

A Study of Early Fish Development.

Experimental and Morphological.

By

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With 35 figures in text and Plates VIII—XII.

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I. Introductory.

The present paper is, in some respects, a sequel to one published about three years ago ('00). Certain conclusions formed at that time have been subjected by me to experimental test. I have also re-examined my former preparations carefully. To what extent the present studies confirm my earlier ones will appear in what follows. For the present it is sufficient to say that as far as direct observations go, I have few corrections to make. Some of my theoretical conclusions, on the other hand, have undergone considerable modification.

In the first part of this paper I have given in tabular form the results of my experiments upon various fish embryos. These experiments were begun in Naples in the summer of 1900 and were briefly mentioned in the work referred to. They have since been greatly extended, so that in all I have a record of 1361 eggs which were experimented upon. This total does not include a large number, several hundred in all, of unsatisfactory experiments which were not recorded. I have not, however, excluded negative or contradictory results.

Exocoetus, of an unknown species, *Salvelinus fontinalis*, *Batrachus* (*Opsanus*) *tau*, *Fundulus heteroclitus* and *F. majalis* were the species whose eggs were employed. As the tables show, *Fundulus* (chiefly *majalis*) furnished the greater part of the successful results. The egg

of *Exocoetus*, on account of its rapid development, is very favorable for the purpose of operations, but unfortunately very few were available. Those of *Salvelinus* and *Batrachus*, by reason of their large size, are easily manipulated, but their extremely slow development renders them less serviceable. This was found to be particularly true of the egg of *Batrachus*, which, though it often survived for several days after the operation, did not commonly advance enough in that time to give conclusive results. Death then occurred, owing, apparently to infection. Of the two species of *Fundulus*, *F. majalis* has a considerably larger egg than *heteroclitus*, the former averaging about 2.5 mm in diameter. Of the eggs recorded in the columns headed »*Fundulus*«, all but 70 or 80 were of the species *majalis*.

Two methods of operation were employed. 1) Glass needles were inserted into a given point of the egg and left in situ while development proceeded. In order to make these needles, sticks of soft glass were heated in a BUNSEN flame and drawn out into slender rods, having the diameter of fine sewing needles. These rods were then broken up into pieces about two inches in length, each of which had to be pointed. To produce the point, a needle was brought momentarily into contact with a stick of glass held in the flame till it had become viscid. By a sudden pulling apart of the two, the desired result was attained, though by no means always at the first trial¹⁾. When a needle was to be used, the pointed end was broken off about 4 or 5 mm from the extremity and this short piece was inserted into the egg by means of a fine pair of forceps having bent tips. A low-power dissecting-microscope sufficed for the purpose. It was soon found that if the needle were withdrawn after the puncture of the egg, the injured spot would usually heal, in those cases where the egg survived, and leave no traces of its whereabouts. Consequently the needle-point was left in the egg.

2) Electro-cautery was also successfully employed. The cautery points were of the type used by physicians for throat and nose work, etc. They required first to be finely pointed with a file. The current for most of the experiments was furnished by a battery of 8 »Mesco« dry cells, connected in »parallel circuit«. It was necessary to obtain a bright red heat.

In the case of *Exocoetus* and of *Fundulus*, the operated eggs were immediately replaced in sea-water. The eggs of *Salvelinus* and

¹⁾ This method of making needles I owe to Prof. E. B. WILSON.

Batrachus, on the contrary, were kept in moist chambers on damp cotton-flannel. It was found by experience that the eggs of these fishes, after operation, lived much longer out of water than in it.

The eggs were fixed by the sublimate-acetic-formalin method (CHILD's) described in my former paper. In this way the natural appearance was beautifully preserved, the yolk remaining translucent, while the embryonic portion was rendered whiter and more distinct. The fixation of the cells was found to be on the whole sufficiently good. »Control« material was in each case preserved, both at the time of the operation and at the conclusion of the experiment. Valuable sections were obtained in many cases, though unfortunately not in every case where they would have been instructive. Many embryos of interest were damaged, owing to the difficulty of cutting the yolk, or to other causes, so that my record, in this respect, is far from complete.

I wish here to express my great obligation to the officers of the United States Commission of Fish and Fisheries, and to Dr. HUGH M. SMITH in particular, for the assistance which I have received at all stages of this investigation. The present liberal policy of our Fish Commission, in supplying the facilities and material for biological research, deserves the highest praise. I have already recorded my indebtedness to the staff of the Naples Zoological Station for help rendered during the earlier experiments of this series.

II. Key to the Tables (Plates VIII—XII).

In the first column is indicated by diagram the character of each operation. It must be remembered that these diagrams merely represent average cases. There was necessarily a considerable range of deviation, both in the position of the spot that was punctured or cauterized and in the stage of development of the egg at the time. Those eggs were always rejected, however, that departed too widely from the standard. In attempting to represent the eggs of all these species by a single set of diagrams, a serious difficulty arises owing to the fact that with different fishes the rate of development of the embryo does not bear the same relation to the rate of overgrowth of the yolk. For example, the embryo of the toad-fish (*Batrachus*) is much farther advanced than that of *Fundulus* when equal proportions of the yolk-sphere have been covered. Accordingly, in the diagrams showing the operations, I have represented

in each case the condition in *Fundulus majalis*. The embryo and blastoderm in these figures are, however, for present purposes, correct enough representations of any of the species concerned, although the yolk-sphere is proportionately much too small for the eggs of *Salvelinus* or *Batrachus*. Of course the preceding remarks apply only to the figures in the first (left hand) column. In addition to the use of diagrams, I have adopted a system of notation, by which to indicate the stage of the egg and the position of the needle or spot cauterized. This system will facilitate future references to these operations, but no description is necessary, as it is self-explanatory. Let me repeat that many eggs have been assigned to one or the other »stage« which really were intermediate between two of those figured. This is true in less degree of the position of the operated spot.

The other four columns give the results of the various operations for the various species employed. It will be seen that there are no cases in which any one experiment was performed upon all four genera of eggs. The »Time« or »Average time« denotes the interval which elapsed between the operation and the preservation of the egg. »Number [of] eggs« of course denotes the number of any species subjected to a given operation. The numeral opposite each diagram naturally indicates the number of resulting embryos which may be approximately represented by that diagram. Here again, considerable deviation must, in some cases, be allowed for. The »Unclassified« eggs were so recorded for various reasons. The word »uninstructive« might have been substituted in most cases. Some of these eggs had recovered completely from the effects of the operation, or were but slightly affected by it. Others were so badly mutilated that nothing could be made of them. No eggs were included under this heading merely because they did not seem to be in harmony my other results. The »dead« eggs although dead at the time the lot in question were preserved, had, in many cases, survived long after the operation and shown instructive effects.

III. Discussion of Results.

Experiment 1: »Stage A, needle in centre«.

The results, in the case of six eggs of *Fundulus*, obviously support the view that the centre of the germ-disc corresponds in position to the future anterior end of the head. There is nothing

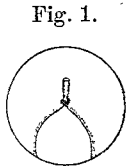
here to prove, however, that the head end may not, at the outset, have been formed at some lower latitude on the egg, and have subsequently grown forward. This point will be discussed later. It is beyond doubt that the elongation of the embryo has been for the most part in a backward direction. It also seems plain that up to the end of the experiment, the spread of the blastoderm has been concentric.

In two cases, listed as »unclassified«, decidedly »skew« embryos resulted, similar to some which will be spoken of later (Exp. 34).

Experiment 2: »Stage B, Op. a — needle«.

This experiment proves clearly, as do many recorded later, that the blastoderm as a whole may, under exceptional circumstances, be shifted freely over the surface of the yolk. Normally the expansive movement of the latter is equal in all directions, the centre remaining a fixed point. An obstacle such as this needle, however, prevents the blastoderm's advance in one direction, though, as will be seen at a glance, it does not hinder its symmetrical growth. The centre must consequently shift its position relatively to the yolk. As a result of this, the head end of the embryo develops at a point far removed from the original »animal pole« of the egg, or more strictly, of the yolk-sphere. It is to be recorded that in nearly every egg of this lot the spread of the blastoderm has been slower than normal.

In one egg (Fig. 1) the blastoderm, on each side of the needle, has continued to advance somewhat, producing a sharp indentation in the margin at this point. Such an indentation in quite similar to those that have been described in the hinder margin of various early fish embryos, and attributed to »concrecence«. In the present case, the effect is obviously due to a retardation



of growth at one point.

Experiment 3: »Stage B, Op. b — needle«.

Apparently the same final result is obtained as in Exp. 1. The needle (at the time the eggs were preserved) was found to lie at the anterior end of the embryo. These two experiments (1 and 3 — compare also with 34) taken together, would seem to prove that the material destined to form the cephalic end of the embryo is at first located at some distance from the »animal pole«, but that it normally attains the latter position through forward growth. In the present experiment the needle has served to prevent this forward direction of the

growth, relative to the yolk, but it has not hindered the growth itself. This interpretation, as will appear later, seems to be in harmony with some observations which I have made upon living eggs.

Experiment 4: »Stage B, Op. b — cantery«.

In two eggs of *Batrachus*, the cauterized spot, at the close of 69 hours of development, appeared at some distance in advance of the head end of the embryo. This result at first glance seems to be contradictory to that obtained in Exp. 2. But it is important to consider that the visible scar, in these eggs, is largely a spot on the yolk, while the injury to the blastoderm, in some cases, appears to have healed completely. Accordingly, I am inclined to think that the spot figured in the diagram does not represent the position of the cauterized point of the blastoderm, but that the blastoderm as a whole has shifted upon the yolk. Further evidence of such a shifting will be offered below. A needle would of course have prevented any such movement.

Experiment 5: »Stage B, Op. c — needle«.

Here the needle was passed through the centre of the embryonic widening of the germ-ring. The spread of the blastoderm, though greatly retarded, has not been arrested, but the formation of an embryo has been prevented, the latter being represented by a seemingly formless thickening¹⁾.

A comparison of Experiments 3, 5 and 6 (below) leads to the conclusion that in 5 we have injured a critical point in the blastoderm, a sort of »noeud vital«, whose integrity is in some way essential to the formation and growth of the embryonic body. Operations upon later stages fully confirm this view; the present experiment is interesting in showing at what an early period this »noeud« appears.

Experiment 6: »Stage B, Op. d — needle«.

In the *Fundulus* column, it is apparent that normal embryos have resulted in spite of the firmly planted needle at the posterior end. Since we know, on other grounds, that the embryo normally elongates for the most part in a backward direction, it is evident

¹⁾ Miss PEEBLES ('98) similarly found that the formation of an embryo in the hen's egg was prevented by inflicting an injury directly in front of the primitive streak. She concluded that here was a »region of greatest growth«.

that the direction of growth, relatively to the yolk, has in these eggs been reversed. We have another instance of that shifting of the blastoderm as a whole which was discussed under Exp. 2. It may be objected that the needle may have been pushed backward through the yolk, while the embryo elongated in the usual manner. This I consider quite unlikely: firstly, because the resistance offered to the needle by the contents of the yolk-sphere would probably be much greater than that offered to the blastoderm by its surface; and, secondly, because, with a single recorded exception, the needle, in all of these experiments, has remained perpendicular to the surface of the egg; whereas a process of pushing, exerted at the point of entrance, would have bent it back and given it an oblique position.

To say that the direction of growth, relatively to the yolk, has been reversed, in no way implies, however, that growth has occurred in a different region of the embryo from that of a normal egg. I believe this region to be the same in the two cases.

This one experiment offers, it seems to me, almost conclusive evidence against the view that the fish embryo grows by such a process of concrescence as was postulated by His and others. If this latter process occurred, the two halves of the germ-ring ought to continue to coalesce behind the needle, leaving the latter at some point near the anterior end of the fully formed embryo.

The condition of the single egg of *Batrachus* which is figured leads to the belief that the needle was, by mistake, inserted somewhat in advance of the posterior margin of the blastoderm. Only the anterior part of the embryo has developed. This terminates abruptly at the needle. A double line, leading from the needle backward to the indented blastoderm margin, strongly suggests a fusion of the two lateral halves of the latter, and I think it likely that this has actually occurred. It is nevertheless obvious that the hinder half of the embryonic body has failed to appear. The case is quite similar to that of Exp. 38, which will be fully discussed in its proper place. The normal behavior of the toad-fish blastoderm will also prove of interest.

Experiment 7: »Stage B, Op. d — cautery«.

Here the position of the scar is not such as we might have expected. It lies completely in front of the embryo. To account for this, I only need repeat the explanation given for Exp. 4, namely, that the blastoderm as a whole has shifted backward, carrying the

embryo with it, and that the visible scar represents a cauterized spot on the yolk which cannot be regarded as indicating the position of the original injury to the blastoderm. This shifting would here be in the same direction as was assumed in the case of Exp. 4. I have not been able to detect the occurrence of such a shifting in the normal development of *Batrachus*, but it is in no way improbable.

Experiment 8: »Stage B, Op. 1e — needle: 15° — 25° to left«.

It was my object in this case to sever the germ-ring at the point impaled. But regeneration took place, and the blastoderm continued to spread normally, leaving the needle far in the interior. Situated close beside the embryo, but free from it, the needle has served as an excellent point of reference by which to determine the direction of the embryo's growth. This, it is seen, has been mainly backward, but possibly slightly forward as well.

Experiment 9: »Stage B, Op. 1e — cautery«.

Here we have much the same result as in the preceding experiment, but with a characteristic difference. The scar is relatively further forward than was the needle point, i. e. the embryo has shifted backward as in Exp. 4 and 7.

Experiment 10: »Stage B, Op. 1f — needle: 45° to left, ca.«

In each of the cases figured, the needle lies not far from the level of the caudal end of the embryo. The embryo, accordingly must have elongated mainly in a forward direction, relatively to the needle and the yolk. This has doubtless been due to the same cause as in Exp. 6 (*Fundulus*). The needle, in large measure, prevented the backward movement. Other points illustrated by these embryos are far better shown in cases described later.

Experiment 11: »Stage B, Op. 1f. — cautery«.

In the case of the *Batrachus* eggs, the yolk blastopore has nearly or quite closed. The germ-ring completely regenerated itself after cauterization, and the only scar lies in the yolk. Why, in all these three embryos, does the head end slant toward the injured spot? It is possible that it was drawn in that direction by an outflow of yolk through the puncture, but there is no trace of such an outflow.

Of the *Fundulus* eggs, those represented by the first diagram show a marked curvature of the hinder end of the embryo toward the injured spot. This may have been partly due to a shortening

of the germ-ring, owing to the destruction of a portion of its substance. But it has probably been also due to another cause (see below Exp. 12 and others). The lower of the *Fundulus* diagrams shows a case in which the germ-ring has been held back at one point of its margin, a deep indentation being the result. Nevertheless, the embryo, although obliquely placed, is seemingly normal. (See below: Exp. 19 — *Exocoetus*.)

Experiment 12: »Stage B, Op. 1g — needle: 90° to left«.

We have here what seems to be a satisfactory demonstration that the material of the germ-ring concentrates toward the median line of the embryo. The needle, originally placed 90° from the mid-caudal point, is later found to lie not far from the edge of the embryonic shield. It is very unlikely that the needle has been drawn toward the embryo. Consequently, we must suppose that the embryo has moved toward the needle. This movement has been accompanied by — perhaps it has been caused by — the passage of the intervening segment of the germ-ring into the embryo. To what extent the elongation of the embryo is due to the addition of material derived from the germ-ring will be discussed later. I will remark here, however, that I regard the chief source of this growth to lie within the embryo itself, rather than in the germ-ring.

It will be observed that this axiad movement of the lateral portions of the germ-ring have here occurred before the equator of the egg has been passed, i. e., while the germ-ring has been increasing in girth. In other words, although the margin of the blastoderm has appeared to be advancing uniformly, in concentric circles, its constituent cells have undergone a movement far less simple. The movement of any one part has been the resultant of at least two separate forces. (See p. 133 below.)

Experiment 13: »Stage B, Op. 1g — cautery«.

Here the scar, at the end of the experiment, is a quadrant removed from the embryo. There has apparently been no diminution of the original angular distance. If however, the scar be confined to the yolk, this is nothing more than we should expect. (See discussion of *Batrachus* embryos above.)

Experiment 14: »Stage B, Op. 1f—rf — Needles«.

Here, although the physiological continuity between the incipient embryo and the remainder of the germ-ring has been nearly or quite

destroyed, a nearly normal »embryonic shield« has resulted. Of especial interest is the strongly convex hinder margin of the latter. Here a thin layer of cells, perhaps only the pavement layer of the epiblast, has grown far beyond the limit of the other cell-layers.

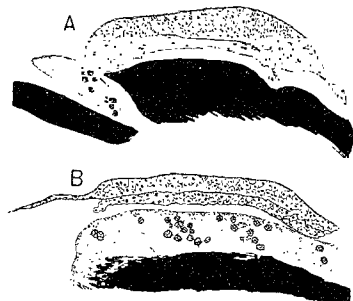
Experiment 15: »Stage B, needles within germ-ring«.

To what is the advance of the blastoderm margin due? Is it caused mainly by the multiplication of its own cells, or by the expansion of the interior region of the blastoderm? That it is due, in considerable degree, to the latter cause is shown by the present experiment. The deep bay produced in the germ-ring beyond each needle is proof that the movement of the latter has been retarded at these points, and this in spite of the fact that the germ-ring itself has not been injured by the needles. Such an indentation, occurring normally in the embryonic area, is met with in the development of some fishes and has been offered as evidence of »convergence«. (See Exp. 2.)

Experiment 16: »Stage B — large area destroyed by cautery«.

This, perhaps, is the most »sensational« of the present experiments. The area destroyed was an extensive region on the embryonic side side of the blastoderm. A semicircular gap was thus formed in the margin. In the course of a few hours, perhaps less, the borders of this gap drew together, and the circular form of the now smaller blastoderm was restored, a slight scar remaining to indicate the position of the injury. After a period of time ranging from 18 to 52 hrs. (average 33 hrs.) a new »embryonic shield« of nearly or quite normal appearance resulted. The blastoderm was generally considerably smaller than a normal one of this degree of development. The embryonic shield, in cases where this could be verified, appeared on the same side of the blastoderm as the portion destroyed. In several embryos which have been sectioned only two layers are distinguishable, the upper of which (epiblast) is thickened to form a well developed neural plate, though this is not concentrated into a

Fig. 2 A and B.



Longitudinal and transverse sections of embryos of Experiment 16.

»keel«. There is no trace of a chorda, and the lower layer is scarcely thickened in the middle line of the embryo (Fig. 2, *A* and *B*). In two other specimens, however, a distinct axial rod (neural axis or latter + chorda) is visible in surface view (Fig. 3) but unfortunately sections were not obtained.

Fig. 3.



An Embryo
of Experiment 16.

This experiment was repeated many times, as at first I was naturally skeptical regarding such striking results. The only possible source of error lies in the difficulty of distinguishing just where the embryonic region of the early germ-ring is situated. At the stage of the present operations, this is not very sharply indicated in the living egg, and usually lies directly over the dense cluster of oil-drops, which render it yet more obscure to view. This difficulty is further increased when the egg is removed from the water, as must be done when it is to be cauterized. I have thought it but fair to state these difficulties to the reader, though I have, myself, very little doubt as to the significance of the experiments. The greatest care was taken to locate the embryonic region, before the removal of an egg from the water, and those eggs were rejected in which this was indistinguishable. The presence of the oil-drops was in one respect an advantage, as they occupied a fairly constant position relative to the embryo. As large a region as possible was destroyed by cauterization in order that the embryo should be included in the area cauterized. Turning to the tables, we see that of 18 eggs which lived up to the time of preservation, 10 had regenerated the embryonic shield. This regeneration was not always as complete as represented in the diagram, but no mere formless thickening of the blastoderm was included under this head. The remaining 8 were uninformative, but not contradictory. For the most part, they were badly mutilated.

It is worthy of especial interest that extirpation of the entire embryonic region should be followed by complete regeneration, while injury to particular a spot within it results in complete arrest of development (Exp. 5).

Experiment 17: »Stage C, Op. c — needle«.

Here the needle was inserted $\frac{1}{3}$ to $\frac{1}{2}$ way in front of the caudal extremity of the »embryonic shield«. Both of the surviving eggs have developed normally, the body elongating behind the needle, but not appreciably in front of it. In the second egg (not figured) the needle

was found relatively further back at the close of the experiment than in the first case.

Experiment 18: »Stage C, Op. d — needle«.

In this experiment, the needle was inserted at the middle point of the hinder margin of the embryonic shield. As in the case of Exp. 6, where the same thing was done at an earlier stage, perfectly normal embryos have resulted in the great majority of *Fundulus* eggs, which have survived the operation. Of course the direction of elongation, relatively to the needle and the yolk-sphere, has been forward (cephalad) as in the previous case.

The *Exocoetus* embryos offer some interesting peculiarities. There are evidences that the resistance to the (relatively) cephalad elongation of the embryo has been sufficient to cause a considerable backward pressure upon the needle. In the egg represented by the lower of the two diagrams, the needle has apparently been bent backward by the growing caudal end. In this case the hinder end of the embryo projects strongly beyond the margin of the blastoderm. Here, again, we have evidence against the view that the embryonic body arises by a coalescence of the two sides of the blastoderm margin, and in favor of the view that its elongation results, in great part, at least, from the activity of growth centres contained in the embryo itself. In the other cases (upper figure) the material resulting from growth in this region, being unable to expand in the normal backward direction has accumulated to form a two-lobed mass, wider and thicker than the normal caudal end at this stage.

Experiment 19: »Stage C, Op. 1e — needle: 15°—25° to left«.

In three of these eggs (upper diagram), the blastoderm has advanced, unimpeded by the needle, and normal development has occurred. The needle has remained as a fixed point by which to measure the growth of the embryo. In another case (lower diagram) the presence of the needle has had the same effect as when inserted at the middle point of the embryonic margin. The elongation has been entirely forward. In yet another egg (middle diagram) a very interesting result has come about. The germ-ring, on the left side, has been severed from physiological continuity with the embryo. The latter has, nevertheless continued to elongate. Behind the needle extends a region of uncovered yolk, continuous with the »yolk-blastopore« and bounding the embryo on its left side. Sections show that

this embryo has developed nearly normally behind the needle. From the level of the latter to a point about a third way to the caudal end, the sections are seen to be nearly, though by no means completely,

Fig. 4.



Transverse section of an *Exocoetus* embryo of Experiment 19 (middle diagram) passing through region formed subsequently to injury.

symmetrical, the left side, especially the mesoblastic mass (somite?) being defective (Fig. 4). Beyond the 10th section behind the needle, the sections are unfortunately so damaged that I am not prepared to say how far back development on the injured side has extended. The condition of this embryo is similar to those in Exp. 30 which will be more fully discussed in their proper place.

Experiment 20: »Stage C, Op. 1e — cautery«.

In the single *Batrachus* egg surviving this operation, no injury has been done to the embryo, and the germ-ring has completely regenerated. The scar is where we should expect to find it if we remember that the formation of the definite hinder end of the embryo occurs, in this fish, at a relatively early period. After this happens, the blastoderm continues to spread backward, and the yolk-blastopore closes far behind the caudal end of the embryo, much as in the elasmobranch egg.

Experiment 21: »Stage C, Op. 1f — needle: 45° to left«.

Here the axial concentration of the broad embryonic shield to form the much narrower definitive embryo has resulted in the caudal end of the latter being drawn up close beside the needle. In seven of the cases the embryo has been bent, in two others it has swung around as a whole, remaining straight. This is a phenomenon of the same sort as that already described for Exp. 12.

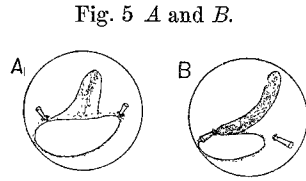
Experiment 22: »Stage C, Op. 1g — needle: 90° to left«.

The result here has also been an approximation of the caudal end of the embryo to the needle. The greater part of this may be accounted for by the narrowing of the embryonic shield as in the preceding experiment. But it is probably in a measure also due to the incorporation of a portion of the intervening germ-ring into the embryo. That such a process does actually occur has been already demonstrated (Exp. 12).

Experiment 23: »Stage C, Op. 1f—rf — needles«.

The attempt was here made to destroy the physiological continuity between germ-ring and embryo on both sides. In the first of the cases figured, this severance was evidently maintained throughout the entire period of the experiment. The embryo has none the less elongated greatly and developed in a seemingly normal manner. The yolk blastopore has narrowed down to a transverse slit, with the needles at its extremities. The caudal end of the embryo projects strongly beyond the adjacent margins of the blastoderm. Here again is evidence that the power of elongation is resident in the embryo itself. How much of this is due to cell multiplication and how much to the rearrangement of cells already contained in the embryonic shield cannot of course be determined by the present experiment.

In the case of the second embryo figured, it is impossible to say just what has occurred. It seems likely, however, that the intermediate (unobserved) stages were somewhat as I have represented in Fig. 5, *A* and *B*.

Fig. 5 *A* and *B*.

The third diagram presents little difficulty. The embryo and germ-ring have early broken away from the right-hand needle and the caudal end has been drawn toward the left in a manner quite similar to that observed in Exps. 12 and 21.

In the fourth diagram we have a case where the embryo has broken away from both needles. The inclination of the body, however, shows that the presence of the needles has exerted some influence.

Experiment 24: »Stage D, Op. c — needle slightly in front of caudal knob«.

Here the centre of growth or »noeud vital«⁴⁾, referred to under Exp. 5, has been transfixed. The result, in the first two eggs (upper diagram), has been to completely check the further elongation of the embryonic body. In the last case (lower diagram) the embryo has apparently continued to elongate, though its growth has been impaired. The condition of the yolk-blastopore, in all of these cases, is interesting. I shall, however, defer a discussion of this class of embryos until Exp. 38 is considered.

⁴⁾ I shall later state reasons for regarding this as the »primitive streak«.

Experiment 25: »Stage D, needle $\frac{1}{3}$ way in front of caudal knob«.

In all of the surviving eggs, the embryo has continued to elongate behind the needle as in Exp. 17, the needle, at the close of the experiment, being situated not far from the middle of the embryo. It must be remarked, however, that in some of these the region caudad to the needle seems to be less strongly developed than in the normal.

Experiment 26: »Stage D, Op. d — needle«.

The result has been a normal elongation of the embryo, but in a direction opposite to the usual one, as in Exps. 6 and 18.

Experiment 27: »Stage D, Op. d — cautery«.

The caudal end, including the growing region of the embryo, has been destroyed, and further elongation has been completely arrested. Between the embryo and the

Fig. 6.



Transverse section, posterior to injury, in trout embryo of Experiment 27.

closing yolk-blastopore is a region, which, throughout part of its extent, appears in section as shown in Fig. 6.

I conclude from this that the blastoderm margin, on each side of the embryo, has continued to spread backward, but that the two halves have remained widely separated. The pavement layer of the epiblast has extended over the intervening strip of exposed yolk, as it frequently does prior to the full closure of the yolk-blastopore in a normal egg. (Compare in this respect with the abnormal embryos of RAUBER, '80, and KOPSCH, '99.)

Experiment 28: »Stage D, Op. 1e — needles: 15°—25° to left«.

In the first case, the needle has checked the growth of the embryo, though a great increase in the mass of cells forming the caudal end has apparently occurred. (See Exp. 18.)

The second diagram represents a condition almost identical with that shown in Exp. 26.

Experiment 29: »Stage D, Op. 1f — needle: 45° ca. to left«.

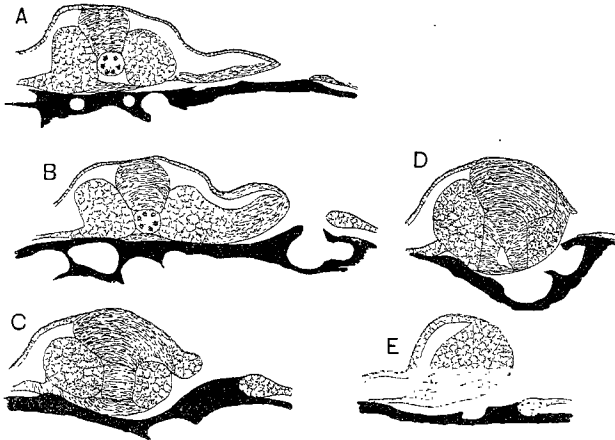
It is evident that the distance between the needle and the caudal point of the embryo has greatly diminished, and in the lower diagram the embryo is seen to have been considerably bent. The question arises: is the distance between the needle and the caudal end of the embryo diminished merely in proportion to the shortening

of the whole germ-ring during blastopore closure, or is it diminished much in excess of this general shortening? The latter seems to me to be the more probable alternative. The present experiment lends support to this view, though much stronger evidence was afforded by Exp. 12 (above) in which the embryo approached the needle while the germ-ring was actually elongating.

Experiment 30: »Stage D, Op. rf — cautery: 45° or less to right«.

One fact of greatest importance becomes evident from a study of these embryos, namely, that far behind the level of the injury there is a quite symmetrical development of the two sides of the

Fig. 7 *A, B, C, D, E.*

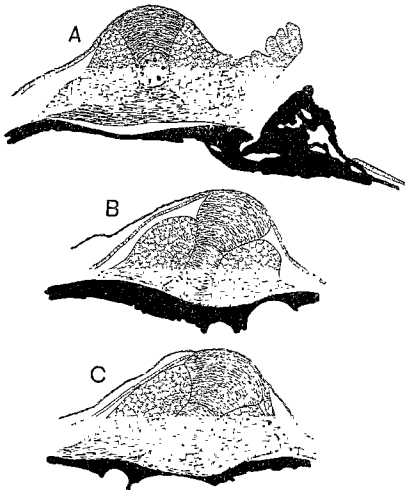


Respectively the 37th, 31st, 21st, 15th and 10th sections (counted from caudal end) of the upper embryo figured in Experiment 30.

body (Fig. 7 *A*, Fig. 8 *A* and *B*). In the first of these embryos (Fig. 7) this completely normal development of the right half ceases at some distance in front of the caudal end. In sections *C*, *D* and *E*, it will be seen that the mesoblastic somites (somites, that is, if the mesoblast is segmented as far back as this) of the right (injured) side are noticeably smaller than those of the left. (See MORGAN, '95, and KOPSCH, '96; also Exp. 19, above.) In the other two embryos, however, no such defective mesoblast on the side of the injury is apparent, unless possibly this be true of the extreme posterior end of the third (Fig. 8 *C*). This appears in only four sections and may be due to the oblique plane in which they are cut.

No addition of material from the germ-ring on the injured side has been possible in any of these embryos. In the first one figured, the segment of the germ-ring lying between the injured point and the caudal end seems actually to have elongated. Previous experiments would have led us to expect that it would have decreased in length. We should have anticipated that the caudal end would have approached the point of injury, provided that the latter were not free to move, as seems to be true in the present experiment. Such an approximation of these two points, where it occurs, is of course due to a force acting at about right angles to the direction

Fig. 8 A, B, C.



Respectively the 19th, 7th, and 5th sections (counted from caudal end) of lower embryo figured in Experiment 30.

of growth. We have to deal with a parallelogram of forces. The result, in some previous experiments, was a curvature of the embryo toward the needle or injured spot. If the force of backward growth were greatly in excess of the traction toward the point of injury, the curvature would be slight. This may explain the condition of the uppermost of the embryos figured. In the second is shown a greater curvature and closer approximation to the point of injury. Of course the traction toward the injured spot might very well have been due to a mere impediment of that side of the embryo, the

latter being »tied up« as it were. In other words, it might be due to the lack of extensibility of the connecting segment of the germ-ring, rather than to an actual tendency of the latter to shorten. That the curvature is due to the lesser growth of the concave side of the embryo, I do not believe, since this defective development is confined to the posterior part of the body.

In the third embryo of the series, the caudal end is entirely free from the germ ring, though when this separation took place it is impossible to say. The injured spot has plainly shifted toward the embryo, or vice-versa. The condition of this egg is hard to explain. But one fact seems plain, namely, that symmetrical development has

taken place during a considerable period, quite independently of at least one side of the germ-ring.

These experiments corroborate the results of MORGAN and KOPSCH, who similarly succeeded in severing the germ-ring on one side. The embryo may elongate, quite independently of the germ-ring, through growth occurring within its own body. This is quite another thing from denying that normally substantial increments are received from the germ-ring. That such is the case both of the above-named authors admit, and I think that I have proved it in the case of *Exocoetus* and of *Fundulus*. The relative share of the two factors in growth will be discussed in connection with Exp. 38.

MORGAN has already met the objection that the continued development of the embryo on the side of the injury may be due to regeneration occurring on that side. As MORGAN points out, the regenerated half would surely lag behind the normal half in degree of development.

Experiment 31: »Stage D, Op. lf—rf — needles:
each 35° to 45°«.

It is doubtful whether in either of the cases figured there has been any appreciable elongation of the embryo. Such a result is quite in harmony with views which I have expressed above. I do not, however, lay much stress upon this case, since many of the control embryos of this lot were abnormal and undersized. Then, too, development has advanced but slightly between the time of operation and of preservation.

The bending of the hinder end of the lower embryo figured, toward the left-hand needle, agrees closely with the condition which has already been discussed in connection with several other experiments.

We now come to a table comprising certain experiments carried on during the summer of 1902, after the results of the preceding summer's work had been provisionally compiled. As far as possible, the results of these later experiments were incorporated into the other tables, but this was impossible with those under consideration.

Experiment 32: »Stage A, centre destroyed by cautery«.

Here a large gap was burned in the centre of the blastodisc at a time when this was still of considerable thickness. An undersized, though otherwise normal, blastoderm and embryo resulted in one case,

after nearly two days of development. It cannot be doubted that much of the material destroyed by cauterization would normally have taken part in the formation of the embryo.

Experiment 33: »Stage between A and B — center destroyed by cautery«.

This experiment differs from the preceding only in respect to the stage at which the operation was performed. The results are identical in the two cases.

Experiment 34: »Stage between A and B — needle in centre«.

Here we have practically the same experiment as Exp. 1, differing only in the stage at which the needle was inserted. The four embryos represented by the upper diagram are strictly comparable with those resulting from the earlier experiment. The lower diagram represents a condition which I had previously met with (Exp. 1) but relegated to the »unclassified« group. It apparently has resulted from a failure on my part to find the centre of the blastoderm with the needle. The interesting feature of these eggs is the bending of the cephalic end of the embryo toward the needle. This has been doubtless due to the same cause as that already assigned (Exp. 21 and some others) to account for the relative positions of needle and embryo, though of course the needle is differently situated in this case.

Experiments 35 and 36 (next two columns).

Here we have merely the repetition, at slightly later stages, of the operations included under Exps. 34 and 3 respectively. In the upper of the two diagrams illustrating results, we have, in each column, a complete agreement with the former results. In the lower diagrams, however, we have something very different. Although the yolk-blastopore is not far from closed, there is as yet no definite embryo formed, but merely a diffused opacity. Of such eggs there are four in one column and two in the other. These results do not seem reconcilable with those of my former experiments, in which the presence of a needle in such a position in no way interfered with the normal formation of an embryo. However, I may add that these were the last experiments of the season and that I could at the time obtain very few fertile eggs of *Fundulus*. Only a very small fraction of those which I attempted to fertilize developed, the remainder having apparently spoiled. Under these conditions abnormalities were to be expected.

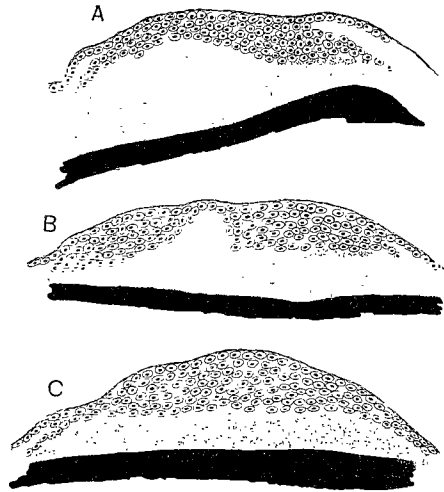
Experiment 37: »Stage C (late) — large area destroyed
by cautery«.

This area lay at the caudal end of the embryo and was intended to include the »centre of growth«. The differences among the three results figured are probably due to the extent of the injury. In the first case (upper diagram) we seem to have a splitting of the hinder part of the embryo, similar to that produced by KOPSCH, '99. Each half is continuous with the corresponding half of the germ-ring. In the second case the embryo has undergone a normal axial concentration, but probably no growth has occurred after the infliction of the injury. The remaining eggs were apparently little damaged, or perhaps they have regenerated.

Experiment 38: »Stage C (late), Op. c — needle«.

This I regard as one of the most instructive experiments of the series. The attempt was made to transfix the region of active growth which we have already shown to be situated a little in advance of the caudal end of the embryo. In the seven eggs represented by the first diagram this attempt seems to have been completely successful. One might conclude from a superficial inspection of one of these embryos that a considerable region of the body had been added behind the needle, and that each half of this later-formed body region was directly continuous with the thickened border of the blastopore (germ-ring). It may be noted, however, even in total preparations, that this posterior region is much thinner than is the embryo in front of the needle, and that the neural axis is continued but a short distance into it. Sections throw important light upon the formation of this region. Fig. 9 *A*, *B* and *C* represents transverse sections of an embryo of the first class, passing

Fig. 9 *A*, *B*, *C*.

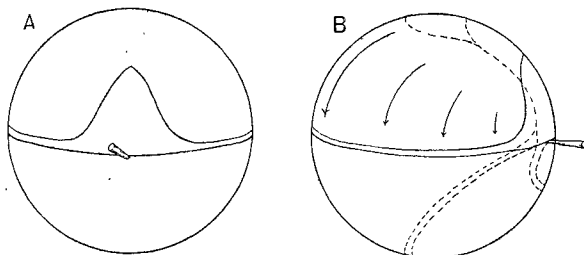


Sections of one of first class of embryos in Experiment 38, *A* being a short distance behind needle; *B*, through region of incompletely closed blastopore, and *C*, behind latter.

Fig. 9 *A*, *B* and *C* represents transverse sections of an embryo of the first class, passing

through the three planes indicated by the dotted lines in the diagram of the entire embryo. The sections passing through the region immediately behind the needle are lost in this series, but doubtless they would resemble Fig. 12 *B*. Farther back, section *A*, Fig. 9, we have

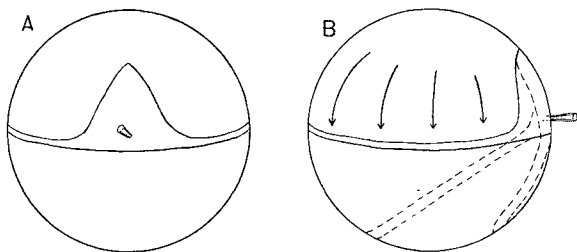
Fig. 10 *A* and *B*.



Illustrate supposed mode of blastopore closure in an egg in which needle is inserted at caudal end of embryo. The relative rapidity of growth in different places is denoted by the length of the arrows.

Passing back still farther, we can trace this undivided strip into a pair of such strips, which, however, are not completely separated from one another, but are connected above by a thin layer of epiblast cells¹). Finally, in the hindmost of the sections, these two strips are seen to reunite. My interpretation of these appearances is as follows. The needle, passing through the centre of growth, has completely

Fig. 11 *A* and *B*.



Mode of blastopore closure in an egg in which the centre of growth of the embryo has been transfixed.

checked the backward elongation of the embryo. The embryo proper extends only as far back as the end of the neural axis. Behind this follows a region formed by the complete, or further back yet, by the partial coalescence of the two halves of the germ-ring. Here we have, then, a rough measure of the share which the germ-ring takes

a region of nearly the same width as the embryo, but of far less depth, and quite undifferentiated in its central region except for the pavement layer. Laterally, however, two germ-layers are distinguishable.

checked the backward elongation of the embryo. The embryo proper extends only as far back as the end of the neural axis. Behind this follows a region formed by the complete, or further back yet, by the partial coalescence of the two halves of the germ-ring. Here we have, then, a rough measure of the share which the germ-ring takes

¹) It is to be remarked that the embryo-sectioned presented little or no trace of an open blastopore. In the one figured as a type, the slit-shaped remnant of the blastopore may have actually been open as it appeared to be in surface view.

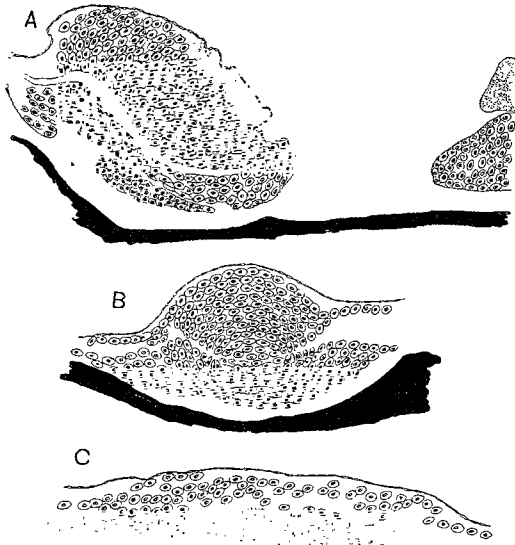
in the production of the embryo. A section through this fused strip presents less than one third of the area of a section through the hinder part of the normal embryo. (Compare with Fig. 12 *B* although, of course, this embryo is not strictly normal.) It is needless to say that I do not pretend to give here an even approximately correct numerical ratio.

While I regard this amount of material as being normally contributed to the growing embryo, I think it entirely disproved that any such coalescence .

normally occurs as has been shown in the case of the present experiment. Coalescence (concrecence) has here resulted from a retardation of one point of the margin (i. e. the caudal end of the embryo). Had the latter been free to grow, no such result would have been shown. It may be asked why, if the above explanation be true, a coalescence of the blastoderm margin did not occur in Experiments 6, 18 and 26, since there also the caudal end of the embryo was prevented from travelling backward. We must remember, however, that

in these latter cases the growing zone of the embryo was left intact, and that the embryo elongated with normal rapidity but in a forward direction. The blastopore, during closure, retained its circular form, instead of being converted into a longitudinal slit, because its ventral lip (former anterior margin of blastoderm) moved with a rapidity equal to that of the lateral portions. This could not have taken place had not the anterior end of the embryo travelled forward at a rate as rapid as that in which the posterior end would normally have travelled backward. We have already seen that the progress of

Fig. 12 *A, B, C.*



Transverse sections of one of second class of embryos in Experiment 38. *A*, through region transfixed; *B*, through perfectly formed region extending for some distance back of this; *C*, region probably formed by coalescence of blastopore lips.

the blastoderm margin is in great part due to an expansive force acting from the interior of the blastoderm. Figs. 10 and 11 *A* and *B* will illustrate my idea. The relative rapidity of movement of the parts is denoted by the length of the arrows¹⁾. This same explanation appears to me to be equally applicable to the normal behavior of the blastoderm of the elasmobranchs as well as to that of those teleosts in which the caudal end of the embryo parts company with the margin of the blastoderm before the yolk-blastopore closes. (See discussion of *Batrachus*, p. 138 below and of the paper of SCHMITT, on p. 135.)

In the second class of embryos resulting from this operation (shown in section in Fig. 12 *A*, *B* and *C*) we find that the embryonic axis has continued to grow and differentiate for some distance back of the needle. This can only be due to a lesser injury having been inflicted upon the growth centre, the needle, in this case, having been inserted to one side of the middle line (see section *A*). Growth has been retarded but not immediately arrested.

The last of the embryos figured does not seem to be especially instructive.

Experiment 39: »Stage C (late) — large area destroyed by cautery«.

Axial concentration, together with considerable elongation, have occurred. It was not determined whether the head end was in any degree regenerated. The destruction of the latter does not seem to have retarded growth, though possibly the elongation of the embryo has been entirely the result of the rearrangement of cells already present.

IV. Summary of Results and Conclusions From Experiments.

1) The anterior end of the embryo, when definitely established, occupies a position corresponding to the original animal pole of the egg (Experiments 1, 34, 35).

¹⁾ It is but fair to state that this view is not supported by the behavior of some eggs, not listed in the tables, in which both ends of the embryo were transfixed. In these the blastopore retained its circular form until the end of the experiment. Since development had not progressed very far in any of these embryos (three in number) at the time of preservation, I do not regard this negative evidence as very damaging to the theory. The experiment, however, deserves repetition.

2) Growth is mainly caudad in the normal egg, i. e. the caudal end of the embryo travels backward over the yolk (Experiments 1, 3, 4, 8, 9, 17, 19 — 1st two cases —, 25, 36).

3) This direction of growth, relative to the yolk-sphere, may be reversed by placing an immovable obstruction at the caudal end (Experiments 6, 18, 19, — 3^d case —, 23 — 1st case —, 26, 28).

4) Growth (elongation), from the first definite appearance of the embryo, up to the closure of the blastopore, occurs chiefly in a limited region situated a short distance in front of the caudal end. If this region be injured, growth will be retarded or arrested (Experiments 5, 24, 27, 37, 38, 39. — Also compare 3 with 6, 17 with 18, and 25 with 26).

5) The head end also grows, or at least moves, forward, though to a much smaller extent (compare Experiments 3 and 36 with 1, 34 and 35).

6) The spread of the blastoderm, for a considerable period, is nearly or quite concentric, the embryonic side traveling neither faster nor slower than the opposite side (Experiments 1, 34).

7) For some time prior to the closure of the blastopore, the ventral lip of the latter (former anterior margin of the blastoderm) travels much faster than the dorsal lip. This is necessarily true in the case of an embryo which spans an arc of less than 180° — that is, if we grant that the head end occupies a position corresponding to the original centre of the blastoderm.

8) The movement of the margin of the blastoderm (germ-ring) over the yolk is, to a considerable extent, at least, due to the expansion of the thinner, interior region of the blastoderm (Experiment 15).

9) The germ-ring normally passes continuously into the embryo at a rate more rapid than would be accounted for by its uniform contraction during the closure of the blastopore (Experiments 12, 22, 29).

10) This is not, however, a process of »concrecence«, in the sense of a coalescence or fusion of the edges of the blastoderm (Experiments 6, 18, 26).

11) An actual concrecence of the blastoderm margins (germ-ring) behind the embryo may be brought about artificially in some cases by arresting the growth of the latter, but the resulting strip is different in structure from, and far less massive than the true embryonic region (Experiment 24 — 1st case —, 38).

12) Thus the germ-ring alone furnishes but a relatively small

portion of the material of the embryo, which, in some eggs at least, may develop nearly normally after the germ-ring has been disconnected on one or both sides. The chief source of growth is the multiplication of cells in the special zone of growth, referred to above (§ 4) (Experiments 11 — 2nd case —, 14, 19 — 2nd case —, 23, 30, 38).

13) The entire embryonic region (so far as visible) of the early blastoderm may be destroyed by cautery, and yet an apparently normal »embryonic shield« may arise by a process of regeneration (Experiment 16).

14) A considerable portion of the central region of the blastodisc or early blastoderm may be likewise destroyed by cautery and yet a normal, though smaller, embryo may appear (Experiments 32, 33).

V. Structure of the Blastoderm and History of the Germ Layers.

In discussing the formation of the fish embryo, the »embryo« cannot be dealt with as a homogeneous mass which is being moulded out of a certain quantity of plastic material. To begin with, it is composed of three germ layers which act, to a considerable degree, independently of one another. These germ layers are early differentiated into the various embryonic organs: neural axis, notochord, somites, etc. An understanding of the organization of the early embryo is necessary to an intelligent discussion of its mode of formation and growth. It is my purpose in a following section to attempt something of an analysis of the complex system of movements undergone by the various cell-masses of the blastoderm which are to share in the formation of the embryonic body.

Let us first turn to a consideration of the gastrula stage with its germ layers. It is well known that a lower layer of cells (commonly regarded as the primary hypoblast) arises under the margin of the blastoderm and grows in a centripetal direction. This lower layer is much more fully developed on the future embryonic side of the blastoderm, and here grows farther toward the centre than elsewhere. It has been commonly supposed, of recent years, that this lower layer gives rise to the enteroblast (gut epithelium) as well as to the mesoblast and to the chorda. In a previous paper (SUMNER, '00) I described a factor which had been quite overlooked by most students of fish embryology. I now find that my paper has likewise been quite overlooked by some of these same students.

ZIEGLER, for example, in his new Lehrbuch ('02) reiterates the time-honored statements relating to the fish blastoderm, and fails to mention the facts which formed the chief basis of my memoir¹). After a thorough reexamination of my former preparations, as well as of many new ones, I still feel absolutely certain of the existence of the »New Factor« previously discussed by me. (loc. cit. — pp. 50, 51.) But the theoretical conclusions then offered were, I now think, obscurely stated and in part false.

In the case of every teleost egg which I have studied at appropriate stages, longitudinal median sections reveal a characteristic mass of cells²), lying at the posterior margin, which seems to arise as a thickening of the pavement layer of the epiblast, (»Deckschicht«) or, more properly perhaps, arises in continuity with the latter. Later it becomes free from the pavement layer, and for a while is bounded with considerable distinctness from the rest of the blastoderm (Figs. 16, 21B). In the blastoderm shown in Fig. 16, this problematic cell-mass appears in about 45 out of a total of about 100 sections. It is most massive in sections near the median plane. The appearances under consideration I find to be very pronounced in the development of *Salvelinus fontinalis*, *Abramis crysoleucas*, and in two species of *Siluridae*³). In the large pelagic egg of an unknown species of *Muraena* (Fig. 13) the »prostomal thickening« is very distinct, and its continuity with the superficial layer of the epiblast is beyond doubt. Its continuity with the gut-hypoblast beneath will be discussed later. It is to be noted that there is a fairly deep depression at the extreme hinder end of the embryo. I find strong indications of this collection of cells in *Fundulus heteroclitus* and *Ctenolabrus* (*Tautogolabrus*) *adspersus*, but I cannot speak with the same

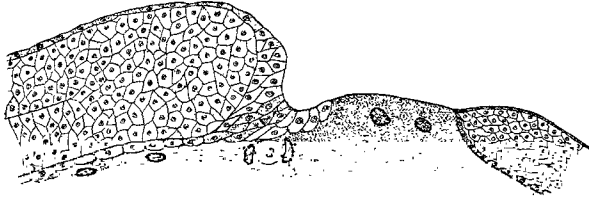
¹) That ZIEGLER has failed to read my paper I feel sure from the fact that he offers a theory regarding the function of KUPFFER's Vesicle, identical with my own and supported by much the same arguments. These suggestions are offered by him as original. (Compare ZIEGLER, p. 200, foot-note, with SUMNER, 1900, pp. 76 and 77.)

²) Referred to in my former paper as the »Prostomal Thickening«. This name I shall continue to use although I do not now regard it as especially appropriate.

³) *Schilbeodes* (*Noturus*) *gyrinus* was referred to in my former paper simply as »*Noturus*«. The eggs which I previously regarded as those of *Amiurus*, I now think it probable were those of *Noturus flavus*. They were sent by a collector who had labelled them »*Amiurus*«, but in response to a request for a specimen of the adult fish, I was sent *N. flavus*.

degree of certainty in the case of these latter fishes. KOWALEWSKI ('85 and '86) has described and figured for *Carassius* and *Gobius* appearances almost identical with those which I have just been discussing, but his work has met with little acceptance. BERENT ('96) has also observed certain stages in the history of these cells. His

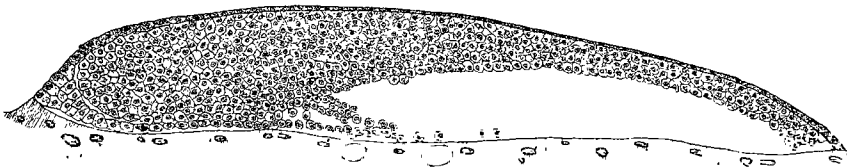
Fig. 13.

Sagittal section of hinder part of *Muraenoid* embryo shortly before blastopore closure.

description, as well as his account of their fate, I regard as substantially accurate.

The fate of the cells composing the »prostomal thickening« is extremely difficult to determine with certainty. I have already alluded to its continuity with the gut hypoblast, at a certain stage, in the egg of the *Muraenoid* (Fig. 13). Fig. 14 shows a very similar appearance in a trout blastoderm of a much earlier stage. The suggestion at once offers itself that the »secondary hypoblast« arises independently out of this early-differentiated mass of cells. This

Fig. 14.

Sagittal section of *Salvelinus* blastoderm of a stage intermediate between Figs. 16 and 17.

was the original conclusion of KOWALEWSKI, though he later modified it. BOEKE ('02) who, in general, confirms my account of the condition in the *Muraenoid* egg, also maintains this independent origin of the secondary hypoblast, holding that the mesoblast and chorda are derived from the overlying »invaginated« layer. He thinks it possible that the periblast contributes to the formation of the secondary hypoblast. BERENT has also contended for the derivation of the enteroblast, in the trout at least, from an early differentiated

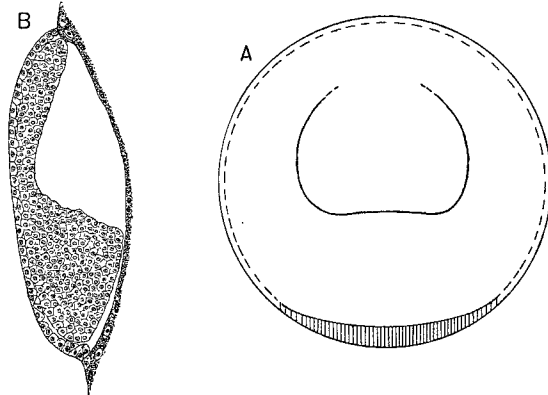
group of cells. These three writers are, as far as I know, the only ones who have traced the enteroblast to its true place of origin.

I now purpose to give a rather full account of the state of the germ layers in the brook trout, since my observations differ considerably from any previous ones made upon the *Salmonidae*.

From a study of sections, I have constructed four diagrams (Figs. 15 A, 16 A, 17, 19) exhibiting the extent of each germ-layer at four stages of development. The shaded areas represent the germ-layers, only in so far as they are present as distinguishable layers, and not in regions where they are fused together or are not yet differentiated. The area shaded with horizontal lines represents the generally recognized »invaginate layer« (primary hypoblast, according to most writers); and, in later stages, the structures (mesoblast and chorda) which, alone, I believe to be derived from it.

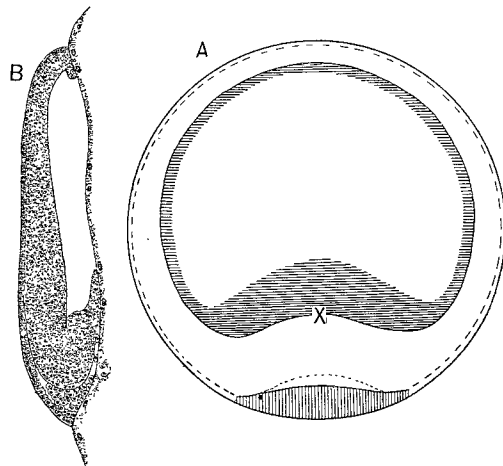
The vertically shaded area represents, in the first two diagrams (15 A and 16 A), the problematic group of cells (»prostomal thickening«) which I have described above as arising on the posterior margin. In Figs. 17 and 19 the vertical shading denotes the extent of the

Fig. 15 A and B.



Reconstruction diagram of *Salvelinus* blastoderm of which a sagittal section is shown in B.

Fig. 16 A and B.



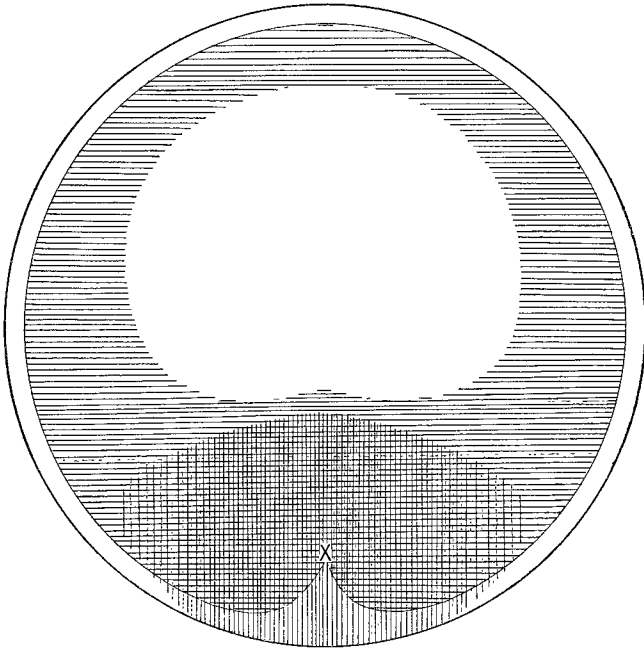
Reconstruction and sagittal section of a somewhat later stage than preceding, drawn to the same scale as the last.

single-layered epithelium which has almost certainly been derived from the prostomal thickening.

Unshaded areas represent, in some cases, epiblast, in other cases, regions where none of the germ-layers are distinguishable, viz.: near the hinder end in some embryos.

Each of these reconstructions is based primarily upon measurements made from the sections of a single embryo, but in every case

Fig. 17.



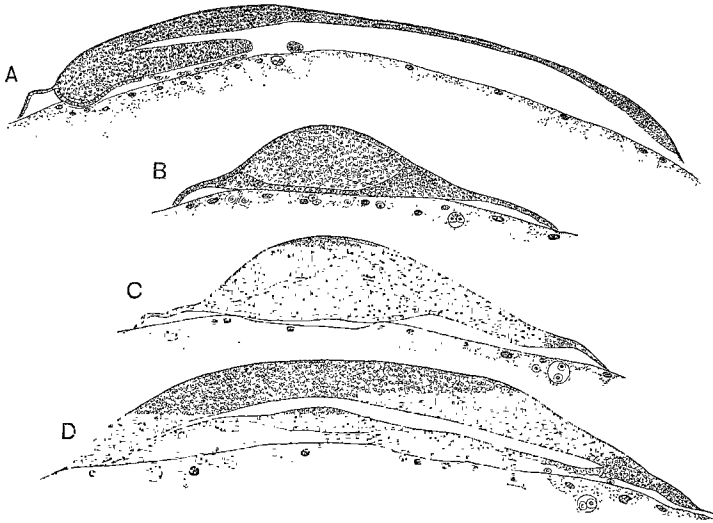
See Fig. 18.

its accuracy has been tested by comparison with other embryos of the same stage.

In the earliest trout blastoderm figured (Fig. 15) what appears to be merely a thickening or proliferation of the pavement layer of the epiblast occurs along the hinder margin, having about the extent represented. It will be seen that thus far no marginal invagination of the blastoderm has occurred, although its periphery, especially on one side, is very much thicker than the central region.

In the blastoderm of Fig. 16, however, invagination is in progress around the entire periphery. A distinct collection of cells, wedge-

shaped in section, is to be observed on the posterior border, occupying the same position as the thickening of the pavement layer in the preceding stage. Indeed the pavement layer is still merged indistinguishably with it in many sections. On its anterior face, this mass of cells is pretty sharply bounded in most sections, though it is to be recorded that in places the boundary does not clearly appear. In some sections, a one-layered extension projects forward beneath the other layers of the blastoderm, as figured.

Fig. 18 *A, B, C, D.*

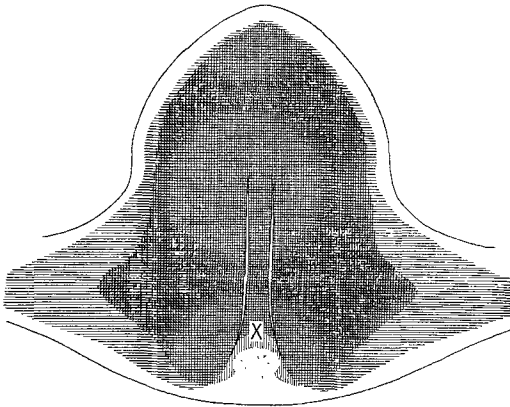
Longitudinal and transverse sections of blastoderms of stage shown in reconstruction in Fig. 17.

A stage intermediate between this and the next one shown in reconstruction is to be seen in Fig. 14. This has been already alluded to and demands no further explanation.

Fig. 18 *A, B, C, D* represent sections of blastoderms at the stage shown by Fig. 17. In the longitudinal section (*A*) it is to be noted that a continuous, one-layered sheet of cells is distinguishable beneath the embryonic region of the blastoderm, extending back to its extreme posterior margin. Although this condition is unmistakeable in a number of blastoderms of this stage, which I have cut, it must be admitted that the lower epithelial layer is not in every section as coherent and as sharply delimited as in the one represented. In some sections it seems to coalesce indistinguishably with the overlying layer, only to reappear again a little further on in the series.

In some sections its cells are detached and scattered. My transverse sections of this stage show this lowermost layer even less clearly. In many sections it cannot be distinguished at all, owing either to its cells being scattered, through faulty fixation, or to a complete coalescence with the overlying layer. But here we must bear in mind that every collection of cells which is morphologically independent is not under obligation to remain distinct to the eye. The more posterior sections show this layer more clearly than the anterior ones, and in those near the hinder margin (*B*) its close connection with the pavement layer may be observed. A little further forward (section *C*) the lower layer is not so evident. The upper

Fig. 19.



Reconstruction showing extent of germ-layers
in «embryonic shield».

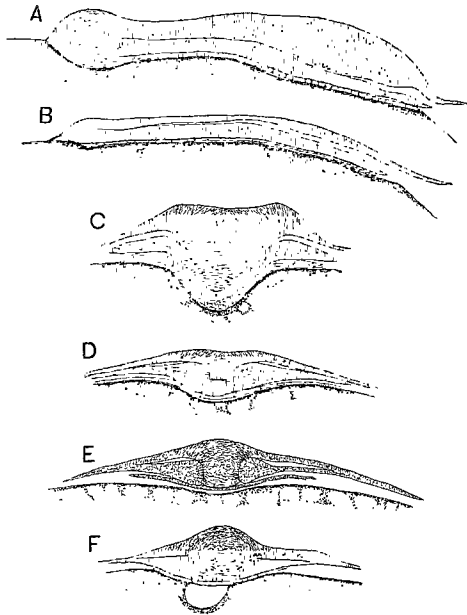
and middle layers are separate except in the median line. Fig. *D*, representing a favorable section at a level still further cephalad, shows us that the epiblast is concentrating to form the neural axis, while the middle layer is likewise concentrating to form the chorda. Mesoderm and chorda, at this stage, form a continuous layer, while the gut-hypoblast extends beneath, and quite independent, of it. One feature of interest is the presence of groups of cells imbedded in the yolk. These are of quite general occurrence throughout a considerable period in the early development of the trout's egg. The cells appear to be typical in every respect, having quite normal looking nuclei, far different in appearance from the enormous free nuclei of the yolk-syncytium. It does not seem probable that these cells have been derived from the yolk-syncytium, but that

their origin is to be traced to an early cleavage period, when the blastoderm had not become completely delimited from the yolk-sphere, i. e. before the syncytium, as such, existed. Whether or not these cells ever emerge from the yolk and contribute to the formation of the gut-hypoblast, I am unable to state. Some appearances strongly favor this view. On the other hand, in the two species of cat-fish which I have studied, these imbedded cells are very rare indeed, and certainly cannot play any such important rôle.

Passing to the next stage of our series, Fig. 19 represents an embryo in the condition often referred to as the »embryonic shield« stage. A median section (Fig. 20 A) shows us that the gut-hypoblast no longer extends back to the caudal end, but that it ceases to exist as a distinct layer immediately in front of the commencing KUPFFER'S Vesicle. Since the latter probably represents a precociously formed region of the embryonic gut (probably post-anal), having a definite function to perform in the embryonic life (nutritive?) the cells destined

to form its walls must be regarded as belonging to the hypoblast. Indeed I have already contended that these cells also owe their origin to the »prostomal thickening«. However, at this stage, the walls of KUPFFER'S Vesicle have not become clearly differentiated and it must be remembered that the reconstruction represents the germ-layers, only in so far as they are distinguishable to the eye. The section 20 B, cut parallel to A but passing some distance to the side, shows us that the gut-hypoblast extends here nearly or quite back to the margin. The cleft separating the epiblast from the middle layer also extends much further back than in the median section.

Fig. 20 A—F.

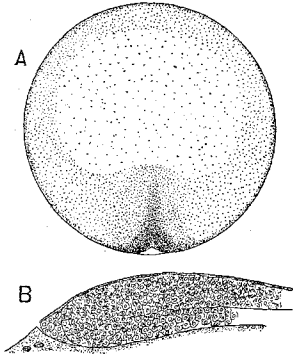


Longitudinal and transverse sections of embryos of same stage as Fig. 19.

Passing to the transverse sections, the most anterior one (*C*) reveals the presence of two thin layers, lying beneath the greatly thickened neural axis of the brain region. Since this section passes through the pre-chordal region, the middle layer represents mesoblast alone. The section *D* demands no explanation. In *E* it is seen that the boundary between neural axis and chorda has disappeared, but that mesoblast and gut-hypoblast remain distinct. The section *F* passes through the incipient KUPFFER'S Vesicle. The figure explains itself.

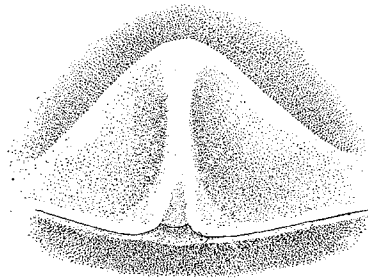
In the development of *Schilbeodes* we find certain well marked differences. Fig. 21 represents a blastoderm of about the same rela-

Fig. 21 *A* and *B*.



A Blastoderm of *Schilbeodes*; *B* hinder part of sagittal section of last.

Fig. 22.



Embryonic shield of *Schilbeodes*.

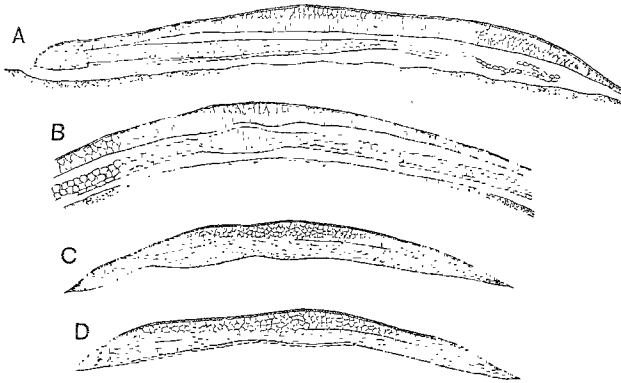
tive stage as the trout blastoderm of Fig. 17. It is to be noted, however, that the underlying epithelial layer of the latter is wanting in the former. The (in section) wedge-shaped mass of cells at the posterior border is very evident.

In Fig. 22 we have a rather early »embryonic shield« stage. The notched condition of the caudal end is to be noted. Fig. 23*A*, shows a longitudinal section of a closely similar embryo, passing some distance to one side of the median line. The wedge-shaped mass of cells is no longer to be seen, either here or in median sections. An underlying epithelial layer is distinguishable throughout only part of the extent of this section, being clearly marked in perhaps the posterior half. Traced forward, it is seen to lose its identity, becoming fused indistinguishably with the overlying layer. It is also lacking

as a distinct layer throughout the entire median region of the embryo, a section thus cut not exhibiting the gut-hypoblast at all. Fig. 23 *B*, (transverse) illustrates this incompleteness of the lowermost germ-layer. These appearances are such as to point to the origin of the gut-hypoblast, mesoblast and chorda by differentiation from a common »primary hypoblast«, as is usually assumed. This question will be deferred for the present, however.

Fig. 23 *C*, represents a section through the extreme margin of the same embryo as *B*. The bilobed condition, evident in surface view, is well shown here. The clefts on each side represent the lines of division between the epiblast and the middle layer. No lower layer

Fig. 23 *A*, *B*, *C*, *D*.



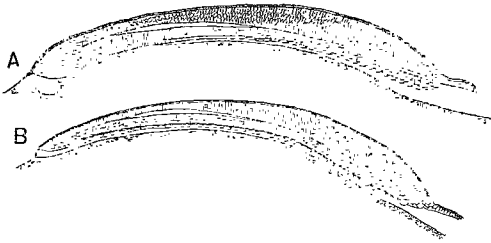
Longitudinal and transverse sections of embryos of stage of Fig. 22.

is observable in this section. In *D*, however, from another embryo of almost exactly the same stage, a distinct lower layer is to be seen throughout almost the entire width of the section. This has also been observed in other embryos, and is not to be regarded as an artefact. The presence or absence of a visible »layer« may not always, perhaps, have such a high morphological importance as is often assumed.

Fig. 24 and 25 represent sections of embryos at a stage a little more advanced than the preceding. In 24*A*, we have a slightly oblique section which is nearly median as regards the posterior portion at least. The gut-hypoblast terminates abruptly at a considerable distance from the posterior end. In the more lateral section *B*, on the contrary, this layer is seen to extend nearly or quite back

to the posterior end. In Fig. 25 *A*, we have about the 18th transverse section from the anterior end of such an embryo. It is to be noted that the neural plate coalesces, in the middle line, with the

Fig. 24 *A, B*.

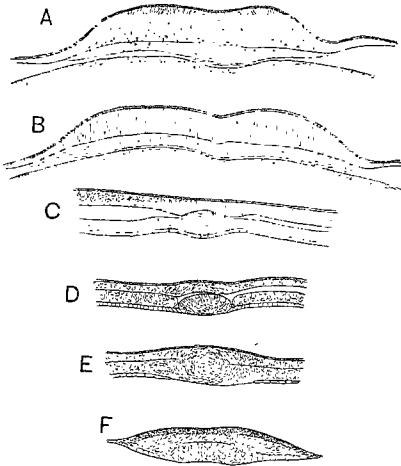


Longitudinal sections of *Schilbeodes* of a stage somewhat later than those of preceding figure.

underlying layer. This condition is found in a considerable, but varying, number of sections in all embryos of this stage which I have examined. The precise limit of the epiblast is indicated in many, though not in all sections, by the arrangement of the

nuclei of the lowermost layer of its cells. I cannot regard the union of these layers as having any morphological significance. It is due to a secondary fusion and not to an original continuity, as is evident when we compare the present with preceding stages. In *B* we have

Fig. 25 *A—F*.



Transverse sections of same stage as Fig. 24.

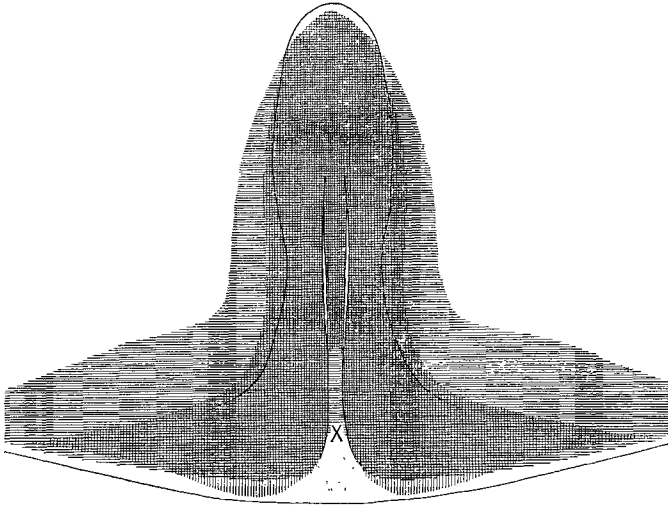
three germ-layers distinct from one another, and here, as in Fig. 18 *C*, it is plain that the foundations of both chorda and mesoblast constitute the middle layer. In *C* the mesoblastic plates have separated from the notochord. In *D*, the 77th section, we find that the lower layer is not to be distinguished beneath the chorda, but that it abuts against the latter on either side. In *E*, all of the germ-layers merge together mesially in an undifferentiated mass (»primitive streak«). In *F*, no separation into layers can be distinguished

with certainty. The transverse line across the centre perhaps represents commencing lumen of KUPFFER'S Vesicle.

Fig. 27 *A—G*, are sections of embryos of the stage which has been reconstructed in Fig. 26. In the longitudinal sections, *A* and *B*, the germ-layers are seen to have the same relative extent as in corre-

sponding sections of the preceding stage. The exact posterior limit of the gut-hypoblast is difficult to fix in the median section, but ends far in front of KUPFFER's Vesicle. Laterally, however, it extends back to the margin. In *C*, the 37th cross-section from the anterior end, the hypoblast and mesoblast are rather vaguely separated from one another, as is true of a considerable part of the head region. Section *D* demands no explanation. In *E* we have the same relation between gut-hypoblast and notochord as has been previously encountered (Fig. 25*D*). This condition extends back to the posterior limit of the chorda, i. e. through more than twenty sections. The

Fig. 26.

Reconstruction of *Schilbeodes* embryo.

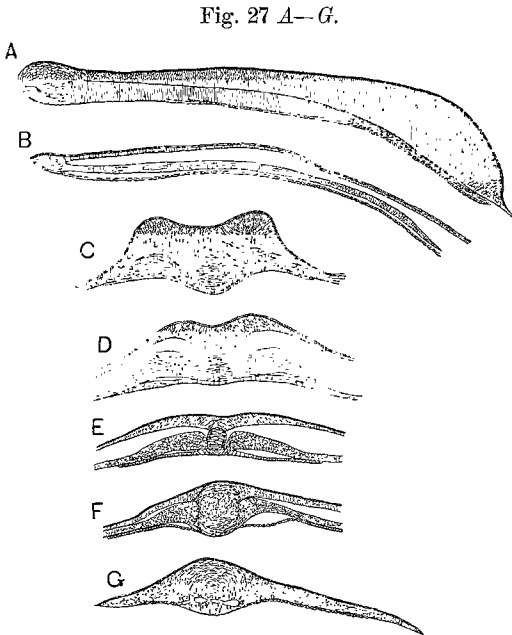
appearance certainly suggests the formation of the chorda in this region, through a concentration of the hypoblast along the middle line. This I do not regard as probable, as will appear later. In *F*, we have the »primitive streak« region, and in *G*, the early KUPFFER's Vesicle, which begins as a collection of small vesicles.

A blastoderm of *Batrachus*, together with two cross-sections, are shown in Fig. 28. The more anterior section (*B*) exhibits an unbroken middle layer, representing both chorda and mesoblast, as in Fig. 25*B*. Further back, it is interesting to note that the lower layer remains clearly distinct, even where the upper and middle layers are fused along the embryonic axis, differing thus from the *Schilbeodes* embryo and agreeing with that of *Salvelinus*, though

the lower layer is here far more distinct than in the egg of the latter.

Sections of a later stage are shown in Fig. 29. The conditions in front of section *A* are closely similar to those represented in Figs. 27*D* and *E*. Section *A*, of the present series, passes through KUPFFER'S Vesicle, which, in *Batrachus*, lacks a cellular ventral wall, at least in earlier stages¹). The walls of the vesicle are directly continuous laterally with the hypoblast layer. Anteriorly (not figured)

its dorsal wall passes insensibly into the notochord, against which the hypoblast abuts on either side, in a manner identical to that which has been previously figured in Fig. 27*E*, and some other sections. In *B*, which is cut at a level somewhat further back, the mesoblast is seen to join the hypoblast in a manner similar to that which has been frequently described for the amphibian and elasmobranch eggs, the appearance suggesting the formation of the middle layer by means



Longitudinal and transverse sections of embryos of stage of Fig. 26.

of enterocoelic pouches. In *C*, all layers are fused in the middle line. A little further back, the embryo thins out rather abruptly, and we have a region (*D*) formed, I believe, by the coalescence of the margins of the blastoderm behind the embryo, as in the case of Experiment 38 (compare with Fig. 9). This matter will be referred to again (Fig. 34, 35).

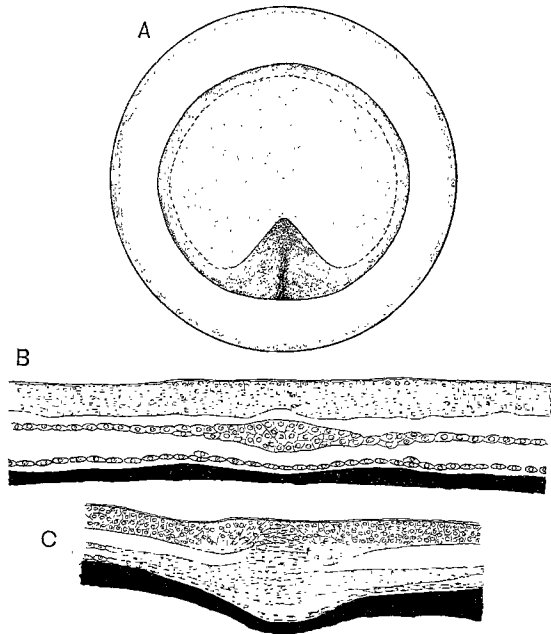
¹) This seems to be true, as a rule, of the embryos of marine fishes; while the fresh-water forms which have been studied commonly have a vesicle which is, from the first, bounded completely by cells. Among the latter are to be included *Salvelinus*, *Abramis*, *Noturus* and *Schilbeodes*, according to my own observations, and *Salmo* and *Carassius*, according to some other investigators.

What, then shall we conclude respecting the origin of mesoblast, chorda and gut-hypoblast in the teleost egg, and what are the significance and fate of the the problematic collection of cells which has been referred, to as the »prostomal thickening«? On comparing the diagrams of the earlier and later stages of the trout blastoderm (Figs. 15 et seq.) it easy to believe, though I do not regard it as altogether proved, that the vertically shaded area has extended itself, from the time of its first appearance, by the multiplication of its own cells. If this be

true, the »secondary hypoblast« has arisen from a foundation entirely distinct from the mesoblast. Its origin is to be traced to an ingrowth from the superficial layer of the epiblast, occurring on the hinder margin of the blastoderm. This continuity of epiblast with hypoblast around the dorsal blastopore lip (Fig. 14) is quite comparable to that shown in the egg of an elasmobranch or that of a frog. The lowermost germ layer is at first confined to the

embryonic region of the blastoderm, but before the closure of the blastopore, it has extended itself around the entire margin (Fig. 13 — I have observed this same condition in the egg of the trout and in that of *Schilbeodes*). It is true that in the »extra-embryonic« region of the blastoderm, the direct continuity between the secondary hypoblast and the superficial layer of the epiblast is not to be observed, and the appearance of an inflected margin is thus lost, but I do not regard this fact as of any real significance, since the »Deck-schicht« everywhere tends to spread beyond the underlying layers

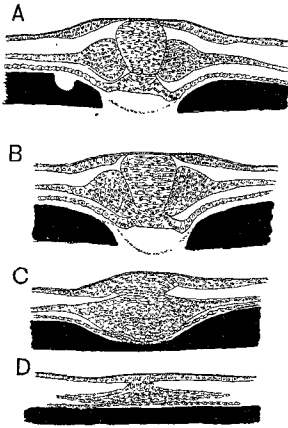
Fig. 28 A, B, C.



Batrachus blastoderm and two transverse sections,
B being the more anterior.

of the blastoderm, as happens eventually even at the caudal end of the embryo. An earlier differentiation of the gut-hypoblast, as of the other layers, is to be expected on the embryonic side of the blastoderm. The greater thickness, in median sections, of the foundation from which it arises is merely a part of the general process of concentration toward the embryonic axis which affects the epiblast and mesoblast as well. Accordingly, I no longer regard the »prö-stomal thickening« as representing an embryonic portion of the blastopore which becomes detached at an early period from the remaining »yolk« blastopore, although I still regard its formation

Fig. 29 A, B, C, D.



Transverse sections of posterior end of *Batrachus* embryo, A being the more anterior.

as due to the unequal mode of blastopore closure, as a result of which the material of the blastoderm margin is continually concentrating toward embryonic axis and passing into the latter. In a sense this might be described as a continual forward detachment of portions of the blastopore, though it would be much nearer the truth to call it a detachment of material which originally formed the lips of the blastopore. But, as I shall attempt to show later (p. 140), this process does not cease until the blastoderm has ended its journey over the yolk.

Now, while I think it extremely probable that in *Salvelinus* the entire secondary hypoblast arises from an independent foundation, I am not at all convinced of this in the case of *Schilbeodes*. In the former egg, this layer is distinguishable at the very commencement of invagination, and appears to grow forward pari passu with the layer which overlies it. In *Schilbeodes*, on the contrary, gastrulation is well advanced, and a very extensive »invaginate layer« has been formed, before the gut-hypoblast is to be distinguished throughout the embryonic region. As I have recorded above, the appearances are all in favor of the origin of this layer in *Schilbeodes* by differentiation from the lower surface of the »invaginate layer«¹⁾, rather

¹⁾ This I do not regard as a very appropriate designation, since I regard the ingrowth of the secondary hypoblast as also belonging to the »invagination« of the egg.

than by spreading from the posterior border. Nevertheless, there is present in the egg of this fish just such a mass of cells as I have described as being the origin of the enteroblast in *Salvelinus*, and I cannot but think that in the former egg also it contributes to the formation of this layer. Indeed I regard it as quite possible that even in *Noturus* the entire hypoblast may have an independent origin, although its cells are for a long time merged indistinguishably with those of the overlying layer. What appears to be a primary process of differentiation may be a secondary separation of elements originally distinct.

It appears from the studies of BOEKE ('02) that the enteroblast develops in the *Muraenoids* quite independently of the layer which is destined to form the mesoblast and chorda. This also seems to be quite likely in *Batrachus*, in view of the extremely early appearance of this layer (Fig. 28), though I have not traced its history in this egg.

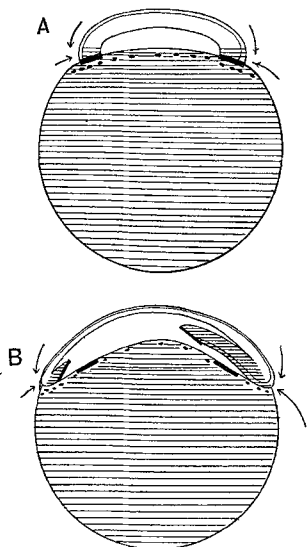
It is obvious from many sections which I have figured and described that, in the anterior regions of the embryo at least, the mesoblast and chorda arise from one continuous layer. This is true of all three of the fishes which I have discussed in detail. But it will be recalled that in a more posterior region, the gut-hypoblast is continuous with the chorda, while the mesoblast is free. In fact the notochord appears here as if it were an axial thickening of the gut-epithelium. That it is actually so formed I think to be quite unlikely, despite all appearances. It does seem probable that the chorda should have a different derivation in two regions of the embryo; but it is far more likely that in the posterior region, just as in the anterior, mesoblast and notochord have been differentiated from an originally continuous layer. This conclusion is not in the least contradicted by the condition of the gut-hypoblast, whose adhesion to the chorda is no more to be regarded as indicating an origin of the latter from the former, in ontogeny, than is its coalescence with the mesoblastic plates (Fig. 29B) to be regarded as pointing to such an origin for those¹).

¹ In my previous paper (SUMNER, '00) I figured these relations between enteroblast, notochord and mesoblastic plates. I did not mean to imply as BOEKE has inferred from a rather ambiguous statement of mine that I regarded chorda and mesoblast as being derived from the enteroblast.

VI. The »Blastula« and the »Gastrula« of the Teleost.

Fig. 30 *A* and *B*, illustrate what seems to me to be the most reasonable interpretation of the blastula and gastrula stages of the teleosts.

Fig. 30 *A* and *B*.

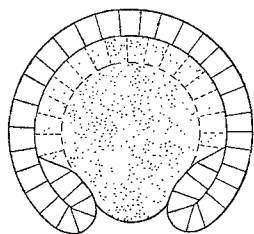


Diagrams illustrating gastrulation in teleost.

A comparison of this blastula with that of *Amphioxus* is easy, and has often been made, the unshaded region in Fig. 30 *A* representing roughly the ectodermal half of the *Amphioxus* blastula, the shaded region, together with the periblast and yolk, being equivalent to the entodermal half. But whereas, in the *Amphioxus* blastula, at the commencement of gastrulation, the »vegetal« half simply invaginates into the »animal« half, in the case of the teleost a much more complicated process occurs. Two factors are involved: firstly, an epibolic overgrowth of the yolk by the blastoderm, and secondly, an ingrowth of the entire margin of the latter. With the commencement of this ingrowth, there occurs, as CUNNINGHAM ('86), points out, a dislocation (»solution of continuity«)

between the cellular and non-cellular parts of the blastula, the former now moving independently of the latter. The heavy black lines in Fig. 30 *A* represent the surfaces of junction between the blastoderm and the

Fig. 31.



yolk, in what may be regarded as the blastula stage. The two are now sharply delimited from one another, though, at an earlier stage, certain of the cells of the blastoderm were in direct continuity with the yolk. These became merged in the latter to form the yolk-synectium (»periblast«) and a segmentation cavity developed between the latter and the blastoderm. In

the stage *B*, the extent of the supposed dislocation is indicated by the separation of the surfaces which formerly were in contact.

According to this view, the entire margin of the blastoderm represents the rim of the blastopore, from which protrudes the enor-

mous yolk-sphere. The latter is not, however, to be viewed as a foreign body, but as a greatly distended part of the inner layer of the gastrula. In Fig. 31, I have represented a hypothetical gastrula in which a mass of yolk has been substituted for the central region of the inner layer. The comparison with Fig. 30 *B*, is obvious, though of course there are but two germ-layers represented in the former. This interpretation of the teleost gastrula, although it seems almost self evident, is contrary to that held by many embryologists.

VII. Formation and Growth of the Embryonic Body.

It would be practically impossible to construct a graphic representation which would take account of the various movements occurring within the blastoderm while the embryo is forming. At least three component forces are to be distinguished, and the movement of many of the cells of the blastoderm is a resultant of all three. We have 1) a centrifugal expansion of the entire blastoderm; 2) a centripetal growth of the lower layer of the germ-ring, especially marked on the embryonic side; 3) a concentration, throughout the posterior part of the blastoderm, toward the embryonic axis.

It is evident that 1) and 2) are in opposition to one another. Which is in excess? I have only determined this by actual measurement in one case. By means of camera drawings of the living egg, of *Fierasfer* (Fig. 32 *C* and *D*) I find that in the lower layer the centripetal movement is, for a while at least, considerably in excess and that the aperture of the germ-ring decreases in diameter, despite the increase in diameter of the blastoderm as a whole. Later, however, it seems certain that the relative extent of these two components is reversed.

The concentric spread of a fish blastoderm makes it appear as if the margin were affected by 1) acting alone. Nevertheless it is certain that a considerable area of the blastoderm is undergoing the movement 3). Thus, to speak somewhat paradoxically, the margin moves uniformly throughout its entire circumference¹⁾, while its constituent cells are travelling in different directions and at different rates. That a continuous concentration of cellular material toward

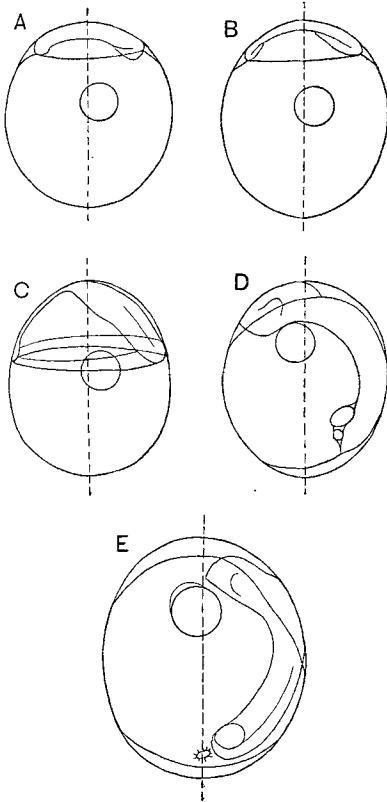
¹⁾ As has already been pointed out (p. 115), this is not strictly true during the later stages of the overgrowth of the yolk. Then, the ventral blastopore lips move much faster than the dorsal, although the circular form of the blastopore is retained.

the middle line is actually taking place no one will question who has ever watched a developing fish embryo. It is more active in the posterior than in the anterior region since the former is wider and must suffer a greater degree

of narrowing. In transverse sections (Figs. 18, 20, 25, 27) it is seen that the axial concentration affects the upper and middle germ-layers independently of one another, while the hypoblast is at first but slightly affected. Some of my experiments (especially 12) have shown that this axiad movement of cells is not confined to the embryonic region proper, but is felt far to each side of the latter, i. e. in the germ-ring¹).

The net result of this heaping up of cells toward the embryonic axis is the continued elongation of the embryo, and it seems possible that during early stages, this is the chief cause of the latter's growth. Now a number of experiments have been discussed above which proved beyond doubt that, after its establishment, the embryonic body elongates mainly in a backward direction. Many previous observers have been led to a like view. Certain of my experiments seemed also to indicate that the head end moved forward somewhat from its point

Fig. 32 A—E.



Illustrating the direction of growth of the embryo in *Fierasfer*, sp. The dotted line indicates the major axis of the ellipsoid. A—D represent stages of the same egg, drawn with camera lucida. E drawn from another egg, and figured on a somewhat larger scale, is intermediate in stage between C and D. The extreme forward position of the head end shown in D was probably acquired subsequent to blastopore closure. Previous to that event, however, the head end, in many eggs studied, lay approximately at the animal pole of the egg — e. g., in E.

(Drawings from camera lucida sketches.)

¹ Special emphasis is always laid upon the germ-ring in these discussions, owing to its greater massiveness. Since, however, it is continuous with the rest of the blastoderm, it is not likely that its movements are independent of the latter.

of origin. Camera lucida drawings of the living egg of *Fierasfer* (Fig. 32 A, B, C, D) an egg having a distinctly ellipsoidal form¹⁾, confirm the experimental results. It is, of course, possible that a shifting of the entire blastoderm, together with the embryo, may occur here, though I think this unlikely, since during a considerable period of its growth, the margin of the blastoderm may be seen to move through concentric circles having their centre at one pole of the ellipsoid.

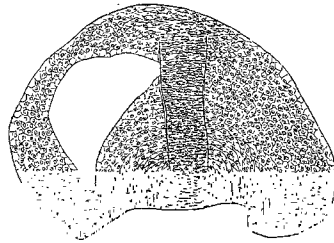
SCHMITT ('02) describes certain abnormal eggs of the trout in which two embryos developed on opposite sides of the yolk-sphere. At the time of their first appearance, the two embryonic shields lay on opposite sides of the blastoderm. As the latter spread, and the embryos elongated, the head ends became more widely separated. At the time of blastopore closure, the two embryos lay on nearly opposite meridians, each extending through an arc of 77.5 degrees, the head and tail ends being about equidistant from the poles. The blastopore, just before closure, extended as a longitudinal slit between the caudal ends of the embryos. From such cases SCHMITT concludes 1) that the caudal end of the fish embryo advances more slowly than the margin of the blastoderm at a distance from it, while points in the latter advance the more rapidly, the greater their distance from the caudal end of the embryo, and 2) that, normally, the head end of the embryo moves to a lower latitude than that in which it first appeared. The truth of the former contention is self-evident when applied to later stages of blastopore closure, but I have already given reasons for doubting its truth during the earlier stages, at least of *Fundulus* and *Fierasfer*. The second contention I believe to be unproved by the condition of the eggs described by SCHMITT. With this writer, I think it likely that the head ends of these embryos have been forced apart by the expansion of the central area of the blastoderm, but I do not think it fair to conclude from this that in an egg having but a single embryo, the head end is normally pushed back from its original position. In the latter egg, the blastoderm margin offers less resistance to this expansive force than does the embryo, and movement here, as everywhere, follows the lines of least resistance. In an egg with two embryos, on the other hand, a like resistance is offered in either

¹⁾ M. KOWALEWSKI ('86a) from the study of another ellipsoidal egg, came to conclusions directly opposite to mine.

direction, and both of the embryos yield. The condition of the blastopore of such an egg, at the time of its closure, might seem to contradict my explanation of the slit-shaped blastopore in Exp. 38 (See Figs. 10 and 11). In the present case, it may be objected, there has been no retardation of the growth of either embryo. There has been no abnormal retardation, it is true, but, as has just been stated, the hinder end of an embryo, for some time prior to blastopore closure, normally advances less rapidly than the margin of the blastoderm on either side. Hence it is that the ends of these embryos are still far from having met at a time when points in the germ-ring which were 90° distant from each embryo have come together.

It has been shown by a number of experiments that the elongation of the embryo is dependent upon the integrity of a region somewhat in advance of the caudal end. It is, I think, generally admitted that in this region a continual process of differentiation is taking place, as a result of which new portions are added to the embryonic body. In a rather late embryo it is easy to locate this zone of growth directly in front of KUPFFER'S Vesicle. The latter, after its establishment, maintains a nearly constant distance from the caudal extremity. In the zone of active growth, the neural and chordal axes are merged together and, in some cases, at least, the gut-hypoblast and mesoblastic plates also lose their identity mesially an undifferentiated mass. With the growth of the embryo, neural axis, chorda and mesoblast constantly become differentiated at the anterior end of this region, the mesoblast segmenting to form new somites, while the gut-epithelium constantly extends itself beneath the chorda. Whether the completion of the gut-epithelium in the middle line is effected by the multiplication of its own cells or by the differentiation of cells on the lower surface of the chorda has been a point in dispute. The former view has a greater inherent probability, and is supported by many sections. On the other hand, appearances are at times strongly in favor of the other alternative. Meanwhile, the zone of growth is continually being replenished at its posterior end. This zone of growth, both in its structure and its relations to the growing embryo, is seen to be analagous to if not actually homologous with the »primitive streak« of the amniota, and I think that the latter name may be applied with perfect propriety in the discussion of teleost development. We have seen that this primitive streak is replenished, partly by its own inherent growth, partly by the addition of cells from the hinder margin of the blastoderm. The

continuity of embryonic and extra-embryonic germ-layers is well shown in the reconstruction, Fig. 26. In Fig. 33, we have a horizontal section through the hinder region of a *Schilbeodes* embryo of the stage of Fig. 21*A*. The arrangement of the cells on each side of the embryonic axis speaks strongly for a progressive massing of these cells toward the latter, and for a resulting elongation of the embryo. This influx of cells from laterally situated regions is much more restricted in its range than at an earlier stage, but I regard it as being merely a continuation of the same general process by which the embryonic axis itself was established.

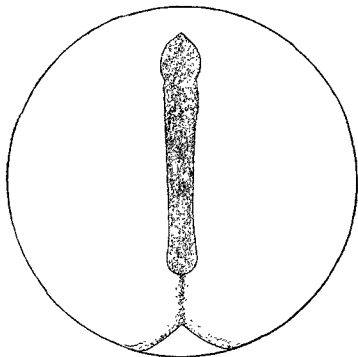


Horizontal section through hinder end of *Schilbeodes* embryo.

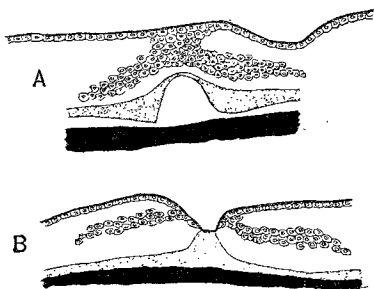
Such a massing of extra-embryonic material at the caudal end must result in a continual forward push exerted along the median line of the embryo. If the hinder border of the blastoderm were fixed, i. e. if the blastoderm were not increasing in diameter, this push would result in an absolute forward displacement along the axis of the embryo. Since the blastoderm is rapidly spreading, however, this displacement is not absolute but relative. Thus, points in the middle line are merely retarded, relatively to points on either side. This may result in an obvious notch on the posterior margin (Figs. 22, 28*A*), though the latter is usually wanting. In the trout blastoderm (Fig. 17 and even as early as 15) we observe that the line of cleavage between epiblast and mesoblast extends much further back laterally than in the median line. Or, to invert the statement, the area of union between epiblast and mesoblast extends much further forward in the median line. I can only account for this by supposing a (relative) forward displacement of the point *X* (Figs. 16 and 17, also 19 and 26). A transverse section *C*, through Fig. 17, passing a short distance behind the point *X*, illustrates just such a fusion of neural and chordal elements as we encounter in a later embryo, e. g. Fig. 20*E*. This is our »primitive streak« region, continually differentiating in front into new portions of the embryo, continually replenished behind by inherent growth and by accretion, until the final closure of the blastopore. The condition of this region has often been attributed to »concrecence«, and indeed if we imagine two halves such as section *A*, Fig. 23, to be united end to end

we should have a condition almost identical with that of 25 *E*, Fig. 23 *C*, certainly appears as if it arose by the coalescence of the lateral margins of the blastoderm. But the same result would be obtained if we supposed that one point on the circumference of the blastoderm had been retarded, e. g., by just such a »push« as has been assumed above. The junction of the various layers would extend farther forward in the region retarded (i. e. in the middle line of the embryo). This axial concentration and its accompanying retardation of the the middle line may, nevertheless, be regarded as a process of bilateral blastopore closure, much modified. This, however, is not »concrescence« in the descriptive sense.

Fig. 34.



Batrachus embryo shortly before blastopore closure.

Fig. 35 *A* and *B*.

Sections through line passing back from embryo to open blastopore. *A* is the more anterior of these sections.

While denying that the embryonic body arises by a coalescence of the two halves of two halves of the blastoderm margin, I freely admit that such a coalescence takes place in certain cases behind the embryo. Such a process was brought about artificially in Experiment 38. A similar process occurs normally in the development of *Batrachus*, as has already been pointed out by Miss CLAPP ('91). Sections through the line connecting the caudal end of the embryo with the dorsal lip of the blastopore show that this line is actually the result of a continued fusion of the margins of the blastoderm (Figs. 34 and 35 *A*, *B*, *C*, *D*).

As far as I am aware, KASTSCHENKO ('88), RUCKERT ('91)⁴,

⁴ The two first named authors describe briefly some cutting experiments upon early elasmobranch embryos. The former was led to believe that the embryo grew independently of the germ-ring; the latter to believe that the germ-ring contributed to its formation.

MORGAN ('93, '95) and KOPSCH ('96, '98a and b, '99) are the only previous students who have operated upon developing fish embryos, and the two latter authors are the only ones who have so treated the eggs of teleosts. The studies of MORGAN and KOPSCH are too well-known to students of embryology to require an account of them here. I will only remark that as far as these authors have performed the same experiments, their results are quite in harmony. The conclusions of KOPSCH may also, I think, be said to supplement, rather than to contradict those of MORGAN.

In the same way, the results of my own experiments, as far as they were anticipated by those of MORGAN, agree pretty well with the latter. The inferior development of the mesoblast on the side of the injury, in cases where the germ-ring was severed, appears distinctly in at least two cases (Exps. 19 and 30). This is a condition described by MORGAN (based upon one embryo) and is certainly the condition to be expected, according to his view and mine that the germ-ring is one source of the building material of the embryo. This last proposition, indeed, I am willing to affirm with much greater certainty than was MORGAN, for I regard it as proved by certain of my experiments. MORGAN's assertion that the head end of the embryo is »formed largely from material that has never been at the edge of the blastoderm« ('95, p. 440) I regard as more than doubtful. The middle and lower layers at least, of this region were unquestionably at first present at the margin, from which they attained their final position through ingrowth. I regard it as highly probable, (see Exp. 1, 3, 34, 35, 36 and Fig. 32) that the primary head end grows — or is pushed — forward from an original position on the margin. MORGAN has here judged from the final result. Eventually, of course, the head-end does lie at the »animal pole« of the egg.

With the work of KOPSCH, also, I find myself on the whole in agreement. On one important point, however, I must dissent from KOPSCH's conclusions. This author describes a critical stage in the development of the teleost blastoderm, during which two definite masses of material, at first situated laterally, are brought together in the median line to form the »Knopf«. The latter »represents a growth centre from which trunk and tail will be formed« and »contains the neurenteric canal« ('98b, p. 75). With its formation, the »Prostomialfeld« is established, and the embryo is in a stage corresponding to that of an annelid after the lips of the blastopore have

coalesced. Thereafter, in both types, elongation occurs through the activity of a growing zone near the posterior end.

With KOPSCH's hypothesis regarding the formation of the »Knopf« I have, in a former paper, expressed my full agreement. I have been led, however, to change my views. I find no good evidence of such a radical change in the manner of growth as is here implied. KOPSCH supports his hypothesis, it is true, with some experimental evidence. For example, he believes that he has been able to destroy or to damage one of these $\frac{1}{2}$ Knopf-Anlagen, before their union, with the result that the embryo, in the trunk region, developed on one side only. An injury further laterad produced no such pronounced defect. I cannot regard this evidence as at all conclusive. The damage done by such an injury we should expect to be the more profound, the nearer we approached to the embryonic axis. Compare, for example, the above experiment of KOPSCH's with my own Exp. 5, in which a needle, passing through the region »K« of KOPSCH figure ($K = \text{Kopfanlage}$), altogether prevented the formation of an embryo.

The formation of the »Knopf« is part and parcel of the general process of axial concentration, which begins at a very early period, on the embryonic side of the blastoderm, and continues until the closure of the yolk-blastopore. The existence of a region in which all three germ-layers are merged together demands no explanation, since this is the condition of the margin of the blastoderm everywhere. In the median line this undifferentiated region extends further forward (see p. 137) and here we have the primitive streak and the »Knopf«¹⁾. The latter (or its homologue) may or may not form a projecting knob. It may actually present a concave margin as in the case of *Batrachus* (Fig. 28 A). As thus conceived, the »Knopf« occurs at a very early period of fish development, indeed from the first appearance of gastrulation.

With KOPSCH's conclusion that the »Knopf« receives a constant addition of cells from the germ-ring, and that these ultimately enter into the formation of the embryo, I am in perfect agreement.

JABLONOWSKI's ('98), in a paper of considerable interest and value, also speaks strongly for the occurrence of a primary and a secondary region of the embryo. (J. does not, himself, use these

¹⁾ The »Knopf« of KOPSCH is not quite co-extensive with the region which I have referred to as the »primitive streak«. The latter does not reach the caudal end, but terminates in front of this.

terms.) The former represents the trochophore of the annelid or mollusc, or the *Amphioxus* gastrula before the appearance of any secondary metameres. (At least so I interpret JABLONOWSKI's view). The latter represents the region subsequently formed by growth and segmentation, this, of course including by far the greater part of the definitive body. He differs from KOPSCH in not regarding the »Knopf« of the teleost as shutting in the neurenteric canal. The latter remains open (ideally) as an »*incisura neurenterica*« until the final closure of the blastopore.

Against these views of JABLONOWSKI I can only restate what I have said in regard to those of KOPSCH, namely that I do not think that there occurs during development any such radical change in the method of growth. In the trochophore, we may doubtless speak of a primary and a secondary region of the embryo, since segmentation and elongation do not commence until after the closure of the blastopore (so far as it does close), but in the teleost, blastopore closure is so slow that the embryonic body undergoes extensive growth, and many metameres are formed, before this occurs. Thus the two phases overlap.

In my former paper dealing with these problems I, myself, argued for the existence of such a critical moment in the development of the teleost embryo, when the »caudal knob« was formed, and I compared it to the moment when the tail-folds of the elasmobranch embryo were united and the neurenteric canal closed in. With this hypothesis was closely associated my explanation of KUPFFER's Vesicle. This I regarded as a precociously dilated post-anal gut, formed in connection with a neurenteric canal, in some cases, and opening through it to the exterior. I have no serious change to make in my interpretation of KUPFFER's Vesicle, though, as I have stated above, (pag. 130) I do not regard the »prostomal thickening« as representing a detached portion of the blastopore. Even the case of the *Muraena* egg, in which KUPFFER's Vesicle is formed by invagination¹⁾, and remains for some time connected with the exterior through an open canal, I should now explain on other grounds. It is to be noted that the lower and hinder walls of the vesicle are, in this egg, incomplete until after blastopore closure²⁾. In most pelagic fish eggs which have been described, the lower wall is at first com-

¹⁾ BOEKE ('02) has confirmed this account.

²⁾ This I admitted in my former paper (p. 74) BOEKE, in his latest work ('03) also describes it thus.

pletely lacking. Later, in some cases at least, the lateral walls draw together and unite on the ventral side, thus forming a cellular floor. The process is thus seen to be not very different from that in *Muraena*. In both cases, this shutting in of KUPFFER's Vesicle on the ventral side is strictly comparable with the closing of the anterior regions of the alimentary canal which occurs later. The passage connecting KUPFFER's Vesicle with the exterior, through the closing blastopore, I still regard as representing a neurenteric canal. The solid condition of the neural axis, as has so often been pointed out, renders the formation of a complete neurenteric canal impossible.

Those who have followed recent work in fish embryology cannot fail to recognize that the Lereboullet-His theory of »concrecence« does not truly describe the formation of the embryonic body¹). Nevertheless it cannot be denied that this theory has proved a very helpful one. The extension of His's law to other groups of vertebrate animals, and the comparison which it has made possible with certain of the invertebrates gave to the theory a strong a priori support. The BALFOUR-SEDGWICK hypothesis of the origin of the bilateral metazoa (BALFOUR '81, Vol. II, p. 317; SEDGEWICK '84; LAMEERE '91, framed a similar hypothesis) was given new life by HERTWIG ('92) who pointed out an actual repetition in ontogeny of the formation of a bilateral from a radial type of organization. I must confess that I still cling to the hope that this hypothesis may, in its general outlines, be saved. It correlates, in a beautiful way, certain developmental processes in widely different groups of animals²), and affords a rational basis of comparison between the coelenterate and the annelid or vertebrate type of structure. Unfortunately for the theory, however, recent embryological evidence does not seem to support it just where it needs support. Most of the later researches upon *Amphioxus*, for example, have not corroborated HATSCHKE's account of the excentric closure of the blastopore in this form (see KEIBEL's admirable review of recent literature of vertebrate embryology in MERKEL und BONNET's *Ergebnisse*, Bd. 10, 1901). Again, DUVAL's account of the formation of the primitive streak in birds,

¹) It is true that EYCLESYMER, in a recent paper ('02), assumes the truth of that theory, as far as teleosts are concerned, but this in no way affects the justice of my assertion. EYCLESYMER has wittingly or unwittingly overlooked the important experimental evidence of recent years, all of which is utterly opposed to his assumption.

²) See JABLONOWSKI ('98) and KOPSCH ('98b).

which harmonized so well with the generalized doctrine of conrescence, appears to be thoroughly discredited by more recent work. In the case of the frog's egg also, although there is still a divergence of opinion, the weight of recent evidence seems to be against the PFLÜGER-ROUX-HERTWIG-MORGAN view that the white hemisphere of the egg is overgrown by the dorsal blastopore lip, an assumption which is necessary if we are to suppose that the embryo is formed by the coalescence of the lateral halves of the blastopore margin, progressing in a backward direction.

I do not regard the facts of teleost development to be inconsistent with the supposition that we have to do with a modified »conrescence« (»confluence«). We have a bilateral closure of the blastopore — this in spite of the latter's remaining circular — accompanied by the backward elongation of the embryo, which occurs partly at the expense of the blastopore lips. Elongation, to be sure, is mainly due to the activity of a growing zone of the embryo, but this is what occurs in the history of any bilateral metazoan after blastopore closure. In the case of the teleost, the two processes occur simultaneously.

VIII. Summary of Conclusions.

A summary of conclusions from my experimental studies has already been given above (p. 114). My other conclusions may be summarized as follows: —

1) Gastrulation, in the fish egg, involves a dislocation of the cellular and non-cellular portions of the blastula. As the former portion invaginates along its margin, the original continuity of the blastula is destroyed.

2) In the eggs of some fishes, at least, two layers arise independently of one another through invagination: 1) a middle layer, formed by the inflection of the margin of the blastoderm (exclusive of the pavement layer) around its entire circumference (»primary hypoblast«, according to most authors); and 2) a lower layer, derived from an independent collection of cells (»prostomal thickening« of my former paper), which first appears as a thickening of the outer pavement layer of the blastoderm, on its posterior margin.

3) From the (at first continuous) middle layer are differentiated chorda and mesoblastic plates. The lower layer (enteroblast) gives rise to the gut-epithelium.

4) As a consequence of its mode of origin, the enteroblast is for a while continuous with the superficial layer of the epiblast at the posterior margin of the blastoderm. This continuity between the outer and the inner layer around the dorsal blastopore lip is quite comparable with the condition to be observed in most vertebrate gastrulae. The ingrowth of the enteroblast is a part of the process of gastrulation.

5) The layer of hypoblast (enteroblast) is at first confined to the embryonic region of the blastoderm, but gradually extends itself, until, just before blastopore closure, three layers are distinguishable throughout the entire germ-ring.

6) The early massing of the primitive hypoblastic cells at the hinder border of the blastoderm is merely a part of the general process of axial concentration which affects the embryonic side of the blastoderm from the first appearance of an embryonic thickening.

7) This process of axial concentration continues uninterruptedly until the final closure of the blastopore. At first it affects the entire embryonic half of the blastoderm. Later it becomes restricted to the marginal region extending on either side of the caudal end of the embryo.

8) The continued massing together of laterally situated material at the hinder end results in a forward push, acting along the axis of the embryo. This causes a relative retardation in the middle line. The undivided region of cells, everywhere present along the margin of the blastoderm, extends further forward along the embryonic axis than it does at points on each side of this. Thus it comes about that in transverse sections we have a region of the embryo in which neural and chordal axes are fused (or rather, not yet differentiated).

9) This region is identical with the »zone of growth« or the »noeud vital« referred to in the experimental part of this work. At its anterior end are continually differentiating neural axis, chorda and somites, and the gut-hypoblast is continually completing itself in the middle line beneath the newly-formed portions. At its posterior end, it is continually receiving new material from the laterally situated portions of the blastoderm margin. Its growth is, however, to a large degree intrinsic and not dependent upon external sources of supply. This region corresponds, in a general way, to the primitive streak of the Amniota.

10) There is at no time an actual »concrecence« in the sense

of a coalescence of the two halves of the germ-ring to form the embryo. Matter originally forming the lips of the blastopore is, however, continually laid down along the axis of the embryo. There is thus a bilateral closure of the blastopore, a process which may be coenogenetic, due merely to the presence of a large amount of yolk; or palingenetic, repeating the primitive mode of origin of the bilateral from the radial (coelenterate) type of organization. Evidence is not at present sufficient to enable us to decide between these two views.

College of the City of New York, May 26, 1903.

Supplementary Note.

After the foregoing work was completed, I received BOEKE's recent admirable piece of work upon the early development of the *Muraenoids* ('03) in which he deals with some of the problems that I considered in my last paper on fish development. It gives me great pleasure to find BOEKE agreeing with me on a number of important points, particularly in regard to the general history of the »prostomal thickening« and the formation of KUPFFER's Vesicle. He has also pointed out certain errors in my work, e. g., in my assertion that in the *Muraena* egg, »at a time when the blastoderm has covered nearly one half the circumference of the yolk, neither prostomal invagination nor gut-hypoblast are to be seen«. (SUMNER, '00, p. 59.) This statement I based upon the examination of sections of two blastoderms, neither of which was cut in a favorable plane. After a more careful study of these, however, I later succeeded in finding what I had previously overlooked. BOEKE attributes the origin of the cells of the »prostomal thickening« to the periblast, though admitting their close relation with the pavement layer of the epiblast, directly after their appearance. Upon this point I must suspend judgment. I have myself seen evidences for this mode of origin in the egg of *Salvelinus* (see p. 122 above), but not in that of *Noturus* or *Schilbeodes*. BOEKE's denial of a »median Lücke« in the hypoblast beneath the chorda (pp. 186, 200) certainly does not apply to all stages of all fish eggs, as I have sufficiently shown above. Again, his contention that the embryo, prior to blastopore closure, grows mainly forward (pp. 141—142) is quite at variance with my experimental evidence. BOEKE, following H. V. WILSON, uses the

oil-drops in the yolk as points of orientation. He rejects EIGENMANN's contention that these do not maintain a constant position. I am inclined to think that EIGENMANN is here in the right. By inverting the egg of *Fundulus heteroclitus* in a compress, I have repeatedly caused the oil-drops to rise through the yolk and assume a position antipodal to their original one, thus completely changing the centre of gravity of the egg. BOEKE's theoretical interpretations of some of the phenomena of Teleost development are extremely interesting, but it is impossible for me to attempt a discussion of them here.

Zusammenfassung.

A. Der experimentellen Ergebnisse (siehe oben pag. 114 IV).

1) Das Vorderende des Fischembryo nimmt nach festgestellter Entscheidung eine mit dem ursprünglichen animalen Pol des Eies übereinstimmende Lage ein. (Versuch 1, 34, 35.)

2) Das Wachstum ist in normalen Eiern ein lediglich caudales, d. h. das Schwanzende des Embryo wandert rückwärts über den Dotter. (Versuch 1, 3, 4, 8, 9, 17, 19 — erste zwei Fälle, 25, 36.)

3) Man kann diese Wachstumsrichtung durch Schaffung eines unbeweglichen Hindernisses am Schwanzende umkehren. (Versuch 6, 18, 19 — dritter Fall, 23 — erster Fall, 26, 28.)

4) Wachstum (Längenwachstum) tritt, von dem ersten deutlichen Auftreten des Embryo bis zum Blastoporuschluss, in einem beschränkten Bezirk kurz vor dem Schwanzende auf. Verletzt man diesen Bezirk, so verlangsamt sich das Wachstum oder hört ganz auf. (Versuch 5, 24, 27, 37, 38, 39; vgl. auch 3 mit 6, 17 mit 18, und 25 mit 26.)

5) Das Kopfbende wächst ebenfalls oder bewegt sich doch vorwärts, wenn auch in geringerer Ausdehnung. (Vgl. Versuche 3 und 6 mit 1, 34 und 35.)

6) Die Ausbreitung der Keimhaut findet in einem beträchtlichen Entwicklungsabschnitt ganz oder nahezu konzentrisch statt, indem die Embryoseite weder schneller noch langsamer als die andere Seite wandert. (Versuch 1, 34.)

7) Einige Zeit vor dem Schlusse des Blastoporus wandert dessen ventrale Lippe (der frühere Vorderrand des Blastoderms) viel schneller als die dorsale Lippe. Dies ist notwendiger Weise so, falls der Embryo einen Winkelbogen von weniger als 180° umfasst, — wenn wir nämlich zugeben, dass das Kopfbende die Stelle des ursprünglichen Keimhautcentrums inne hat.

8) Die Bewegung des Keimhautrandes (Keimringes) ist, wenigstens in beträchtlichem Maße, veranlasst durch die Ausdehnung des inneren, dünneren Keimhautbezirkes. (Versuch 15.)

9) Der Keimring geht normaler Weise kontinuierlich in den Embryo über, und zwar viel schneller, als sich aus einer gleichmäßigen Zusammenziehung während des Blastoporuschlusses erklären lässt. (Versuch 12, 22, 29.)

10) Es ist dies jedoch kein »Concrescenz«-Vorgang im Sinne einer Verwachsung oder Verschmelzung der Blastodermränder. (Versuch 6, 18, 26.)

11) Eine wirkliche Conereszenz des Blastodermrandes (Keimring) hinter dem Embryo kann man manchmal künstlich dadurch zu Stande bringen, dass man das Wachstum des letzteren sistirt, aber der resultirende Streifen unterscheidet sich in seiner Struktur und geringeren Massivität von dem wahren Embryonalbezirk. (Versuch 24 — erster Fall, 38.)

12) Der Keimring liefert somit nur einen verhältnismäßig kleinen Antheil des Materials für den Embryo, welcher sich, wenigstens in einem Theil der Eier, nahezu normal entwickeln kann, wenn auch an einer oder an beiden Seiten die Verbindung des Keimringes unterbrochen wurde. Die Hauptwachstumsquelle ist die Zellvermehrung in der eigentlichen (oben erwähnten) Wachstumszone (§ 4). (Versuch 11 — zweiter Fall, 14, 19 — zweiter Fall, 23, 30, 38.)

13) Die ganze Embryonalregion des frühen Blastoderms — so weit erkennbar — kann mittels Kauterisation zerstört werden, und doch kann noch ein anscheinend normaler »Embryonalschild« wieder entstehen vermittels eines Regenerationsprocesses. (Vgl. Versuch 16.)

14) Ein erheblicher Theil des Centralbezirks der Keimscheibe oder des frühen Blastoderms kann gleichfalls durch Kauterisation zerstört werden und doch ein normaler, wenn auch kleinerer Embryo erscheinen.

B. Der sonstigen Ergebnisse (siehe pag. 143 VIII).

1) Die Gastrulation des Fischeies bedingt eine Verlagerung des cellulären und nichtcellulären Theils der Blastula. Da sich der erstere entlang seinem Rande einstülpt, so wird die ursprüngliche Continuität der Blastula zerstört.

2) In den Eiern wenigstens einiger Fische entstehen zwei Schichten unabhängig von einander durch Einstülpung: 1) eine mittlere Lage, gebildet durch Einbiegung des Blastodermrandes (ausgenommen dessen Deckschicht) rund um seinen ganzen Umfang herum (»primärer Hypoblast« der meisten Autoren); 2) eine tiefere Schicht, abstammend von einer unabhängigen Zellansammlung (»prostomal thickening« meiner ersten Arbeit), welche zuerst als eine Verdickung der äußeren Pflasterzellenlage des Blastoderms an seinem hinteren Rande erscheint.

3) Von der (zunächst kontinuierlichen) mittleren Lage differenziren sich Chorda und Mesoblastsomiten. Das untere Blatt (Enteroblast) dient dem Darmepithel zum Ursprung.

4) In Folge seiner Entstehungsweise steht der Enteroblast eine Zeit lang am hinteren Keimscheibenrande in kontinuierlichem Zusammenhange mit dem oberflächlichen Blatt des Epiblasts. Dieser Zusammenhang des inneren und äußeren Keimblattes um die dorsale Urmundlippe lässt sich durchaus mit dem bei den meisten Vertebratengastrulae beobachteten Verhalten vergleichen. Das Hineinwachsen des Enteroblasts bildet einen Theil des Gastrulationsprocesses.

5) Die Hypoblastschicht (der Enteroblast) beschränkt sich zunächst auf den Embryonalbezirk der Keimhaut, dehnt sich aber allmählich aus, bis man, gerade vor Schluss des Urmunds, im Bereich des ganzen Keimrings drei Schichten unterscheiden kann.

6) Die frühzeitige Zusammenballung der primitiven Hypoblastzellen im hinteren Keimringrande ist lediglich ein Theil des allgemeinen nach der Achse hin gerichteten Konzentrationsprocesses, welcher vom ersten Auftauchen einer Embryonalverdickung an die Embryoseite der Keimhaut ergreift.

7) Dieser axiale Konzentrationsvorgang geht bis zum endgültigen Urmundschluss ununterbrochen vor sich. Zuerst ergreift er die ganze Embryonahälfte

der Keimhaut. Später beschränkt er sich auf einen zu beiden Seiten des Embryoschwanzendes sich erstreckenden Randbezirk.

8) Die fortgesetzte Ansammlung ursprünglich lateral gelegenen Materials am Hinterende hat ein Vorwärtsdrängen entlang der Embryonalachse zur Folge. Dies veranlasst eine gewisse Bewegungsverlangsamung in der Mittellinie. Der ungetheilte Zellenbezirk, überall am Keimhautrande vorhanden, erstreckt sich in der Embryonalachse weiter nach vorn als an irgend einem von ihr seitlich gelegenen Punkte. So kommt es, dass wir auf Querschnitten einen Embryonalbezirk finden, in welchem Neural- und Chordalachse verschmolzen (richtiger: noch nicht differenzirt) sind.

9) Dieser Bezirk ist identisch mit der »Wachstumszone« oder dem »Noeud vital«, auf welchen im experimentellen Theil vorliegender Arbeit Bezug genommen wurde. An seinem Vorderende differenziren sich beständig Medullarplatte, Chorda und Urwirbel, und der Darmhypoblast vervollständigt sich beständig in der Mittellinie unterhalb der neugebildeten Theile. Er empfängt an seinem Hinterende beständig neues Material von den seitlich gelegenen Partien des Keimhautrandes. Sein Wachsthum ist jedoch größtentheils ein innerliches und nicht abhängig von außen gelegenen Hilfsquellen. Dieser Bezirk entspricht im Allgemeinen dem Primitivstreifen der Amnioten.

10) Zu keiner Zeit giebt es hier eine »Concrescenz« im Sinne eines Zusammenwachsens der beiden Keimringhälften zur Embryobildung. Jedoch wird allerdings Bildungsmaterial der Blastoporuslippen fortwährend entlang der Embryonalachse deponirt. Es findet also ein bilateraler Urmundschluss statt, ein Vorgang, der coenogenetisch, durch die Anwesenheit einer großen Dottermenge bedingt sein kann; oder palingenetisch, indem er die primitive Entstehungsweise des Bilateral- aus dem Radial-(Cölenteraten-)Typus wiederholt. Die bekannten Thatsachen lassen zur Zeit noch keine Entscheidung zwischen diesen beiden Auffassungen zu.

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(It is needless to say that no attempt has been made to compile a general bibliography of this subject.)

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