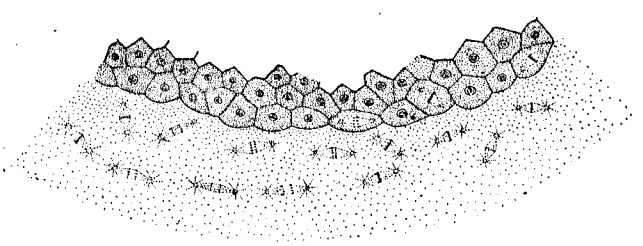

Fig. 24

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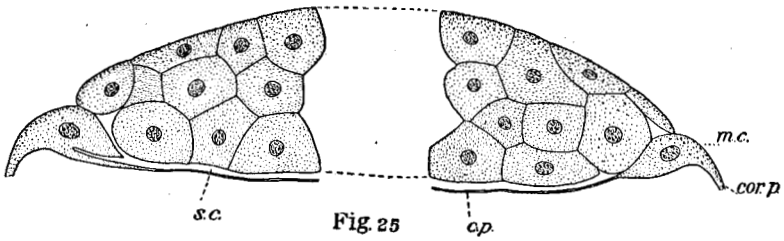

Fig. 25

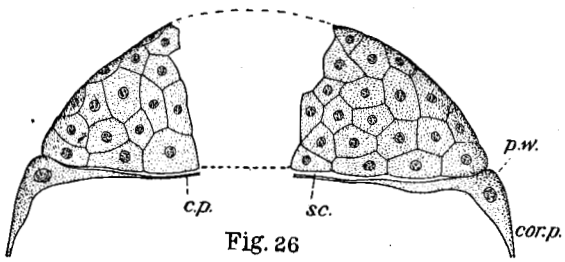

Fig. 26

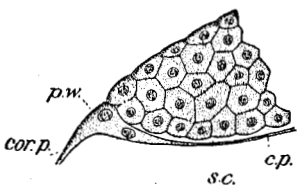

Fig. 27

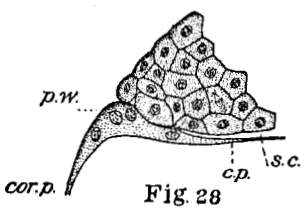

Fig. 28

Fig. 29

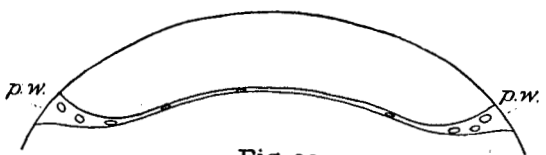

Fig. 30

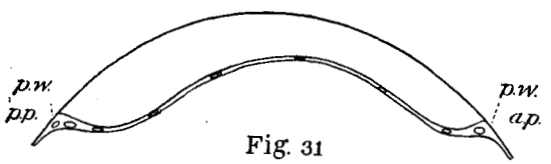

Fig. 31

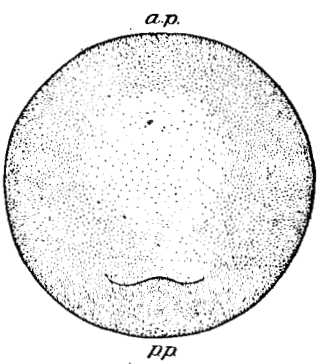

Fig. 32

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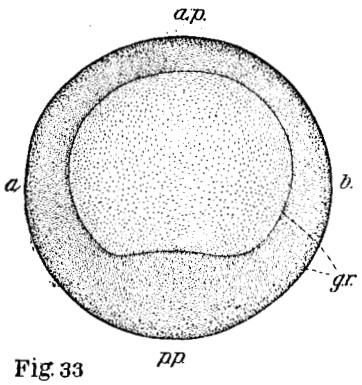

Fig. 33

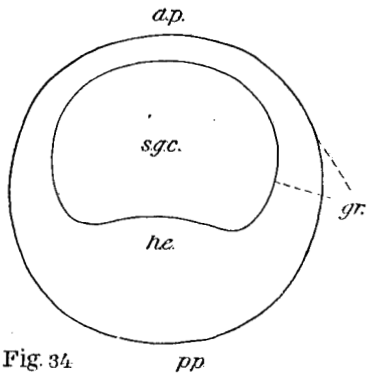

Fig. 34

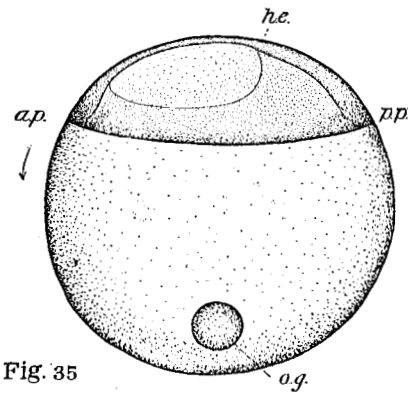

Fig. 35

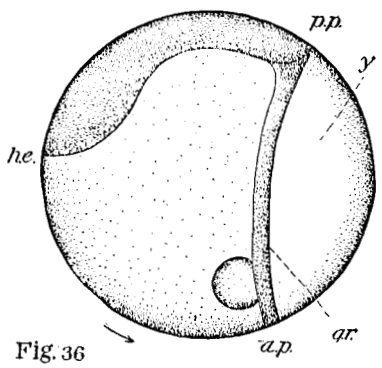

Fig. 36

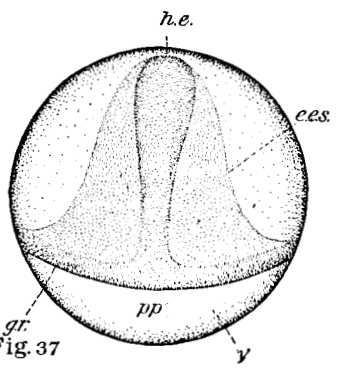

Fig. 37

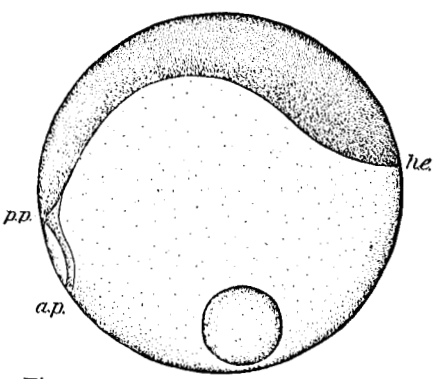

Fig. 38

H.V.W. DEL.

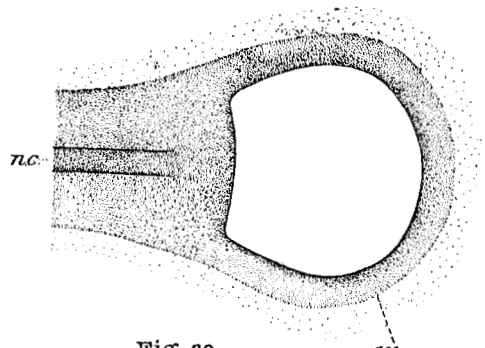

Fig. 39

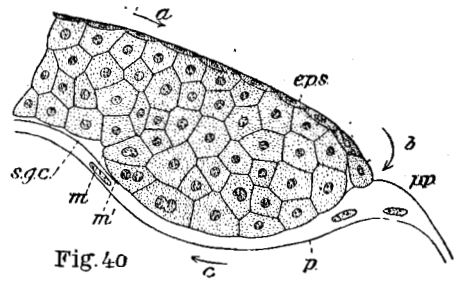

Fig. 40

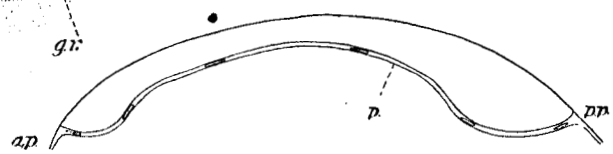

Fig. 42

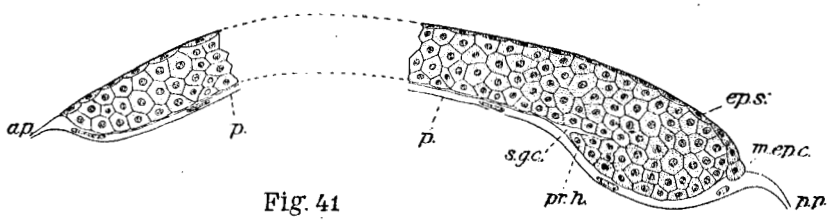

Fig. 41

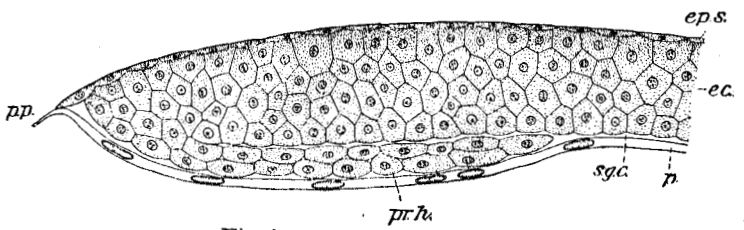

Fig. 43

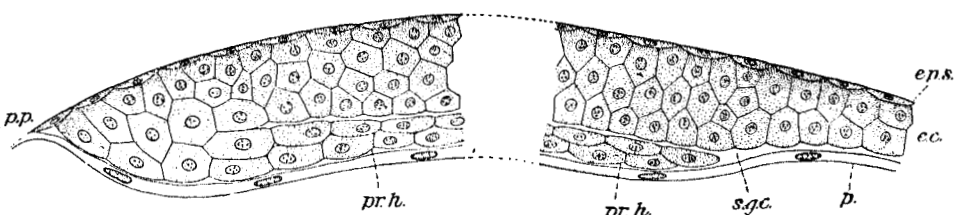

Fig. 44

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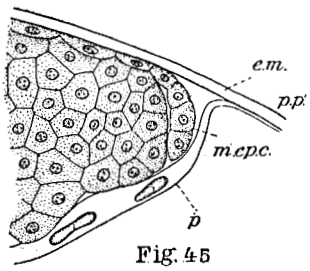


Fig. 45

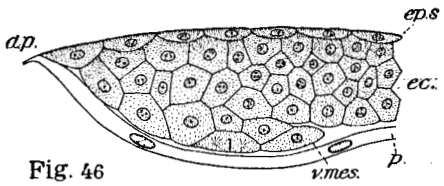


Fig. 46

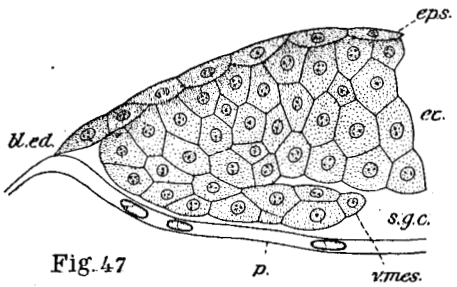


Fig. 47

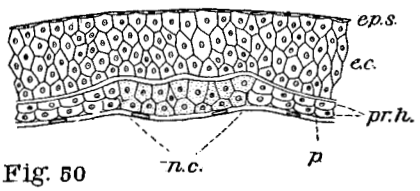


Fig. 50

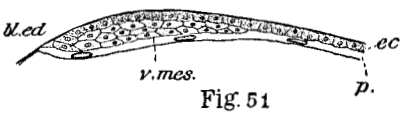


Fig. 51

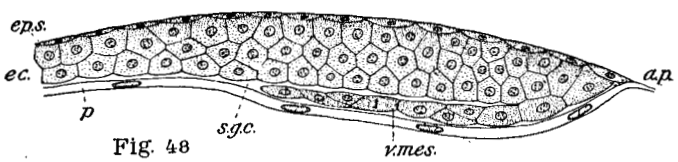


Fig. 48

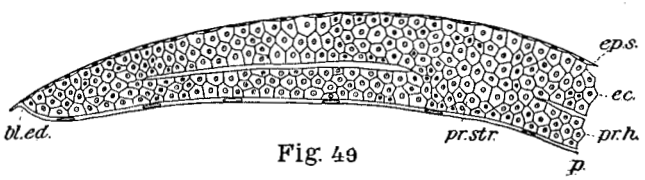


Fig. 49

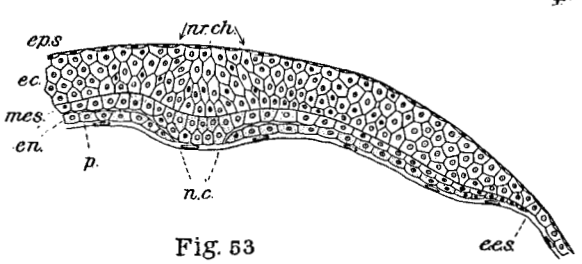


Fig. 53

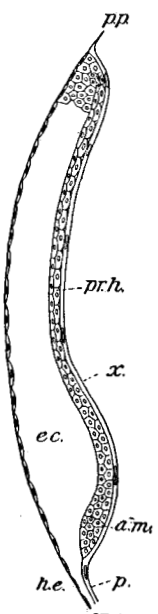


Fig. 52

H.V.W. DEL.

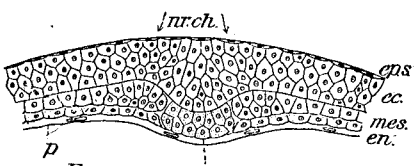


Fig. 54

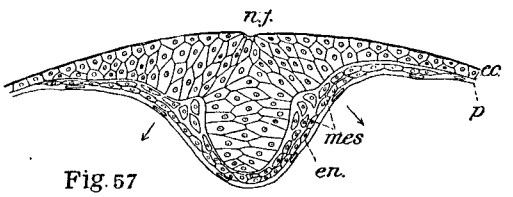


Fig. 57

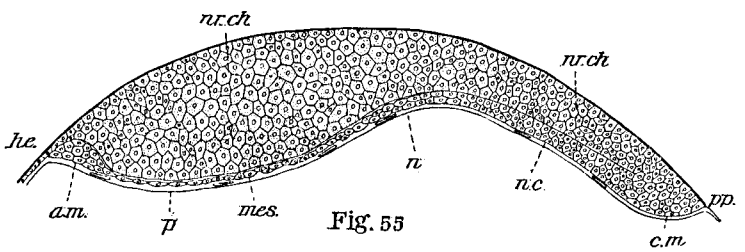


Fig. 55

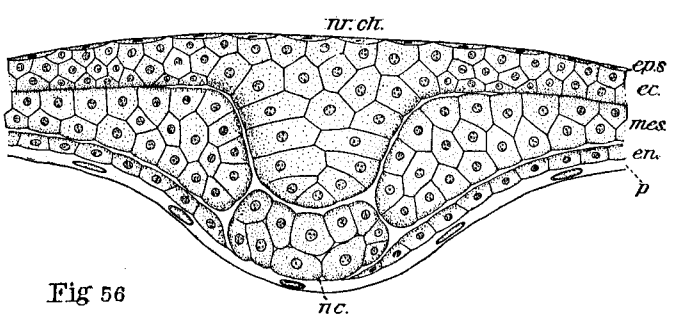


Fig 56

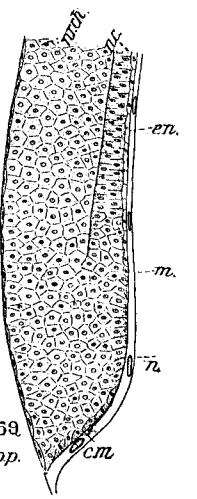
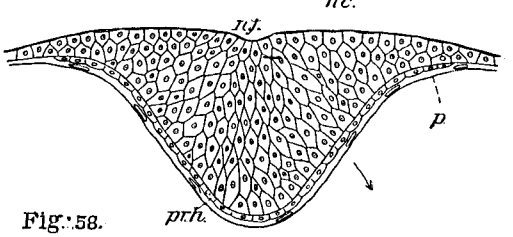


Fig 59



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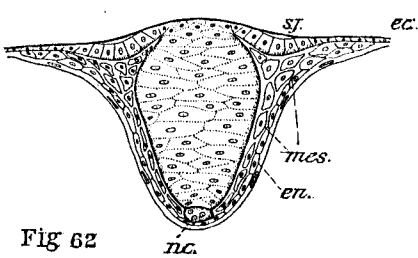

Fig 62

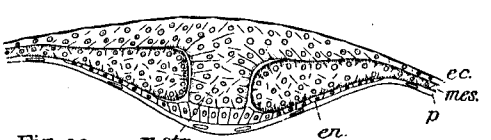

Fig. 60

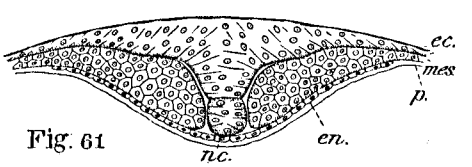

Fig. 61

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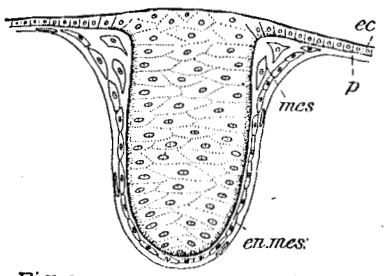

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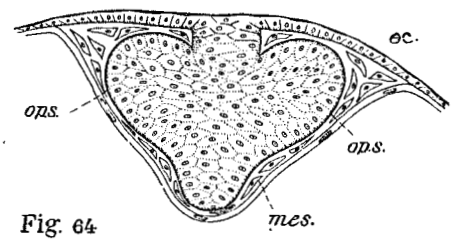

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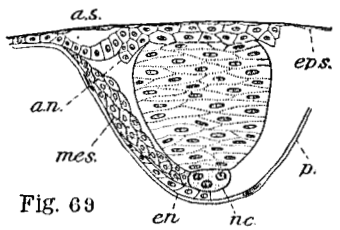

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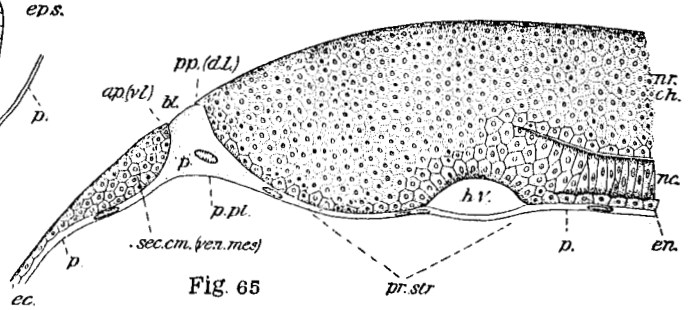

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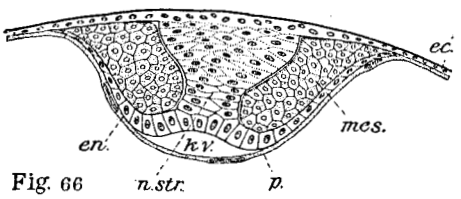

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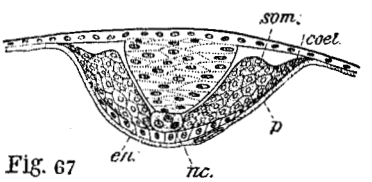

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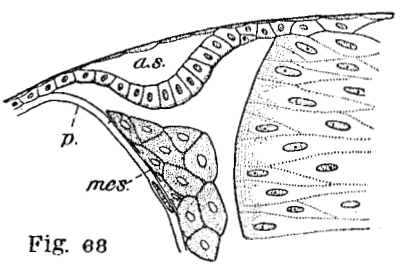

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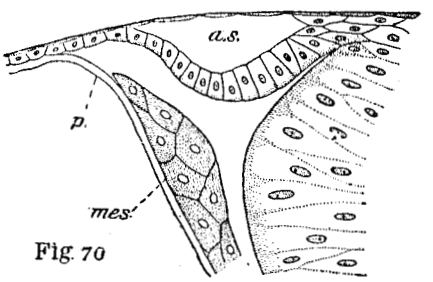

Fig. 70

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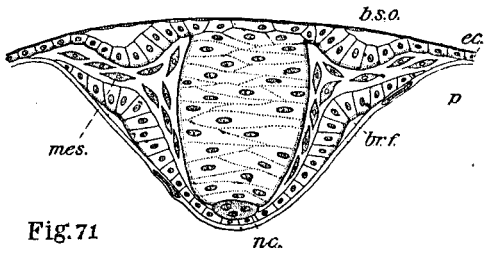

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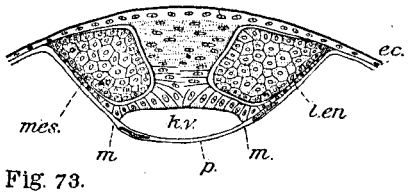

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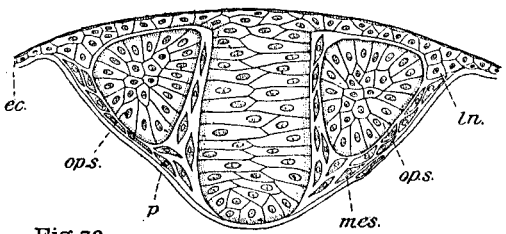

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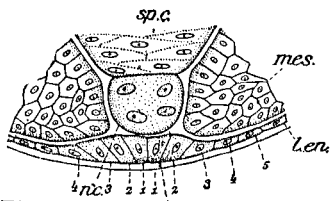

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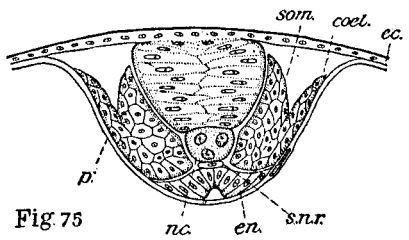

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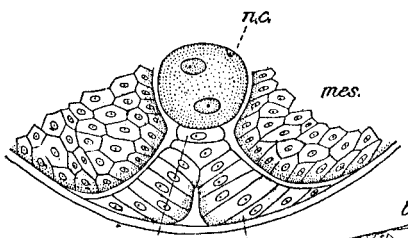

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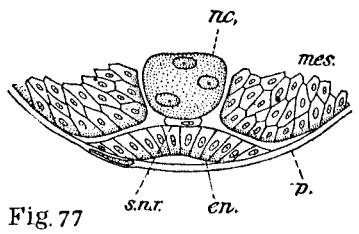

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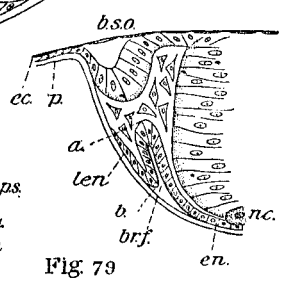

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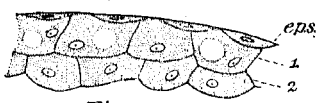

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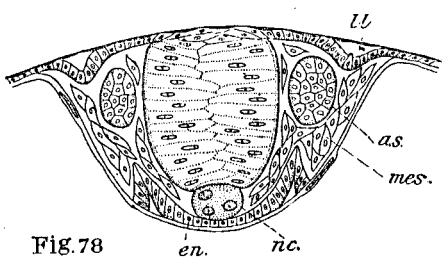

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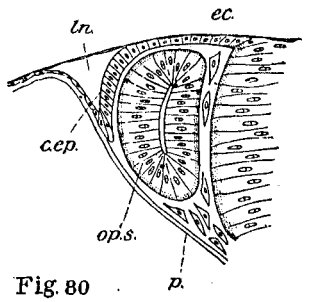
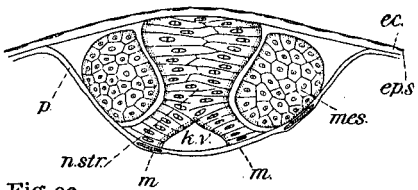


Fig. 80

H.V.W. DEL.



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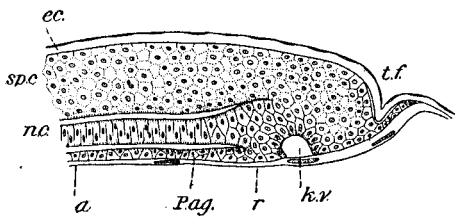

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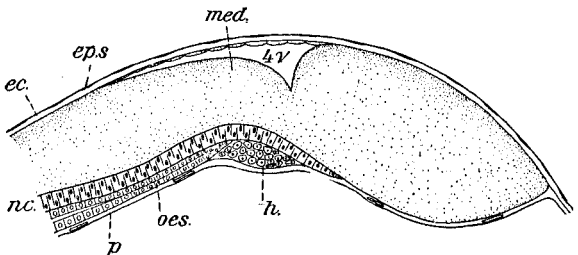

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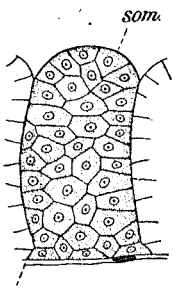

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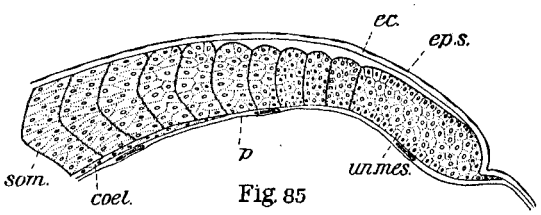

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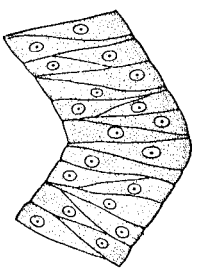

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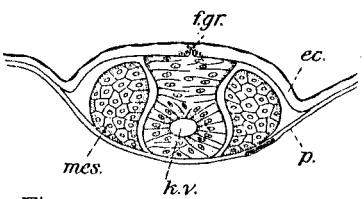

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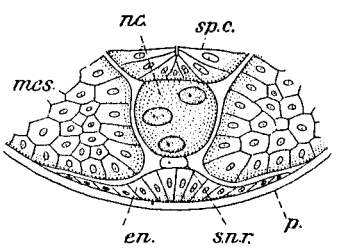

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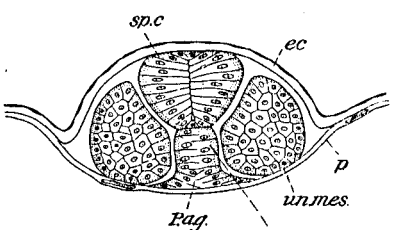


Fig. 90

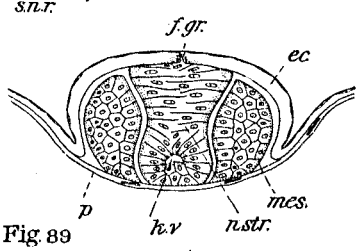


Fig. 89

H.V.W. DEL.

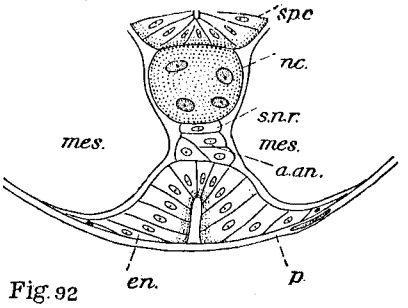


Fig. 92

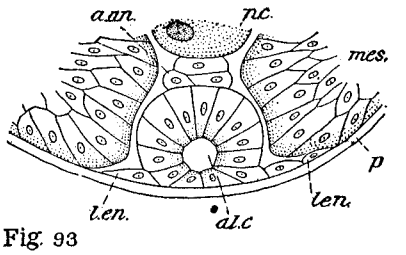
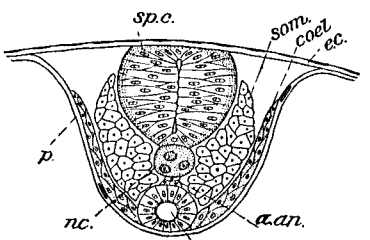


Fig. 93



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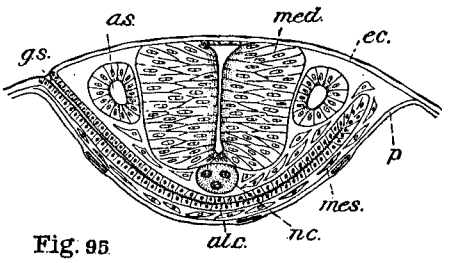

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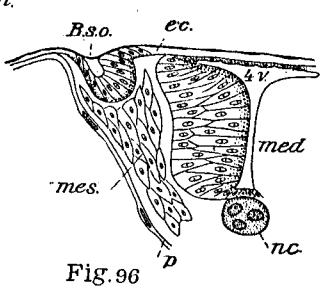


Fig. 96

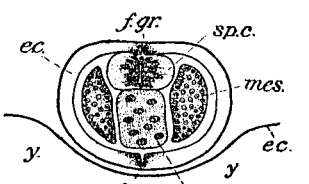


Fig. 99

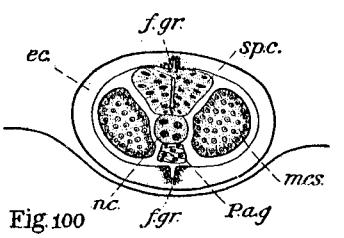
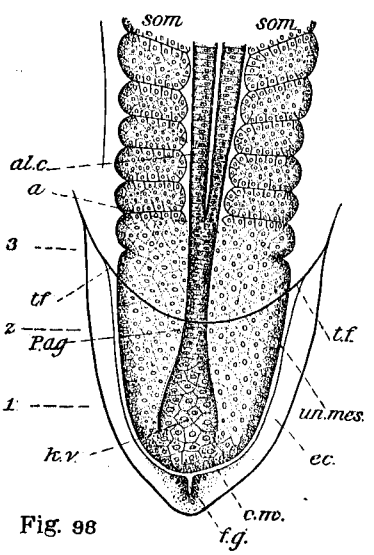


Fig. 100



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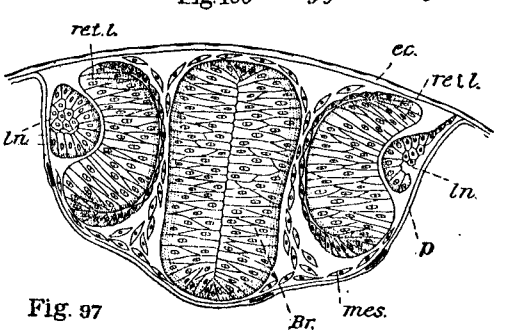

Fig. 97

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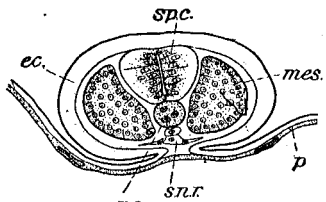
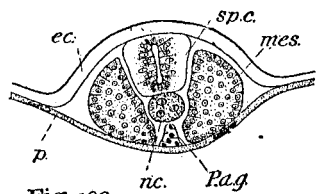


Fig. 101



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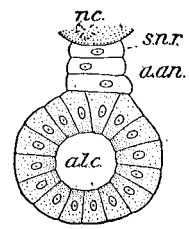


Fig. 104

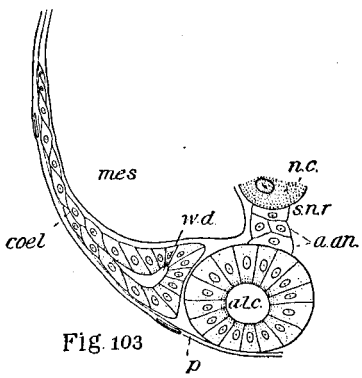
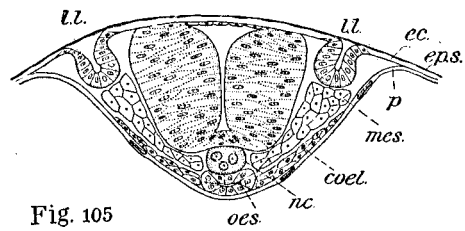


Fig. 103



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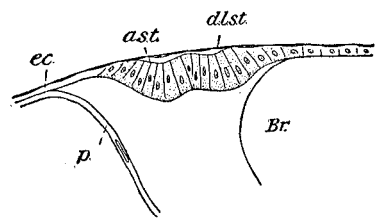


Fig. 107

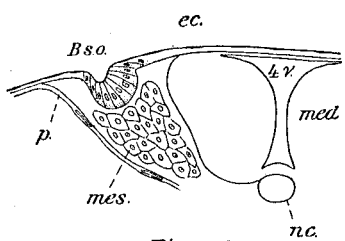


Fig. 106

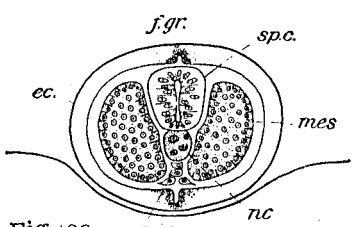


Fig 109

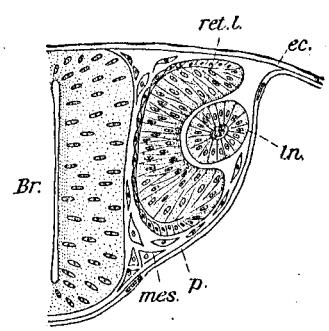


Fig. 108

H.V.W. DEL.

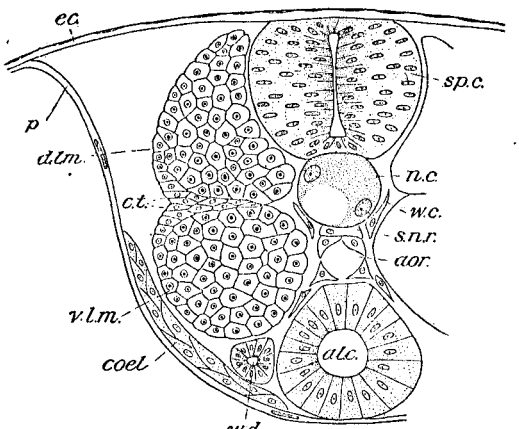


Fig. 110

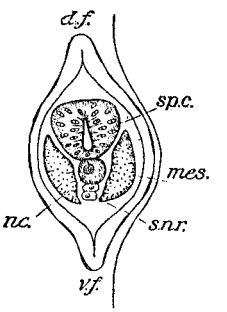


Fig. 111

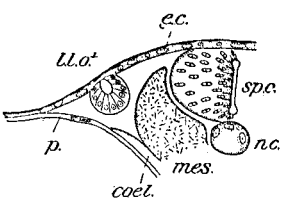
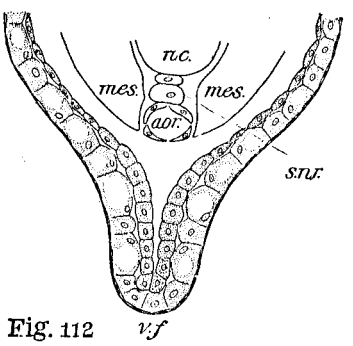


Fig. 113



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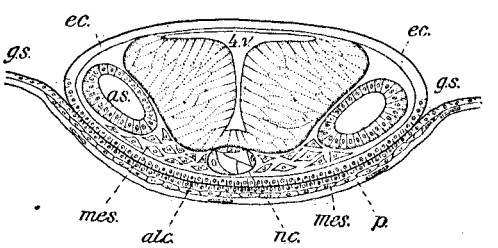

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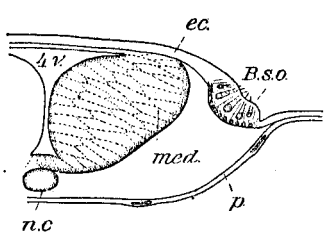

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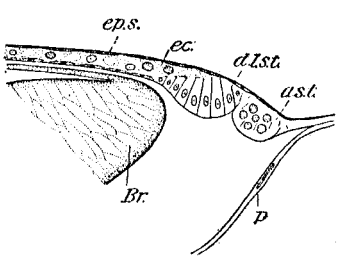
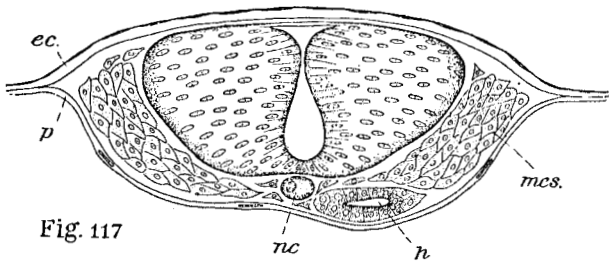


Fig 116

H.V.W. DEL.



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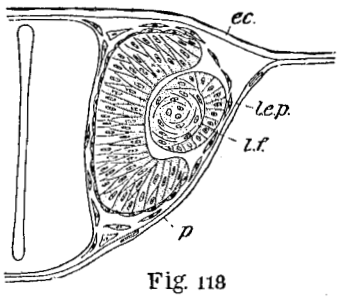

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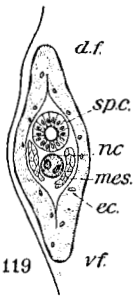

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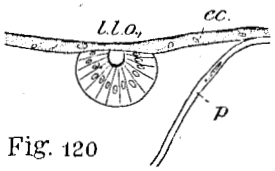


Fig. 120

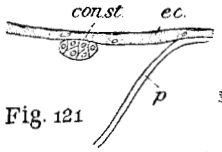
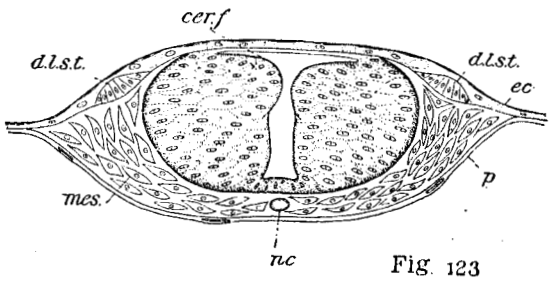


Fig. 121

Fig. 122



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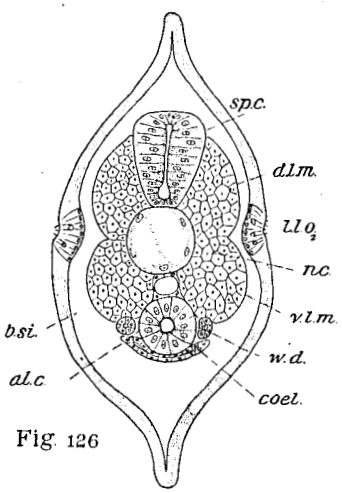

Fig. 126

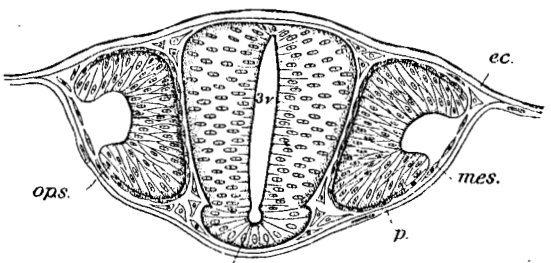

Fig. 124

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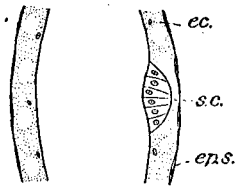

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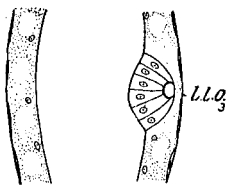

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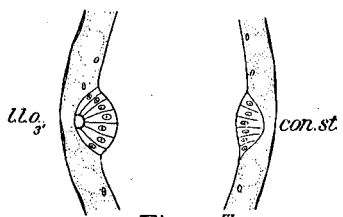

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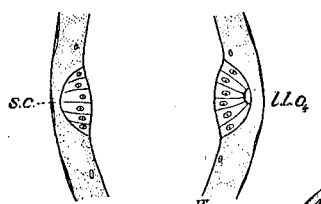

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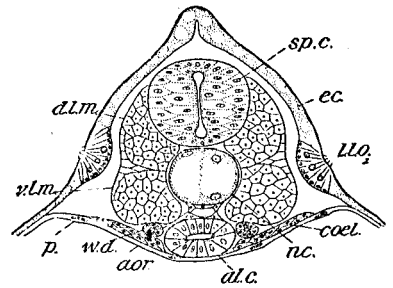

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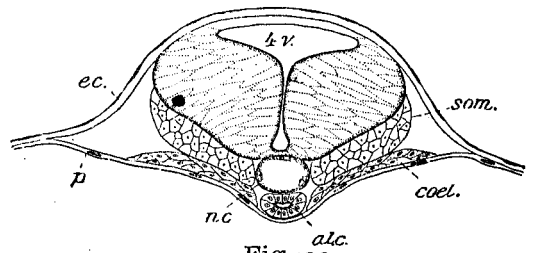
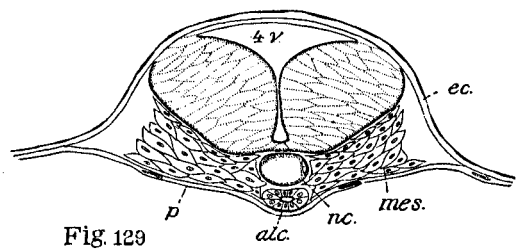


Fig. 128



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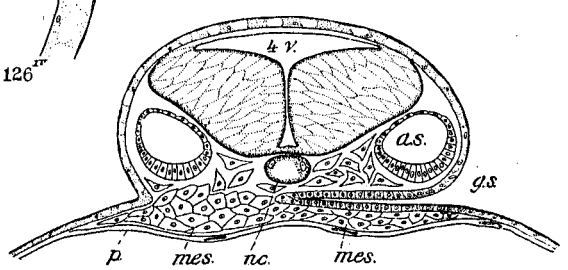

Fig. 130

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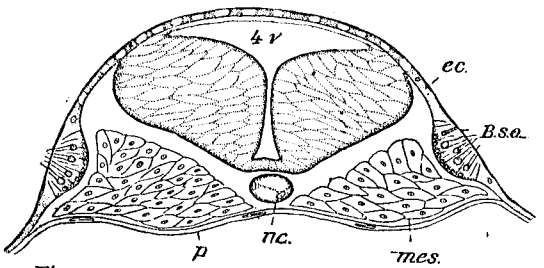
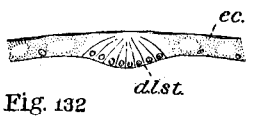


Fig 131



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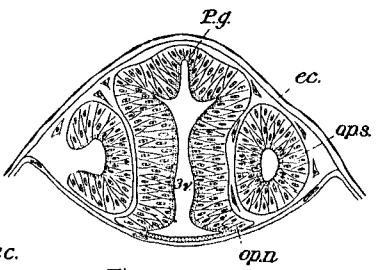


Fig. 135

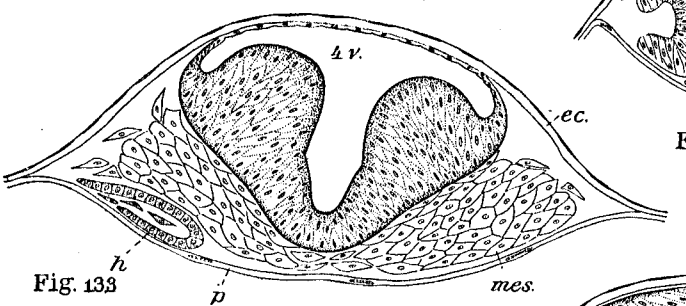
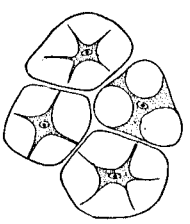


Fig. 133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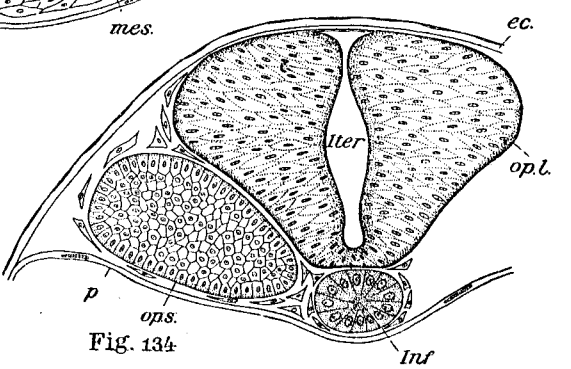

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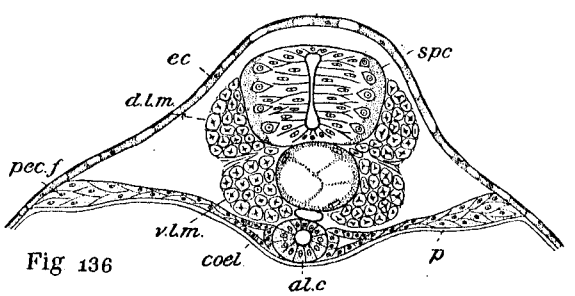
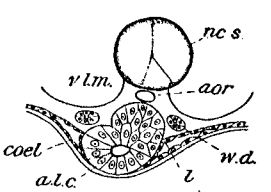


Fig 136



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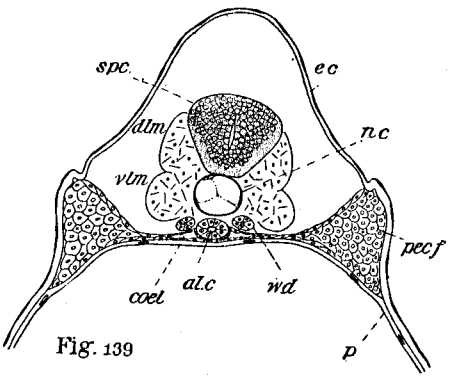


Fig. 139

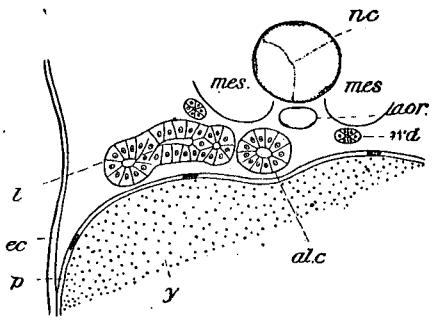


Fig. 140

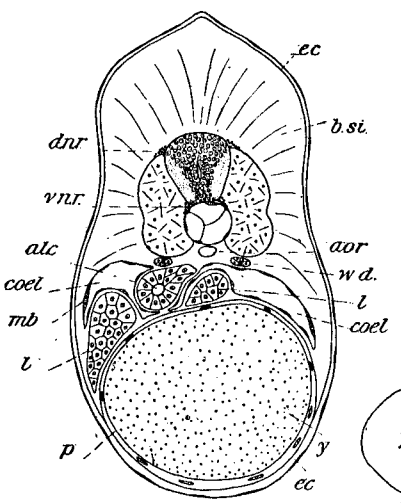
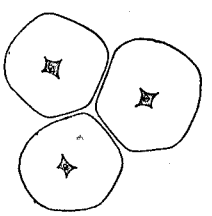


Fig. 141



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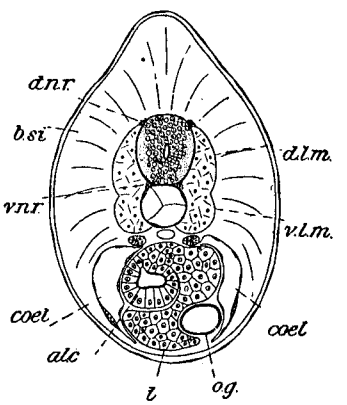

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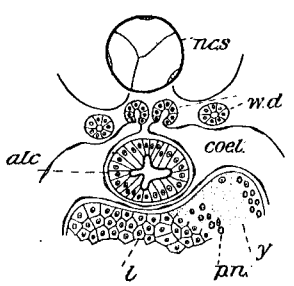

Fig. 144

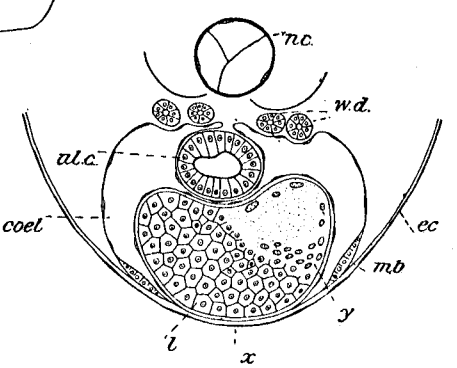

Fig. 145

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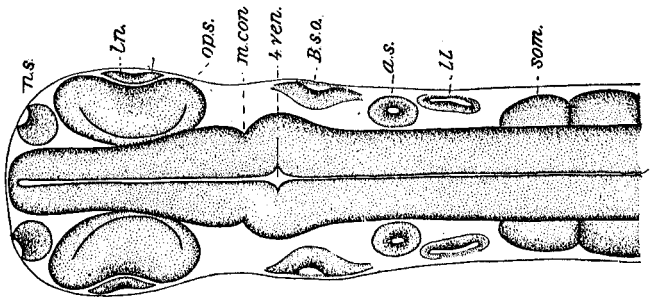

Fig. 148

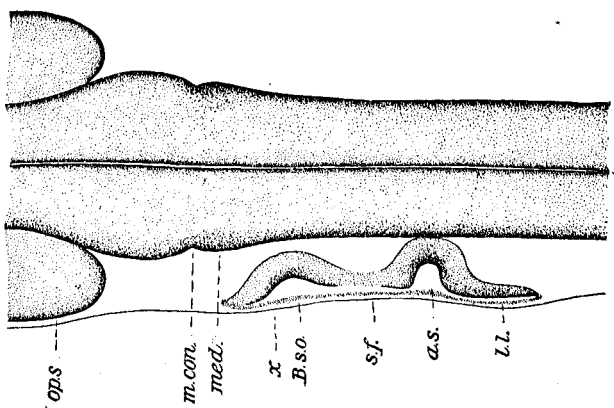

Fig. 147

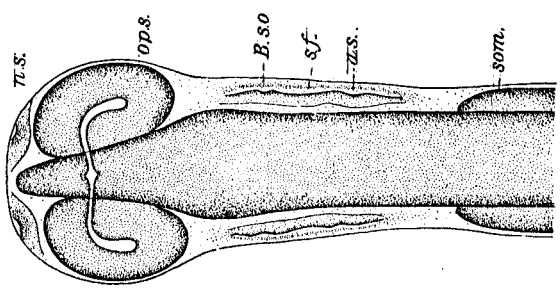

Fig. 146

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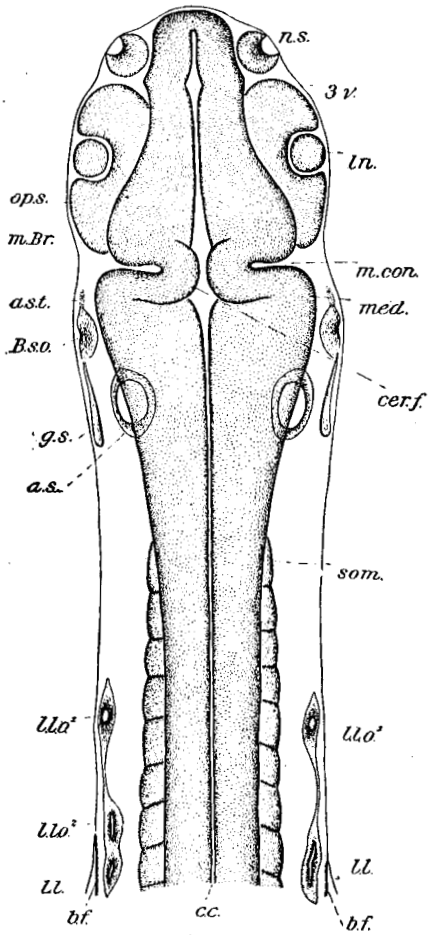

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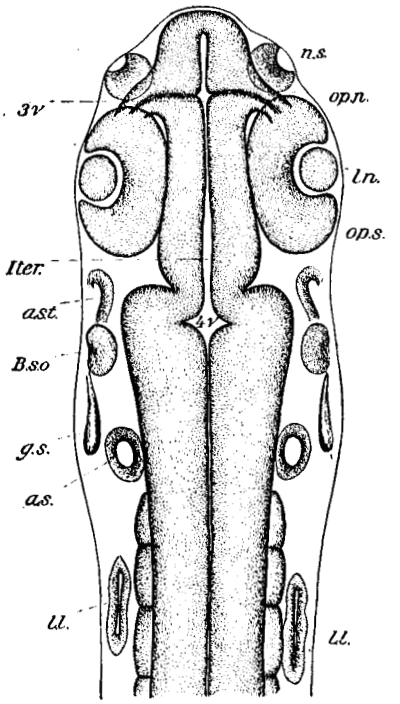

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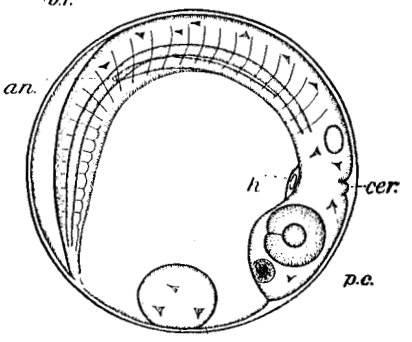

Fig. 151

H.V.W. DEL.