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SOME OBSERVATIONS ON THE EMBRYOLOGY OF THE TELEOSTS.

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VI. Some Observations on the Embryology of the Teleosts.

By J. S. Kingsley and H. W. Conn.

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THE following observations on the development of a marine bony fish were made at the Summer Laboratory of the Boston Society of Natural History at Annisquam, Mass., during the months of June, July and August 1881. Most of the facts here recorded have been witnessed by both authors and in the majority of cases have been repeated many times. The composition of this article is the work of Mr. Kingsley and when the pronoun "we" occurs in the following pages it indicates the fact that both of us are responsible for the statements presented, the phenomena which were witnessed by Mr. Conn alone are indicated by the use of his name, while the "I" which will be frequently met in the course of the article indicates that Mr. Kingsley alone is responsible for the statement or interpretation presented. As we separated soon after the conclusion of the observations herein recorded, it has fallen to the lot of Mr. Kingsley to make the comparisons with the work of other authors; and the whole discussion of previous results, with the exception of a portion of the work of Oellacher, has been done by him. The bibliography which follows has been wholly the compilation of Mr. Kingsley and embraces only those papers which have been consulted during the preparation of this article. Nevertheless it is hoped that it may prove of use to other students of Vertebrate Embryology. I have adopted the method of referring to these various papers which is used by Dr. Mark in his valuable memoir on the Maturation, Impregnation and Segmentation of Limax; viz: the name of the author followed by the date of the article in full faced type. Where two or more articles by the same author were published in the same year they have the additional letters [a], [b], [c], etc.

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[1] Owing to the long delay in the publication of this article I have had an opportunity to add extensively to it, having gone over much of the ground again during the summer of 1882. The additions, however, will all appear as foot-notes each with the date 1882. For the opportunities enjoyed for pursuing my investigations I would return thanks to Professor Hyatt and Mr. Van Vleck for their kindness and the facilities afforded me during the two summers spent at Annisquam.

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The eggs on which we worked were obtained by surface skimming and were usually found in about equal abundance at day and in the evening, and as a rule were rather more abundant when the tide was coming in than when it was running out. One or two evenings however proved an exception to the rule, as once with about half an hour's skimming we found over 700 eggs by actual count. This large number however was exceptional, one hundred or one hundred and fifty being near the average.

The most numerous form of egg and the one on which our observations were principally made was perfectly spherical, about a thirtieth of an inch in diameter, and perfectly transparent. The shell enveloping it (*chorion* of older authors) was extremely thin and only under high powers showing a double contour. A Tolles one-tenth objective revealed no traces of any structure in it, nor were there visible any pores such as exist in the egg shells of many Teleosts.[1] The various preparations of carmine and haematoxylin as well as the anilines very quickly stained the shell, but a prolonged immersion in any staining fluid colored the contents of the egg but very imperfectly and very slightly, thus offering a striking contrast with the eggs of Merlucius which were studied at the same time by Mr. Van Vleck, and which stained easily and well. As our eggs were obtained by skimming and were mature and fertilized, there existed between the shell of the egg and the egg proper a narrow space filled with a transparent fluid in which the yolk floats freely. This space is the breathing chamber of Ransom. The food yolk or deutoplasm comprises the greater portion of the egg over one side of which the formative yolk, or protoplasm, is spread as a thin layer. The deutoplasm is perfectly colorless, free from all oil globules or granules of any sort and of nearly the same refractive index as the salt water in which the eggs were kept, rendering it an operation of some difficulty to pick the eggs out of the water in which they were kept, on account of their being nearly invisible. The protoplasm was in the early stages relatively very small, composing less than a twentieth of the bulk of the egg. Like the deutoplasm it was perfectly free from granules or globules of oil or food-yolk but it was of a very pale straw-color and was more refringent than the deutoplasm. With eggs of such transparence one could easily watch most of the changes going on, even in the interior of the egg, while it was on the stage of the microscope, thus affording in this respect a more favorable object than the egg of the trout which has been the subject of so much investigation in Europe.

Upon the above described egg most of the observations were made which form the basis of this paper. When any other form is used the fact will be stated in the text.

We greatly regret that we are unable to identify the eggs on which we worked,[2] but all attempts to rear the young fish beyond a few hours after hatching proved futile. We tried keeping them at the ordinary temperature of the room, keeping them in an ice chest, and in breeding boxes, allowing a free circulation of water, or placed in the water from which the young were taken, but in vain. Mr VanVleck had no better success with the young of Merlucius.

From the numbers of eggs which we found we supposed that they must belong to some abundant shore fish, and the following observations may aid in approximating the species. The flounder (*Platessa americana*) is, according to a letter to Mr. G. Brown Goode, a win-

[1] Conf. His. '73 pl. II. Aubert '54 pl. VI, fig. 1.

[2] During the summer of 1882 I was able to identify the eggs, which turned out to be, as suspected, those of the Cunner (*Ctenolabrus coeruleus.*)

ter spawning fish. The eggs of the smelt (Osmerus) are over twice the size of those on which we worked. The eggs of Merlucius, Microgadus, Motella, and possibly of all of the Gadidae have one or more conspicuous oil globules in the deutoplasm. The eggs of the Cunner (*Ctenolabrus coeruleus*) taken from the living fish, very closely resemble our specimens in size and appearance, and it seems to us that the probabilities are in favor of our eggs belonging to this species.

On the other hand one would expect to find (if there be any correspondence between closely allied fish and their eggs which, with the possible exception of the Gadidae mentioned above, has not yet been shown[1]) a similarity between our eggs and those of the perch as described and figured by Lereboullet and Ransom. Lereboullet describes the egg of the European perch ('54 p. 241–242) as transparent vesicles containing "globules graiseaux et un grosse goutte huileuse" and also as agglutinated. These features were seen by us neither in the ovarian egg of the Ctenolabrus nor in the ripe, unsegmented egg which we studied. Neither were there present any filiform appendages. Ransom has given a separate description of the egg of the same species, but we saw none of the pores, the rythmic contractions, nor the micropyle[2] which he figures and describes, nor any such tubes as he shows ('67[a] Pl. XVI, fig. 26 etc.). The mode of development, especially in the early stages, is also far different from that of the perch as epitomized by Lereboullet.

The other eggs upon which observations were made were:

I. An egg of similar size and appearance with a large reddish colored oil globule in the deutoplasm.

II. An egg of the same size with several reddish oil globules and a slightly granular protoplasm and deutoplasm.

III. A larger egg, about a twentieth of an inch in diameter with one large oil globule.

III. A large egg, about a fifteenth of an inch in diameter (twice that of our form) and like it in being perfectly transparent and in possessing no oil globule, though both its protoplasm and deutoplasm were slightly granular. In all of these eggs the relative amount of food and germinative yolk were about the same.

These eggs all floated at or near the surface of the water and presented a marked contrast to those of an Elasmobranch, Batrachian, Reptile or Bird (and which I have never happened to see mentioned as a characteristic of fish eggs) in that *the germinative portion is invariably downward, or on the lower surface of the egg while the deutoplasm is uppermost.* This peculiarity renders it very easy by inclining the microscope, to rotate the egg into almost any desired position with one exception: it does not permit us to obtain a surface view of the blastoderm. Nor could this be obtained by confining the egg, as the slightest pressure almost immediately killed it, rupturing the vitelline membrane and thus contracting the blastoderm so that nothing could be made out of it. The bad eggs always sank.

[1] Ryder's papers should be consulted in this connection.

[2] During the summer of 1882 I was more fortunate, having twice seen the micropyle. This is shown in Pl. XIV, figs. 1 and 2. A shallow depression surrounds the micropyle which itself, when seen in optical section, is a funnel-shaped tube, its walls extending in some little distance beyond the rest of the egg membrane. Near the micropyle the membrane is thickened and in surface views is seen to be permeated by very minute tubes (or possibly only surrounded by punctae, though from what is known of other forms, the former view would seem the most probable). I did not see these tubes except very near the micropyle. The micropyle itself more closely approximates that of the Herring as figured by Hoffman ('81, Pl. I, fig. 9) than it does those of the more nearly related Julis and Crenilabrus as figured by the same author on his third plate.

The small size of the eggs and their great contraction in any hardening medium (osmic, chromic, and Kleinenberg's picric acids, bichromate of potash and alcohol) prevented the cutting of any satisfactory sections though many attempts were made. It was also found very difficult to keep the eggs alive for twenty-four hours, and to these two causes must be attributed the fragmentary condition of these notes, of whose shortcomings no one can be more cognizant than the writer. Still in many ways, notably in witnessing the invagination of the hypoblast and the formation of the notochord, the extreme transparence of the eggs rendered our information on these points far more satisfactory than any sections could have done, as every step of the process could be clearly seen.

In the following pages the development of the egg will be taken up by stages, each of which is characterized by some prominent feature or by the appearance of some important organ. From the fact that the several portions of the body are undergoing development at the same time, a perfect chronological arrangement in treating of the subject cannot be maintained, but it is hoped that the general features may be followed in nearly their proper order.

These stages may be briefly indicated and epitomised as follows:

I. The maturation of the ovum. We have been unable to obtain anything on this point in our eggs, but introduce some observations upon the eggs of the "Old England Hake," Merlucius.

II. The phenomena of segmentation until the formation of the germ layers.

III. The formation of the three primary layers, the segmentation cavity, the invagination of the hypoblast, and the appearance of nuclei in the intermediary layer of Van Bambeke.

IV. The formation of the notochord and neural cord. During this stage the invagination is completed.

V. The formation of the optic bulbs and the segmentation of the muscle plates into proto-vertebrae. During this stage the first appearance of what we call "Kupffer's vesicle" and what Balfour regards ('81 p. 61) as the post-anal vesicle, is seen. In the later portion of this stage the splitting of the mesoblast into somatoplure and splanchnoplure begins, while the epiblast in the cephalic region thickens to form the lens of the eye.

VI. In this stage the ears and nasal pits make their appearance and undergo a portion of their development; the lens of the eye is segmented from the epiblast and the first traces of blood vessels were seen; the segmentation of the muscle plates still continues.

VII. The heart and pericardial cavity begin to be differentiated and the former to beat. The blastoderm at this stage completely envelopes the deutoplasm and in subsequent stages will be spoken of as the yolk sac. The gills bud and the gill-arches and gill-arteries appear in the later portion of this stage. In this stage the first contractions and movements of the embryo are seen; simultaneously with the first beating of the heart.

VIII. During this stage the development of the organs previously outlined progresses while the outgrowth of the tail and the formation of the anus are the new features. The fore and hind gut also become prominent and the lumen in the latter is readily seen.

IX. This stage is characterized by the hatching of the embryo and is reached in from forty-eight to fifty-six *hours* after the first segmentation furrows make their appearance, a

slight difference in time being noticeable with a change in the temperature. The yolk sac has rapidly decreased in size.

X. The formation of the mouth, the complete disappearance of the yolk sac and the deposition of pigment in the eye.

Though the processes above outlined and now to be described present many striking differences from those which are found in fresh water fishes, they are essentially similar, not only in all the eggs of the marine forms which we have studied, but they also present many resemblances to those of other marine forms as described by Van Beneden, Haeckel, Kupffer and others, though seeming to indicate that there are two distinct types of teleost development, one for the fresh and the other for the salt water forms. However, we are not yet possessed of sufficient material on which to base any generalizations, since we know less about the development of the Teleosts than of any other Vertebrate type with the exception of some of the lower groups of the old class of fishes.

The description which we give is purposely detailed and may be even prolix, and for these reasons: but few forms of marine fishes have been studied and so detailed observations are necessary to serve as a basis for future comparison; the second is that our paper may be of more aid to American students who as a rule have no such facilities for consulting books as have their co-workers in the old world. Besides the works of Alexander Agassiz, Drs. Brooks, Garlick, Lockwood, Putnam, Ryder, and Wyman, referred to in the bibliography, I do not know of a single paper by an American on the embryology of the Teleosts, while all papers embracing any original investigation on the embryology of the other Vertebrates will not exceed two dozen and of these fully one-half were not at their time of publication any contribution to knowledge. With such a poor showing surely anything which may incite to better work may be pardoned.

I. Maturation of the Ovum.

We were unable to make any observations on the maturation of the eggs which we studied,[1] nor to witness the phenomena connected with the impregnation, but the following

[1] During the summer of 1882 eggs were taken from the living cunner some being fertilized and others not, and from the study of the latter I am able to add a little to the account of the maturation of the egg and to say a word concerning the formation of the polar globule in addition to the statements of the previous year. I would, however, state that it is barely possible that the supposed unfertilized eggs were in reality fertilized, as, for obvious reasons, I cannot say that there were no spermatozoa in the water in which they were kept. These eggs in general appearance have been described at the beginning of the second section of this article, and hence the description need not be repeated here. The features of maturation witnessed were a disappearance of the nucleus and of the strongly refractive globules; then the protoplasm began gathering itself together as shown in the figures 9, 10 and 11. A slight constriction appears around the central portion of the protoplasm which, cutting down and then in, separates the germinal portion of the egg from the yolk giving it eventually the shape of a button. It is to be noted that this operation does not include all of the protoplasmic portion of the egg, a thin layer (exaggerated in the figures) extending down over the yolk and in all probibility giving rise to the intermediary layer. While this segregation of the protoplasm is taking place the aster appears, followed by the formation of the polar globule. This aster appeared, each time it was seen, as a true aster, not an amphiaster, but this may be the result of the position of the egg, for were an amphiaster viewed in the direction of the axis it would present this appearance. At the centre of the aster there appeared the polar globule in a manner almost exactly similar to that so often described in the eggs of invertebrates. Once on rotating the egg the polar globule was actually witnessed in its passage through the micropyle as shown in figures 6 and 7, its connection with the egg being completely severed. At another time I saw a projection which I am inclined to regard as a polar globule attached to one of the resulting cells of the first segmentation (fig. 8, *p. g*), but as the process of formation was not witnessed I am not positive

account of the changes undergone by the eggs of Merlucius may partially fill the gap. The eggs in question were taken from the fish and some were fertilized while others were not. We studied only the latter. Mr. Van Vleck spent his time on the development of the former, and it is sincerely to be hoped that he will soon publish his results in detail as many of them are very interesting and important.

When first seen the proloplasm of the egg of Merlucius was collected at one pole (the lower) and covered about a third of the surface of the deutoplasm as a thin layer. At this time no nucleus was visible, though carefully looked for. Soon there appeared, at about the centre of the germinative disc, the well known aster so familiar to embryologists (fig. 3). After about five minutes changes were noticeable in the aster; its rays grew shorter, were less distinctly defined, and finally the whole disappeared and no trace of the star could be seen. The length of time from the first appearance of this aster until its complete disappearance was thirteen minutes. Five minutes later the aster reappeared, this time at the centre of the outer surface of the germinal disc where during an interval of about ten minutes it presented the same appearance and went through the same changes as before, at last disappearing as before leaving no trace of its former presence. After twenty minutes more had elapsed it was again seen near the edge of the disc and on its outer surface, where after remaining in sight for about five minutes it again faded from sight. Several additional appearances and disappearances were witnessed but with no differences worthy of note and no further records were kept. These asters made themselves visible with comparative suddenness, while their disappearance was more gradual and is best described by the term "fading out". Close watch was kept for the formation of polar globules but without success.

The foregoing account is by Mr. Conn, but what interpretation to place upon the numerous appearances and disappearances of the aster I do not know. In another egg of the same lot I saw the following phenomena:

The appearance of only one aster was noticed and this at the outer surface of the germinal disc and close to its outer margin. It was apparently composed of granules of protoplasm radically arranged. At the same time of its appearance slightly marked amoeboid movements of the whole protoplasmic area were seen and which were the most prominent in the neighborhood of the aster and which seemingly proceeded in a slow wave-like manner toward the aster as a centre from the circumference. This appearance was noticed for about five minutes, and as the time progressed the aster gradually faded from the sight as has been described above by Mr. Conn. After its disappearance two very small globules or granules were seen on the surface of the germinal disc in the exact spot formerly occupied by the aster (fig. 2). There exists in my mind considerable doubt as to whether these granules were the polar globules, arising chiefly from the following reasons: their very minute size, and also from the fact that neither by myself nor by Mr. Conn was there witnessed anything comparable to the amphiaster (Archamphiaster of Whitman) which

of my identification. It may be that the exclusion of the polar globule from the egg is the normal method and this would account for the fact that it has so unfrequently been seen in the fishes. With the evidence of the present year I feel tolerably confident that the polar globule of the text (fig. 5 *p. g.*) was in reality such. Hoffman ('81) gives an essentially similar account of the formation of the polar globules and their extension from the egg and, more fortunate than I, saw all the stages of the operation, which were similar to those described by Fol and others in the maturation of the eggs of the Invertebrata. He however does not appear to have seen the polar globules retained within the egg.

accompanies the formation of the polar globule in the Invertebrates. The star was always single, nothing comparable to the "Kern-platte" was seen,[1] and in my egg the elongate globules, arranged in the direction of their longer axes, marked the exact centre of the aster. Though watched for a considerable time no further changes were witnessed, and as the egg was not fertilized no segmentation could be expected. No other eggs of so early a stage were obtained.

II. Segmentation.

But two eggs were obtained by skimming before segmentation had commenced, the larger portion having the blastoderm well segmented indicating a development of from four to six hours. In one of these eggs which I found (fig. 4) the protoplasm was collected around one pole of the egg, imparting to it a very pale yellowish tinge and gradually fading out so that at about a third of the distance around the egg it was invisible. In this yellowish protoplasm the germinative vescicle or nucleus was visible and in these a single nucleolus. The egg measured .0375 in., the nucleus .0037 in., and the nucleolus .00055 in. Soon several strongly refractive bodies appeared in the nucleus, similar in appearance to, but smaller than the nucleolus. An unfortunate pressure of the cover glass then killed this egg, preventing any further observations upon it.

Mr. Conn however found a single egg of the same species just as the first segmentation furrow was appearing. He describes the egg as essentially similar to the one which I had, the protoplasm extending down over the yolk in a similar manner. The nucleus, however, was not visible. The segmentation furrow made its appearance at the centre of the surface of the protoplasm and gradually progressed outward and downward until the germinal portion of the disc was divided into two blastomeres. During the later stages of this segmentation the line of demarcation between the protoplasm and deutoplasm became more distinct, and at the close of the segmentation the protoplasm is gathered up at one pole of the egg as a thick two-celled cushion, as shown in figs. 12 and 13. These changes occupied about a quarter of an hour.

We made more detailed observations upon the first segmentation of the eggs of Merlucius, but as Mr. Van Vleck is intending to publish on the development of this form we do not here relate our results, simply saying that they fully agreed with the description above. This segmentation we regard as the first segmentation of the egg. That of separation of protoplasm and deutoplasm cannot be considered as segmentation, since it is one of the features of the maturation of the egg and is accomplished before impregnation.

It is to be noticed that in *all* the eggs which we studied all of the segmentation furrows, including the first, pass completely through the germinal area and in all the segmentation of the protoplasm of the egg is complete. Similar results were noticed by Van Beneden in the egg of an unknown Teleost, and by Haeckel in the egg of ? Motella. On the other hand Œllacher, Stricker (*Trutta fario*), Van Bambeke (Leuciscus), and Carl Vogt (Coregonus) describe the segmentation planes as at first passing only part way through the germinal disc.

Returning to our original egg; from the stage with four blastomeres many observations were made and all features of segmentation were verified and reverified by both of us.

[1] See preceding note.

A description of the changes undergone by a single egg will be given in detail while the variations presented by others will be noticed as occasion demands. At the close of the section on segmentation, a tabulated account of a few eggs, all apparently of the same species, will be found, with the periods of time occupied in the various phenomena of segmentation.

This egg with two blastomeres when first placed under the microscope had nuclei in each cell, though, judging from the analogies presented by the later stages as well as by the eggs of Merlucius at the same period of development, the nuclei probably did not reappear until some minutes after the first segmentation furrow was completed. Soon after this the nuclei disappeared, and in six and one half minutes afterward they were no longer visible, the first external features of cell division were noticed. A slight furrow appeared in the surface of each blastomere, their direction being at right angles to the original plane of segmentation (fig. 13). At first these furrows existed only at the junction with the primary one and were also superficial. They then gradually extended outwards and downwards from this place until in thirty seconds from their first appearance they had completely separated each blastomere into two. Two minutes later the nuclei reappeared.

In some eggs, between the completion of the segmentation furrow and the reappearance of the nuclei in the four resulting blastomeres, marked amœboid movements were observed. In other eggs from the same lot these movements were not noticed until the blastoderm had eight blastomeres. These amœboid motions were very marked and similar ones were noticed in connection with each segmentation from the third onward. It is difficult to describe or illustrate these motions. Processes were sent out by the cells, and furrows appeared cutting into the blastomeres, conveying the impression, the first time that the phenomenon was witnessed, that the cells were about to divide again immediately without the reappearance of the nuclei or the intervention of the usual period of rest. On the contrary, with the reappearance of the nuclei, or very soon after, these motions ceased and the blastomeres acquired their regular cellular appearance and an interval of rest intervened before further cell division began.

As was mentioned above, the same amœboid movements were witnessed at each segmentation and in all eggs studied, and, though we are by no means positive, it seemed to us that connected with these movements was an increase in the amount of protoplasm and that particles of the yolk or deutoplasm were taken into the blastoderm. Certain observations which we made, but which are not easy to describe, seemed to admit of this and only this interpretation; and the fact that the blastoderm grows, not only in superficial extent, but also in volume, shows that the amount of protoplasm is in some way increased and thus adds additional weight to the view which we have taken. On the other hand the fact that the cells, after they have reached a quiescent state and have regained their usual smooth contours, exhibited no traces of globules or granules of yolk as would have been expected with bodies of such different refractive indices, would seem to be against this idea. It may be, however, that the deutoplasm undergoes a gradual change, and that portions of it are transformed into the substance of the intermediary layer and that the nourishment of the cells is in turn derived from the intermediary layer. That this inter-

mediary layer contains a large proportion of protoplasm is shown by the free cell formation which subsequently occurs in it, to be described farther on.

It has been impossible to give any adequate representation of these amoeboid movements as they utterly surpass any efforts of the artist, but figs. 15 and 17 may serve to convey some slight idea of the appearances. Both are taken from blastoderms of eight cells. In fig. 17 only two cells are shown, each of which is about to segment while two processes are shown arising from one cell and *uniting* with the protoplasm of its neighbor. In this case the union was not broken until after the segmentation was completed. The other (fig. 13) represents an entire blastoderm of eight cells after the segmentation furrows are complete but before the reappearance of the nuclei.

At the time of the reappearance of the nuclei in the blastoderm of four cells, grooves were noticed extending down from the blastoderm a short distance on the surface of the yolk (fig. 14); but they soon faded out, not lasting over four or five seconds. This phenomenon was noticed at two subsequent stages; once when the blastoderm was composed of sixteen and once when of about sixty blastomeres. It may have occurred at other times but was not looked for; in fact the times when it was seen were the result of accident, it being incidentally noticed while other changes were being watched.

Concerning the internal features of the segmentation we regret that we can say nothing. Many times the nuclei of the blastoderm were carefully watched, but all that can be said is that they gradually faded away from the sight, growing less and less distinct until at last they were invisible and had utterly disappeared before the segmentation of the protoplasm began. After the cells had divided the nuclei again became visible but rather more rapidly than they had disappeared. In this disappearance and reappearance there did not appear to be any change in the size of the nuclei, but rather their optical properties more and more approximated that of the surrounding protoplasm until at last the microscope was unable to differentiate them. At no time while the segmentation fissures were being formed were the nuclei to be seen. In vain we looked for those interesting features connected with cell division which have been described in such detail by Bütschli, Flemming, Klein, Peremeschko, Schleicher, Strasburger and others. Except in the case of the maturation of the egg of Merlucius described above, we have but twice seen anything in the eggs of Teleosts which in any way even approximated an *aster*, *amphiaster* or "*spindel-kern*".[1] Once asters were seen in a large proportion of the cells of a blastoderm composed of about one hundred segments. The other time a single aster was seen among the free yolk nuclei or rather, as I prefer to call them, the free nuclei of the intermediary layer. In both cases circumstances were such as to prevent any detailed observations upon them, while the little which was seen is of no value standing by itself and hence is not described. It is sufficient to say upon this point of internal features of segmentation that in the earlier stages of division nothing comparable to the phenomena I have seen in the eggs of other forms could be distinguished without the aid of reagents, which however do not readily penetrate the envelope of the eggs which are studied. The eggs of Merlucius afford in this respect a striking contrast, as in them Mr. Van Vleck obtained by staining well marked spindel-kerne.

[1] The studies of 1882 require the modification of this statement for I was able several times to see these structures, they being well stained by a solution of carmine in acetic acid though they did not stain with the more common reagents. The appearance of both asters (*b*) and spindelkern or amphiasters (*x*) sre shown in pl. 15, fig. 24*.

Returning to the egg whose segmentation we are discussing we have to note the succeeding features of division and first the changes in going from four to eight cells. In six minutes after the appearance of the nuclei in the four blastomeres they again disappear as before, and fourteen minutes elapsed before any further changes were visible. A depression then appeared at the middle of the inner margin of each cell and gradually extended outward and downward to the outer surface and to the yolk. These furrows were parallel to the first and at right angles to the second segmentation furrows and like them cut completely through the protoplasmic portion of the egg. The whole process of division occupied but a few seconds and the nuclei reappeared two minutes later and the blastoderm of eight cells was before us.

In the egg which formed the basis of this description the segmentation furrows appeared simultaneously in each of the four cells of the blastoderm and proceeded at nearly regular rates in all. Other eggs agreed with this, but still others (and they formed a large minority) exhibited at this early period a heterochronous division, as in proceeding from four to eight blastomeres an intermediate stage was observed in which for a few moments the blastoderm consisted of six segments, two of the cells having divided slightly in advance of their fellows. In one egg which I studied three blastomeres divided some little time before the fourth, the first three dividing simultaneously and the result was a blastoderm of seven cells. This egg presented also another peculiarity in that the nuclei reappeared at nearly the same moment of time in all of the cells and before the fourth had divided, and thus the seventh cell had two nuclei and only after the lapse of a minute and a half did this cell divide.

It was at this time, when the egg has eight segments, that we noticed the first traces of Van Bambeke's intermediary layer (figs. 21, 22, 23*i*). It was a very thin layer of protoplasm extending between the blastoderm and the yolk and at this stage was without the thickened margins and the free nuclei which are present in the later stages. No traces of granulations or oil globules could be seen in it. We would not, however, by the foregoing account, be understood to say that the intermediary layer made its appearance at this time, but merely that we first noticed it then. Of its time and method of origin we can say nothing except that it certainly was not present at the first segmentation of our eggs.[1]

As will be seen further on we do not agree with Van Beneden in regarding this intermediary layer as the hypoblast, but I am inclined to believe that he is correct in his idea that the tail-like processes of the cells in Haeckel's figure of an egg with two cells, in reality represent this intermediary layer. I also agree with the Belgian savant in his opinion that both Kupffer and Lereboullet observed this same intermediary layer, the statement of Kupffer to the contrary notwithstanding. Lereboullet ('54 p. 250) says "Il existe sous le blastoderm une membrane particulière, distincte, composée de grandes cellules trés pâles; c'est d'elle que se formeront les organes abdominaux". This agrees perfectly (except in regard to the last portion, the destination of the layer) with the condition of the layer in many of the fresh water fishes with numerous oil globules the "grandes cellules" being either oil globules or vacuoles. But of this intermediary layer we will speak more at length further on.

[1] I now think that this statement will have to be modified, as I regard the thin portions of protoplasm which are left extending down over the egg at the time of maturation (fig. 11), and which are also shown in fig. 12 extending out from the blastomeres, as the first traces of the intermediary layer, and the same investigations confirmed me in my belief that the layer is largely produced by the change of deutoplasm into protoplasm.

At no time after eight blastomeres were reached did the segmentation proceed regularly, and with each succeeding segmentation the irregularity became more and more marked until at last, at about that stage when the blastoderm should theoretically consist of sixty-four cells, every trace of regularity is lost and each cell divides entirely independently of its neighbors, the nuclei appearing in one just as they are disappearing in another, while a third is at the same instant dividing. In some eggs this irregularity is noticed at an earlier stage and is much more marked than in others, but in all it soon reaches such an extent that in any case it is difficult to ascertain when the theoretical segmentation is completed.

We have now to describe the division of the blastoderm from eight to sixteen cells. Nine minutes after the last mentioned reappearance of the nuclei, they again disappeared almost simultaneously, but one or two seconds intervening between the times of the first and of the last. After this disappearance and *before* the division of the cells amœboid movements, similar to those which have been described were witnessed. The cells lost their regular outlines and their smooth contours and became lobulated and furrowed. Fig. 17 before referred to represents two cells from the blastoderm. While this amœboid motion was in progress the segmentation furrows appeared. Fig. 18 will illustrate this division better than it can be described, the two interior cells divided first, then the other two, and lastly the four corner ones. The fissures in the second began before those in the first were completed and those in three before the segmentation of the second was accomplished. The complete segmentation occupied about two minutes. The planes of division were in general times at right angles with those of the preceeding segmentation and the result was a parallelogram with four cells on a side. The nuclei were again seen four minutes after the segmentation was complete and remained in sight for ten minutes. The amoeboid movements after this segmentation were very strongly marked and lasted for considerable time, and the cells did not attain their smooth contours until about the time when the nuclei vanished. The regularity of the parallelogram was far from being constant as frequently one or more cells would segment obliquely and the result would be more like that shown in fig. 19.

From this point onward the segmentation in every egg studied by us was very irregular, and by various stages of 20, 21, 28, 29, and 30 cells the theoretical 32-celled blastoderm is obtained and a short period of rest (which was not timed but which could not have exceeded three minutes) intervened after which the segmentation proceeded but so irregularly as to be beyond description. Figs. 20 and 21 will illustrate some of the later stages.

In the following table are given the results of timed observations of the segmentation of several eggs. Several others were studied but without noting the intervals between the stages. The dash (—) indicates that stage of development when the eggs were first noted and the figures the number of minutes since the last timed stage.

	1	2	3	4	5	6	7	8	9
Nuclei disappear after first segmentation (2 cells)	7			—	—		—		—
Second segmentation (4 cells)	6½			7	7½		10		8
Nuclei reappear	2						3		3
Nuclei disappear	6	—	—		8		6	—	7
Third segmentation (8 cells)	14	13	15	10	11	—	14	4	12
Nuclei reappear	2	5	6				½	4	2
Nuclei disappear	9	5	5		10	10	11	10	8
Fourth segmentation (16 cells)	9	10	11	7			7	12	8
Nuclei reappear	2	5	4	2	9	11	4	5½	3

After this stage the segmentation is so irregular that it cannot be timed.

From the above table, which is based on eggs of apparently the same species,[1] it will be seen that there a considerable variation in the times which were required for the same changes in different eggs but nevertheless in many, well marked periods of rest alternating with stages of activity, may be noticed. These periods of rest and activity have recently been commented upon by Dr. W. K. Brooks ('81) and have also been noticed by many of the older embryologists in the eggs of other vertebrates and also in those of many invertebrata. I am of the opinion that these periods of (apparent) rest are thus to be explained, that at each one of them the deutoplasm, which I believe to have been taken up by the germinal area, is connected with protoplasm, and that while there is an interval of physical rest, the same time is one of chemical activity. There are several reasons for this belief, but before stating them I wish to obtain further evidence and make additional observations not only on the eggs of fishes but also on those of other animals.

The phenomena of segmentation in the eggs of Teleosts have been several times described, and the accounts which we have presented to us agree in the main with what has been given above, though there are several points of more or less importance in which differences are to be noted. The first fact which we would discuss is that the planes of segmentation even at first pass through the germinal area, cutting it completely. This is in strong contrast with the observations of most writers and so far as we are aware occurs in the eggs of all marine teleosts. Every form which we studied presented this peculiarity and the description and figures of Haeckel[1] and Van Beneden of the development of European marine teleosts. (It might here be remarked that the figures of Haeckel are highly idealistic and show many features which certainly do not exist in nature). On the other hand all writers describing the segmentation of the eggs of fresh water fishes agree in that the first cleavage planes pass but partly through the germinative disc, there remaining a portion next the deutoplasm (vitelline globe) which does not segment until much later. These facts are in strict accordance with the ideas of Balfour that eggs undergo total or partial segmentation according to the relative proportions of protoplasm and deutoplasm. In the eggs of fresh-water fishes besides the vitelline globe there is a large amount of deutoplasmic material scattered through the germinal area; in the eggs of Merlucius there is a very slight amount in the same region, while in the eggs which we studied the protoplasm and deutoplasm appeared to be entirely distinct.

According to Vogt ('42 p. 30) the segmentation furrows do not entirely cut through the germinal area of the eggs of Coregonus until a stage with eight blastomeres is reached. To Oellacher we must refer for the most detailed account of the segmentation of the eggs of fresh water fishes which has yet been published. His observations on the segmentation of the eggs of *Trutta fario* (p. 395 et seq. pl. xxxiii figs. 18-20) agree essentially with those of Vogt but from their later date are much more valuable. He lays especial stress upon the fact that the cleavage furrows do not pass at first completely through the germinal portion, and in the later figures (l. c. figs. 22-26) he shows a layer of unsegmented protoplasm underlying the central cells of the blastoderm and continuous with the margin-

[1] These eggs as far as the microscope would show were identical, but there is a bare possibility that No. 4 which was slightly larger than the rest belonged to a different species. This would explain the great difference in time in certain changes which here are very much accellerated.

al ones. At a later stage this lower layer of protoplasm becomes segmented. Aside from this difference, which is to be explained by the presence of quantities of yolk granules, the external features of segmentation present no important differences from our results.

The eggs of Merlucius although containing large numbers of deutoplastic globules in the germinal portion undergo a complete segmentation of the protoplasm as in that of the Cunner. There was to be noticed, however a very marked irregularity in the process of segmentation even from the very first. The blastomeres varied widely in size and the segmentation furrows progressed at varying rates and times in different portions of the germinal area.

Though we found it impossible to obtain any satisfactory sections of our eggs, their perfect transparency enabled us to see, clearly and plainly, that even the first segmentation furrow extended down to the vitelline globe and that the first two cells as well as the subsequent ones were entirely separated from each other. At no time was there an unsegmented basal portion of protoplasm except that presented by the intermediary layer and peripheral cushion of Van Bambeke.

Kupffer mentions some marked irregularities in the eggs which he studied: in some the second segmentation furrow sometimes was eccentric or occasionally was even parallel to the first. We have seen nothing of this sort in the eggs which we studied.

The theoretical segmentation of an egg is first two meridional furrows and then an equatorial one, but frequently this regularity is interrupted, in fishes noticeably so. If we interpret Haeckel aright, the equatorial furrow is the fourth to appear in the eggs of ?Motella as he figures (fig. 58) a section of a blastoderm of sixteen cells and in it lower layer cells are seen. These figures however show in every line that they are wholly diagrammatic and could not have been drawn from either actual or optical sections. In the eggs of the fresh water fishes it is at a somewhat later stage that the equatorial furrow is formed and the lower layer cells produced, but even in eggs of the same species there does not appear to be much regularity. In our eggs lower layer cells did not appear until the blastoderm was composed of about a hundred blastomeres and even then they did not appear simultaneously in all parts.

III. Formation of the Germinal Layers.

In this section we have to consider the extension of the blastoderm over the yolk from the time of the appearance of the lower layer cells until the formation of the notochord and the neural canal. In it also will be discussed the differentiation of epiblast, mesoblast and hypoblast, and also the phenomena of invagination. Certain of the features here to be described belong in part to the next section, but from the fact that they are first noticed before the formation of the notochord they are best treated here.

Until after the blastoderm has acquired a stage with about a hundred cells it consists, as before mentioned, of but a single layer, thus offering a marked contrast from the eggs of most fishes on which observations have been published. This simple condition of the blastoderm at this time was conclusively shown by optical sections in which the outlines of each cell could be readily traced with a power of a hundred and fifty diameters.

Soon after this number was reached, lower layer cells were noticed. We did not conclusively settle the manner in which they arose but are inclined to believe that the greater portion arose from the already formed cell elements of the blastoderm while possibly a small proportion had their origin in the free nuclei of the yolk. With their formation the epiblast becomes differentiated and at first consists of a single layer of cells. These cells in vertical optical section are lens shaped while the lower layer cells are polygonal in outline on account of their mutual pressure.

At first the blastoderm fits as a cap over the yolk but soon by the proliferation of cells it acquires a lenticular shape and is seated in a concavity in the surface of the deutoplasmic portion of the egg. At this time the intermediary layer (Parablast of Klein not of His) is plainly seen and its thickened margins (bourrelet périphérique of Van Bambeke) is very conspicuous. Regarding the origin of this layer we have nothing new to offer. Its first appearance was not noticed either as to the exact stage or as to the method in which it arose.[1] Neither was the time of its disappearance observed; it was visible until the blastoderm nearly covered the yolk. When first seen the layer was clear and transparent without any traces of granules, vacuoles, nuclei or cells, though at a later stage they were visible. The first observer who noticed this intermediary layer was Lereboullet who saw it both in the pike ('54 p. 248) and perch (l. c., p. 250). He describes it in the latter as follows: "Il existe sous le blastoderme une membrane particulière, distincte, composée de grandes cellules très pâles; c'est d'elle que se formeront les organes abdominaux"; and in speaking of the pike he says that the vitelline globules are changed to this layer. Almost all subsequent observers have seen this same layer and have added to our knowledge of it. Our discussion of their results will be taken up in connection with that of the germ layers with which it is intimately connected.

At about the time of the differentiation of the lower layer cells as well as at later stages free nuclei were seen on the *surface* of the yolk. These nuclei were irregularly arranged, in fact no traces of any regularity could be discerned except that all were on the surface and now were to be seen on the interior of the yolk. (The term surface here embraces not only that portion which is in contact with the egg membranes but also that on which the intermediary layer rests). In the eggs of Merlucius at a slightly older stage similar nuclei were seen and around many of them, especially those nearest the blastoderm, the cell walls could be made out, the whole presenting an appearance somewhat similar to that given by Kupffer ('68 p. 217 pl. XVI) in the eggs of Gasterosteus. These free nuclei and cells are not arranged with anything like the regularity of Kupffer's figures. In the Cunner egg I watched the process of cell formation around these nuclei with some care. The nuclei nearest the germinal portion of the egg were the first to become the centres of cells and the formation of the cell boundaries took place in a corresponding direction, that is those portions of each cell wall nearest the blastoderm appeared first and these gradually extended themselves around the nuclei. The whole operation required over half an hour. In the Cunner but comparatively few of these free nuclei and resulting cells were seen.[2]

[1] The observations of 1882 elsewhere detailed alter this statement slightly.

[2] In 1882 by staining, these free nuclei with all their accompanying phenomena were seen and studied, fig. 24*, pl. XV, representing a portion of the blastoderm and the adjacent portion of the peripheral cushion; in the latter there being shown asters, amphiasters, and the process of outlining of the cells.

As time passes the blastoderm gradually extends itself over the surface of the yolk until, at a later stage than that described in this section, it completely embraces it, thus forming the yolk sac. When about one-fourth of the surface of the yolk was thus covered, the segmentation cavity was first noticed. No observations were made regarding its mode of origin but it was doubtless by a lifting up of the blastoderm. In the earliest stages its roof was formed by the epiblast alone, its walls of lower layer cells while its floor was formed by the yolk, or rather by the intermediary layer which rests upon the yolk. The floor at this time was perfectly free from nuclei or cells. At first the segmentation cavity is low, circular in outline, with its lateral margins about equidistant from the edge of the blastoderm. The blastoderm continues its extension over the yolk and increases rather more rapidly in one position of the margin than on the others, and at the same time the lower cells encroach upon the segmentation cavity from one side until the cavity becomes eccentric and is placed nearer one portion of the blastodermic margin than to the others. This pushing in of lower layer cells continues until the cavity acquires an arcuate or reniform outline. At this time free cells are numerous upon the floor of the cavity.

This is the first opportunity we have for the orientation of the egg. The segmentation cavity is farthest from the portion of the blastodermic margin where the first outlines of the germ are to appear and so we may now speak of anterior and posterior portions of the blastoderm, the segmentation cavity is anterior and the embryonic area posterior.

The invagination of the hypoblast now begins. As before stated we were unable to cut actual sections but the extreme transparence of the eggs rendered this almost a superfluity. Our observations on the invagination were made both by surface views and by optical sections, the latter being in almost every respect equal to actual ones while from the fact that the steady progress of the invagination could be continuously watched in the living egg they presented advantages which no product of the section knife could equal.

At all points of the margin of the blastoderm a single layer of cells may be seen pushing themselves inward beneath the rest of the blastoderm and separated from the lower layer cells by a well defined line. Between this hypoblast and the yolk is still to be found the intermediary layer. The invagination progresses much more rapidly from the posterior or embryonic portion of the blastoderm than from any other portion of its margin, and at the anterior portion more rapidly than at the sides. Fig. 22, pl. XIV, represents an optical section on the median line of an egg in which invagination has just begun, while fig. 23 represents the same at a somewhat later stage. In this last figure the extent of the lateral invagination is shown by the shaded area. Fig. 24 gives a view of the lower surface of the same blastoderm showing the rates at which the invagination progresses in different parts. (The dotted line indicates the plane on which the section described is taken.) The invagination continues until it forms a layer entirely separating the rest of the blastoderm from the yolk and intermediary layer. Its later stages and the phenomena accompanying it belong more properly to the next section.

Our attention was not especially directed toward the origin of the mesoblast but we are of the opinion that it arises partly from the lower layer cells and partly from the hypoblast. Whether it arises as two lateral plates, we know not, but at an early stage it forms a continuous layer extending across the embryonic area as shown in fig. 25. With

the progress of the invagination the segmentation cavity is encroached upon by the lower layer cells, its floor becomes covered with cells, some arising from the hypoblast while others apparently originate from the free yolk nuclei, and the cavity is shortly obliterated.[1]

I admit that I am in doubt as to the part played by the intermediary layer and its resulting cells. As before mentioned a portion of the cells apparently enter into the floor of the segmentation cavity and are subsequently either embraced in the hypoblast of invagination or are crowded by it into a mesoblastic position. This however accounts for but a small proportion of the cells of the intermediary layer, and it seems to me probable that the hypoblast of invagination forms only the dorsal wall of the alimentary tract while the intermediary layer furnishes the ventral portion. This seems to be in full accord with the formation of the alimentary tract in other forms (e. g. Batrachia) where the ventral portion of the hypoblast is formed by yolk cells.

Regarding the origin of the hypoblast in the Teleosts there seems to be a diversity of opinion. Henneguy ('80 p. 402-3) describes the invagination in the eggs of the perch and trout, the blastoderm being inflected at its margin and a line or fissure separating the sensorial [our lower layer cells] from the inflected portion. So far we agree with him. He however states that the epidermal layer is not inflected. In this he agrees with two of the figures of His ('75 pl. II, figs. 2 and 3) but not with fig. 1. Our observations were that the epidermal layer of the epiblast alone is inflected. Balfour ('81, 57) says that the yolk cells form the hypoblast in the smaller Teleost eggs but that in the larger as in those of Elasmobranchs only a portion of the hypoblast has such an origin. We should consider our eggs as small. Kupffer with a doubt regards the cells as forming the hypoblast. Professor Van Beneden in his researches on the eggs of an unknown Teleost ('78) arrives at widely different conclusions regarding the origin of the germ layers from those we have formed and we cannot reconcile his results with our observations. We have seen step by step, minute by minute, the progress of the invagination and it scarcely seems possible that any error of observation on this point can have crept in, especially as we witnessed the process many times. Yet Van Beneden totally denies that in his Teleost any invagination takes place. It would seem to me that he is wrong from the very start. On p. 52, he considers the egg before the appearance of (our) two segmentation spheres as follows: "Directly after fecundation the egg of the osseous fish divides into two very unequal cells, very dissimilar, differing in constitution and significance; the one is a germ which segments and from which the blastodisc is derived; the other is formed by the deutoplasmic globe * * *. This cell is the origin of the endodermic layer of the future embryo." To all of this I must express an emphatic dissent. The aggregation of the protoplasm at one pole of the egg and of the deutoplasm at the other cannot in any way be considered as a segmentation, nor can the deutoplasmic portion be considered as a cell. No one would think of regarding a centrolecithal egg, that of a Crustacean for example, as composed of two cells or the central portion as of a cellular character, yet the homology between the two eggs is easily shown. On the same page he explictly says that the germinal portion is the homologue of the ectoderm and the vitelline of the entoderm, a view

[1] Subsequent studies lead me to believe that this statement is an error and seem to confirm the idea of Ryder with regard to this cavity, though I must say that there appear many difficulties in connection therewith.

which is not warranted without considerable qualification. It must be understood that I am not criticising his observations on the free cell formation in the intermediary layer and in the yolk, nor do I deny that a portion of the hypoblast may arise from those portions. This free cell formation we have both witnesed in the eggs of several marine Teleosts and we are willing to accept his account of their formation but I do deny that the presence of these cells and nuclei can be addressed as evidence that the deutoplasmic globe itself can be considered as a cell and the complete homologue of the hypoblast. On p. 56 he considers that the hypoblast described by Haeckel, and which closely resembles in its structure and mode of origin that of our fishes, was in reality "composed of cells derived from the intermediary layer." It hardly seems possible that there should be in the eggs of teleosts such diverse methods of origin of the hypoblast and that in closely allied forms. Professor Van Beneden following Haeckel regards his egg as probably belonging to one of the Gadidae, and with this opinion we are inclined to agree. The eggs of Merlucius and of Morrhua present a striking resemblance to those studied by Haeckel and Van Beneden, and if there be any relation between the characters of the eggs and of the fishes producing them (a point on which we have but slight data) the eggs studied by both probably belonged to the Gadidae. Now as we have observed in an egg with a conspicuous oil globule, and as has been traced through with great care by our friend Mr. Van Vleck in the egg of Merlucius, the hypoblast arises exactly as we have described it above.

It would thus appear that Van Beneden has been led into an error either of observation on his own eggs, or of interpretation of the results of Haeckel, regarding this point and we are inclined to believe that the former is the case, for the reason that it appears from internal evidence presented by the article in question that he did not witness continuously the phenomena presented by his eggs. Still there remain certain statements which we cannot reconcile with what we believe to be the facts of the case. For instance the statement on p. 56. "The blastodisc remains all this time very sharply delimited inferiorly and in no part is there a passage from one to the other (from the upper portion of the blastoderm to the hypoblast). In no part have I found the slightest indication in favor of invagination."

In the earliest stages of the egg the intermediary layer is not present but it soon appears and acquires its maximum development about the time of invagination. It appears to arise by an elaboration of the food yolk into protoplasm. It consists in our eggs as in those of Van Bambeke and Van Beneden of a thin layer extending across the egg between the blastoderm and the food yolk, and having a thickened marginal welt. This welt extends down some distance over the yolk and it may be possible that the nuclei of the yolk mentioned on p. 199 belong in reality to this extension of this layer. Klein ('72) describes this as a ring in the trout as he failed to find the portion extending across between the blastoderm and yolk.

Most observers have considered the mesoblast as arising as a continuous sheet in the Teleosts but Calberla claims that it is in two halves as in the Elasmobranchs.

We found no traces of the segmentation cavity of Van Bambeke and judging from the irregularity of his figures we are inclined with others to regard it as a product of reagents or the section knife. The segmentation cavity of Von Baer which was found in our eggs is clearly homologous with that of other forms of animals.

IV. Notochord and Neural Cord.

The origin of the notochord in the Vertebrata has recently been the subject of some discussion, and though we cut no sections we endeavored to make such observations as would throw some light upon its source and the methods of its formation. All of our observations were made upon the living egg, and as we have in a single egg watched continuously every step in the process, and have several times verified all of our results, we feel confident of their accuracy as far as our fish is concerned. To sum up our studies of this point on which we both agree, *the notochord arises from the hypoblast, at first as a longitudinal median thickening of that layer and subsequently becomes segmented off and takes its place among the mesoblastic tissues.*

A detailed account of the evidence on which we base this statement will now be given. First an account will be presented of the changes witnessed in a view of the lower surface of the blastoderm and afterward a description of the phenomena observed in optical sections.

Fig. 24 represents the under surface of the blastoderm at the earliest stage at which the notochord was seen in a flat view. The segmentation cavity (*s*) posseses the arcuate outline before described while the shaded portion of the figure indicates the extent to which the invagination has extended. The embryonic area (*ea*) has encroached but slightly upon the cavity. Another sketch shows the same embryonic area on a larger scale.[1] In the median line is seen the notochord extending not quite half way from the margin of the blastoderm to the anterior extremity of the embryonic area. Anteriorly the notochord is well marked and clearly differentiated from the surrounding tissues, while posteriorly this distinctness fades out until at last no line can be drawn separating the chord from the adjacent hypoblast. Anteriorly the cells of the notochord have the same polygonal outline as have those of the hypoblast, but they are much smaller, indicating that rapid cell division is taking place. As we proceed in our examination, toward the hinder end of the notochord, we find the cells gradually increasing in size and approximating those of the lower germ layer in magnitude until at last no difference can be observed between them. Sometimes a sharp line may be seen cutting across the extreme end of the notochord and slightly in advance of the margin of the blastoderm and separated from it by a narrow strip of hypoblast, as frequently however this arcuate line was absent, but whether present or not we were never able to trace the notochord quite to the edge of the blastoderm. The margins of the chord at this stage were straight anteriorly but at the hinder end they diverged giving the whole a somewhat spatulate outline. This shape, resembling somewhat the appearance of a paddle, the blade behind, was retained until a comparatively late stage in the development.

At this stage, though the notochord was in a great portion of its length clearly and distinctly outlined, not a trace of cells could be seen extending across the ventral surface of the chord as would have been the case had it been of mesoblastic origin, for then the hypoblast would have extended over the lower surface.

As the embryo increases in size the notochord becomes longer, apparently growing in both directions but much more rapidly posteriorly, keeping pace with the extension of the

[1] In drawing the plate this figure was inadvertently omitted. It is hoped that the description is sufficiently clear to be intelligible without it.

blastoderm over the yolk. At the same time other changes may be noticed, in full accordance with the idea that the notochord arises from the hypoblast, though they are by no means the sole proof which we have to offer, as will be seen farther on. Near the anterior end of the chord, the cells of the hypoblast may be seen extending themselves across (on the under surface of) the notochord until these hypoblastic processes from either side meet and close in the chord. The formation of this bridge occupies but a few minutes and is first completed near the anterior portion, from which it progresses at the same time in both directions, reminding one of the closing up of the medullary groove in other vertebrates. It has been impossible to obtain any satisfactory sketch of this process as seen in a superficial view. The feature of the egg which has already been mentioned, the greater specific gravity of the germinal portion, readily permitted us to trace these various changes on the under surface of the blastoderm.

Soon the cord is anteriorly entirely cut off from the hypoblast and closed in ; this process progresses more slowly posteriorly until finally the notochord is wholly separated from its parent layer and is entirely surrounded by mesoblastic tissues, but at what stage the separation is complete our notes and observations do not show. It is however before the formation of the optic lobes and protovertebrae. For a time the cells of the hypoblast can be distinguished by careful focussing extending across the notochord but soon they become so small that it is impossible to recognize them as such with the highest powers which it is possible to use in such investigations carried on upon the living egg.

This cutting off and closing in of the notochord has been several times witnessed by both of us and it seems as if there were but little chance for errors of observation, but while this observation from the surface would show that the hypoblast plays a part in the formation of the notochord it does not conclusively prove that it is the whole source of the notochordal cells. So far as these already described observations go all three of the germ layers may play a part in its formation and there might be some truth in Rudwaner's statement ('76 p. 161) that the notochord arises from the epiblast. We have however other evidence which proves to us conclusively that the hypoblast alone gives rise to the notochord.

Returning to the invagination described above ; its later stages and its connection with the formation of the chorda dorsalis may now be described. These various steps have been constantly and consecutively watched by us both in several eggs, not only of the Cunner but in several other forms, as well as by Mr. Van Vleck in the eggs of Merlucius and there exists in the minds of us three not the slightest doubt of their general accuracy.

In optical sections it was seen that the invaginated hypoblast was but a single layer of cells in thickness and at all times was separated from the overlying mesoblast by a well defined line. An optical section, transverse to the longitudinal axis of the embryo, was closely watched and at first the hypoblast was but a single layer of cells deep and everywhere of uniform thickness. Soon a thickening was seen in the median line of the hypoblast, extending slightly into the mesoblast and also into the underlying yolk or intermediary layer. This thickening *was clearly a part of the hypoblast and the mesoblast was in no way concerned in its composition*, the line of demarcation between the two layers being as well defined as before. Gradually a sharp line appeared cutting the

thickening away from the hypoblast (fig. 25) and succeeding this the hypoblast was seen to extend itself across beneath the now formed notochord (figs. 26—28 in just the manner which we were led to expect from our observations made from surface views.

It is always difficult to make out cell limits in optical sections of eggs as small as those on which we worked and hence they are omitted in our drawings, but it was seen that in this notochordal swelling of the hypoblast that the cells were smaller than the adjacent portion of the layer just as was seen in the surface views.

We would repeat and lay especial stress upon the fact that we witnessed, by constant and repeated observations on living specimens, every step in the formation of the notochord, from a hypoblast of but one cell in thickness until the chord was segmented off from its parent layer and eventually entirely enveloped by the mesoblastic tissues, and at no time did we witness the slightest appearance that could be regarded as evidence that any portion of the notochord was other than hypoblastic in its origin. The later history of the notochord will be treated under the respective sections of the development of the fish.

Previous to Balfour's first paper (Quarterly Journal of Micr. Science, 1874) all writers on vertebrate development had regarded the notochord as belonging not only in position but in its method of origin to the mesoblastic tissues. Dr. Balfour there and also in his complete monograph ('76[b]) showed that in the Elasmobranchs, at least, the notochord is a hypoblastic structure in its origin. Following him, Hensen, in a paper on the development of the rabbit, gives the same general account of the derivation of the chorda dorsalis with only such variations as might be expected in two diverse classes of Vertebrata. Calberla ('77) studying Petromyzon, Sygnathus and Rana arrives at similar results and was the first to show that in the Teleosts the notochord is an endodermal structure. Rudwaner ('76) in a short paper also treats of the origin of the chorda dorsalis, but his article is of little value and his figures are evidently diagrammatic and do *not* represent the true state of affairs. He derives the notochord (p. 161) from the outer germ layer or epiblast. More recently Braun, in his paper on the development of Parrots in the Arbeiten a. d. zool. zoot. Inst. zu Würzburg (the exact reference to which I have not at hand), still considers the notochord as of mesoblastic origin. All of these observers have worked with and studied sections and hence the discrepancy in their results. On the other hand our observations were made on the living embryo, and hence we *saw* the organ formed and did not have to call upon the imagination to fill up any gaps and also the sources of error in interpretation were eliminated.

In the development of the neural canal the Teleosts present a marked contrast according to all observers with the other vertebrates in that it is first formed as a solid cord in which a lumen afterwards appears.

At about the same time that the notochord was first seen the first appearances of the medullary folds were witnessed. Coincident with the invagination of the hypoblast the edge of the blastoderm increases in thickness and this thickening is most marked at the posterior margin in the median line forming what Balfour has called the tail swelling. When first noticed it presented much the appearance of the "stage A" of Dr. Balfour's Elasmobranch ('76[b] pl. VI, fig. A). This soon became elongate and more and more prominent until a broad shallow longitudinal furrow finally made its appearance dividing it into two lateral halves. These halves are the medullary folds and are low rounded ridges. With the

increase in size of the blastoderm and the growth of the now outlined embryo, the folds anteriorly came closer and closer together and the medullary groove narrower and proportionally deeper. It then grew more and more shallow, its decrease in width also continuing until at last the groove was entirely obliterated. This groove is shown in fig. 28.

As before mentioned it was very difficult to obtain surface views of the blastoderm and hence our surface observations on the closing in of the medullary folds have not that detail which we could wish. Were an egg so held that the blastoderm was uppermost, be the pressure never so slight, it almost immediately died and then contracted so that no consecutive studies could be made. Enough, however, was seen to show that there was none of that infolding and direct formation of a neural canal which is so familiar in the other vertebrates. It was not seen whether the modified closing up took place near the middle and extended both ways or whether from the anterior end backward. From what we know of other forms the former would seem the more probable.

Fig. 28 shows an optical section through the hinder part of an embryo of the stage shown in fig. 34. Here the medullary groove is shown broad and shallow, the notochord has been separated from the hypoblast but has not attained its later quadrate section but still retains the flattened outline; the muscle plates on either side have not yet been differentiated from the mesoblast, while the hypoblast extends across immediately beneath the notochord and is not separated from it by any intervening mesoblast. In this optical section it was not possible to make out clearly the cell boundaries although the limits of the various germinal layers were readily made out as figured.

As to the method in which the neural canal forms, whether in the normal way by an actual enclosing of a tube of epiblast cells, or as maintained by Calberla by a lumen forming in the epiblast which is pushed down and not infolded, our observations will not allow us to decide, though I am inclined to believe that the latter is the method, and for this reason. The earliest optical sections of the neural cord do not show any traces of a medullary canal while at a later stage such a canal is found. The same is also true of the brain and the optic lobes. It will readily be seen that, if either of these methods be the true one, the canal so formed is perfectly homologous with the same structure in other vertebrates and hence the actual manner of its formation has not so much importance. It is however interesting to observe that there is the same formation of medullary folds in our fish as are found in other vertebrates, that they arise the same and only differ in the details of the formation of the canal. As was said above these medullary folds exist, but at no time did we see any closer approach to the formation of a closed tube by a longitudinal union of the summits of the neural folds than that shown in fig. 28. Still I am inclined to believe that a large hiatus in our observations may exist here. In a dorsal view of the tail of an embryo with about twenty protovertebrae a well-marked median line was observed which at the posterior extremity slightly broadens out into a groove, just as would be the case did the neural canal form as in other vertebrates.

Not having witnessed the formation of the neural canal of course nothing definite was seen of a neurenteric canal of the same character as exists in the Elasmobranchs, Batrachia and birds. In figure 30 which represents the first formation of the neural folds a slight notch is seen at the posterior margin of the blastoderm which afterward became much more marked. This notch arises in the same way and to my mind is

homologous with the canal connecting the neural and alimentary canals which has recently been demonstrated to exist in all vertebrates. An unfortunate accident occurred to the egg forming the subject of this figure and hence no consecutive observations were made upon it. No other eggs in such favorable conditions were found. In fig. 30 I have shown the medullary folds extending slightly farther over the deutoplasm than does the rest of the blastoderm, and between them the medullary groove, the epiblast of which of course forms the lining of the neural canal. Continuous with the epiblast which passed down into the notch between the medullary folds is the hypoblast which extends up the under surface of the blastoderm. Whether this notch eventually closes up leaving a tube connecting the neural and alimentary canals I do not know, but it seems impossible to escape the conclusion that it well represents the neurenteric canal of other forms although the enteric canal of the fish does not at this time have the closed condition which obtains in other forms.

According to various observers, before the formation of the neural canal the epiblast separates itself into two layers, an outer or epidermal and an inner or nervous layer, the latter being confined more closely to the embryonic area. This differentiation we were not looking for and hence no allusion to it appears in the foregoing account of the formation of the neural cords; whether it exists or not we cannot positively say though it probably does and was overlooked in our studies. According to those observers who make this distinction, the nervous layer forms the bulk of the neural canal and Calberla ('77) claims that a thin layer of epidermal cells penetrates into the nervous layer and eventually forms the epithelial lining of the neural canal, but Götte ('78) denies this and Balfour studying Lepidosteus and the teleosts ('81) has not been able to confirm Calberla's observations. It would however seem probable that Calberla is right though farther observations are necessary to settle the point.

Schapringer ('71 p. 555) does not afford any information on this point, for he merely states that the medullary canal does not form in bony fishes, as in Birds, Batrachia and Mammals by a folding in of the medullary folds but through a process of separation on the inside.

According to our observations the method of origin of the neural ridges has an almost exact parallel in that of the Elasmobranch as given by Balfour ('78) and also closely resembles Klein's observations on the trout '62 pl. 17, fig. 2. The figures of His, ('75) in text, and which are copied by Balfour, '81 fig. 33, are greatly different from anything which we have seen as will be noticed on an inspection of our plates.

Optic Bulbs and Protovertebrae.

Shortly before the stages shown in figs. 29 and 32 the fore-, mid- and hind-brains are differentiated and almost immediately the optic lobes begin to be segmented off. At this time both the brain and the rudimentary lobes appear to be solid bodies without any internal cavity nor does there appear to be any trace of the neural canal in the spinal cord. A fissure appears on either side of the brain cutting off a portion which forms the optic lobe. This fissure begins above and behind and gradually extends forwards and downwards until at last but a slight connection is left, the rudimentary optic nerve (fig. 36). The fissures progress until at last the nerve going to the right eye is connected only with the left side of the brain while the optic nerve of the left eye arises from the right

side of the same central organ, the condition which obtains in the adult fish. During the differentiations thus described other changes take place, and before the stage represented in fig. 42 is reached the lumen in the brain, optic bulbs, and neural cord appears as shown in figs. 36 and 45.

Synchronous with the differentiation of the regions of the brain, the mesoblast adjoining the notochord (fig. 32) becomes separated from the rest of the layer as the muscle plates, and with the separation of the optic bulbs the muscle plates are divided into protovertebrae. The first of these protovertebrae to be formed is at about the middle of the body, and the fissures which limit it arise simultaneously. They begin close to the notochord above and progress outward and downward and at the same time slightly backward. From this central one the formation of the protovertebrae extends gradually in both directions. At the same time the formation of pigment cells is seen. These arise as black dots and gradually increase and change their shape until at last they assume forms like those shown in fig. 51.

Regarding the peculiar structure which from its first describer we may call Kupffer's vesicle I have but little to say. It first makes its appearance just before the stage shown in fig. 34 on the under surface of the posterior end of the embryo and rapidly increases in size until it acquires a diameter nearly equal to that of the fish. Of its subsequent fate our notes afford no information. Its first appearance is indicated by one or two small globules which soon are joined by others until an appearance like that of fig. 52 is seen. Very soon these globules unite and in two hours the vesicle has the appearance and relative proportion of fig. 53. Fig. 54 shows about the limit of its development and is reached in about five hours from the first appearance of the minute globules.

As mentioned above, this vesicle was first noticed by Kupffer ('68) and by him regarded as a rudimentary allantois. Balfour ('81) regards it as homologous with the terminal vesicle of the post-anal gut of Elasmobranchs. On the other hand Henneguy ('80) studying the perch thinks he saw an opening or traces of invagination of the vesicle and would homologize it with the primitive intestine of the Cyclostomi and Batrachia and its opening with the anus of Rusconi, a view which it seems to me is entirely unwarranted by the previous growth of the embryo and by the method of origin and position of the vesicle as we have seen it. The view of Balfour seems much more probable.

The epiblast over the optic lobe begins to thicken to form the lens of the eye when sixteen protovertebrae are outlined. This thickening increases until soon it acquires the character shown in fig. 49 and almost immediately the thickening begins to be segmented off and to make its way into a depression in the optic lobes and to acquire more and more of a spherical character as shown in figs. 55-57. The manner of the involution of the lens and the features connected with it have so often been described that it is unnecessary to repeat them here.

At about this time the mesoblast begins to split into somatopleure and splanchnopleure. This splitting begins at the head end of the embryo and progresses regularly in every direction. This is a true splitting and not as suggested by Mr. Ryder in a letter to the author an apparent one. He interprets his observations as follows: the portion of the mesoblast which extends down over the yolk is that portion which eventually forms the splanchnopleure and that the somatopleure gradually extends down between this layer and

the epiblast, and he also ('81) regards the space into which the somatopleure thus forces itself as the remains of the segmentation cavity. So far as our eggs and our observations go there is not the slightest evidence in favor of either view, for the epiblast is everywhere in close connection with the mesoblast and nothing could be seen in the envelopes of the yolk which could in any way be interpreted as remains or derivations of the segmentation cavity, a cavity of which all traces are lost at an early stage in the development. In our eggs also there was a veritable splitting of the mesoblast as will be seen by an inspection of fig. 36.[1]

From this point on my observations are exceedingly fragmentary and the account would best be confined to little more than the remaining figures, a course of procedure which will serve to connect the early stages with those of which Mr. Agassiz treats.

In a side view, at first but two of the prominences of the brain are seen (fig. 33) but soon the third, the mid-brain, makes itself visible as shown in fig. 34, and at the latter stage we first find the traces of the pericardium and the heart (*p.* fig. 34). Of the origin of these portions of the anatomy I can say but little. It seems almost impossible to correlate the steps of their development with those found in other vertebrates. At first there appears a mass of mesoblastic tissue arising almost beneath the hind brain and projecting into what has previously been regarded as the cavity produced by the splitting of the mesoblast (and therefore corresponding to the pleuroperitoneal cavity of other vertebrates) but which is regarded by Ryder as the segmentation cavity. (We shall return to this cavity further on). This mass of cells grows downward and when it comes in contact with the lower layer of cells (either hypoblastic or sphlanchnopleuric) a lumen appears, the primitive heart. At first this is a simple tube and indeed for a considerable time retains that character as shown in figs. 40 and 50. When the heart first begins to pulsate the vibrations are very slow and frequently it ceases beating for some time and then begins again. Coincident with the first pulsations, which appear at a little later stage than that represented in fig. 43, the first motions of the embryo are seen, and consist of slight tremors of the whole body. At first the contractions of the heart produce no currents of blood nor in fact are any corpuscles to be seen. In the eggs of the cunner it is extremely difficult to trace the development of the circulatory system after this time, only slight and unsatisfactory views of portions being visible, while only once was I able to see anything whatever of the blood vessels of the yolk sac. Of the formation of the corpuscles nothing was seen nor were they visible until a comparatively late stage.

We have seen in preceding pages the method of formation of the hypoblast of the alimentary tract by an invagination to which are possibly added cells from the intermediary layer. A portion of the hypoblast eventually forms a solid cord extending along beneath the body but of the exact method we are not certain. This cord gradually grows forward and at intervals a lumen appears, as shown in fig. 33 *me.*, the future cavity of the canal; this is shown again at a later stage in figure 42, which represents the hinder end of the mesenteron of a fish about as far developed as shown in fig. 43. Though the exact process of the closure of

[1] The investigations of 1882 which embraced these points lead me to regard Mr. Ryder's conclusions more favorably, and though I am not ready to accept them wholly without some modification, the changes required are far less than those implied in the above paragraph which is based upon some erroneous interpretations.

the blastoderm was not seen, the writer feels confident that the posterior end of the alimentary tract arises in the normal way by an invagination of the epiblast to form the proctodeum (fig. 41) and that the division between the two portion breaks down, the result being as shown in figures 44 and 53. Ryder seems to regard this proctodeum as the neurenteric canal. The steps between these two figures were all seen, but the formation of the anterior end of the canal was not witnessed. There are many points of the alimentary tract upon which additional information is needed: the manner of the formation of the mouth, the connection, if any exists, between the yolk sac and the stomach or intestine, and the relations of what is here called the hypoblast of the yolk sac to that of the digestive portion. There are many points in connection with this latter which are absolutely unintelligible to me no matter how looked upon. In fig. 33 this layer is represented as extending beneath the embryo and nowhere united with the alimentary canal and together with another layer starting off on its course around the yolk. Now if this be the hypoblast from which the digestive tract arises, some connection would be expected between them though none has been found.

The fins which are first seen in an egg about as far advanced as fig. 51, arise in the cunner as a simple outgrowth and not as a continuous lateral fold, as is found in many forms. The first skeletal elements appear as a small body at the base of the fin parallel to the body axis and it is not until considerable later that radial portions appear. This basal skeleton instead of appearing as a pair of rods as described by Ryder was rather a broad plate with a central opening, as if his rods had united at their extremities. The same feature was seen in Lophius.

The remaining features of the development so far as I have clearly made them out can be seen from the plates, and my knowledge is too deficient to say more concerning them than will appear in the explanation of the figures. In about two days from impregnation, the fish hatches with a large yolk sac, which in four days more has almost entirely disappeared as shown in fig. 53. The time of hatching I cannot exactly state, in fact it varies considerably with the temperature. Eggs which were impregnated Friday morning at ten o'clock were found hatched Sunday at eleven A. M. (How much before that hour the actual hatching took place I cannot say). The act of hatching was often witnessed. The membrane of the egg, yielding to some violent struggles of the embryo bursts open and the young fish emerges usually head first. At the time of hatching the young fish is about a tenth of an inch in length and very slender in proportion.

Explanation of Plates.

a. anus.
al. alimentary canal.
ao. aorta.
aud. auditory vesicle.
b. blastoderm (in fig. 24* an aster.)
bc. ductus Cuvierii.
ch. notocord.
ea. embryonic area.
f. fin.
f. b. fore-brain.
f. v. fin vein.
g. gills or gill arches.
h. or *hy.* hypoblast.
ht. heart.
h. b. hind-brain.
i. intermediary layer (in fig. 40 jugular vein.)
j. jugular vein.
l. lens.
l. t. thickening of epiblast for lens of the eye.
lv. liver.
mb. mid-brain.
me. mesoblast (mesenteron in figs. 33 and 41.
n. p. nasal pits.
n. g. neural groove.
o. eye.
op. optic vesicle.
o. g. oil globule.
p. pericardium.
pc. cardinal vein.
p. g. polar globule.
pr. proctodeum.
p. t. protovertebrae.
s. segmentation cavity.
so. somatopleure.
sp. splanchnopleure.
su. v. superior vertebra.
s. v. sinus venosus.
t. s. tail swelling.
u. Kupffer's vesicle.
u. g. urogenital apparatus.
y. yolk.
y. s. yolk sac.

PLATE XIV.

Fig. 1. Micropyle of egg of Ctenolabrus in section.
Fig. 2. Surface view of same with surrounding pore canals.
Fig. 3. Merlucius the germinal disc above showing the archamphiaster of maturation.
Fig. 4. Early egg of Ctenolabrus before maturation and impregnation.
Fig. 5. Portion of germinal area of egg of Merlucius with probable polar globules.
Fig. 6. Formation of polar globule and expulsion of same through the micropyle.
Fig. 7. The same a few seconds later; in these two figures the details of micropyle are omitted.
Fig. 8. A polar globule attached to one cell of a blastoderm of two segments.
Fig. 9-10-11. Three successive steps in the formation of the biscuit-like germinal area, shown in section, the shaded portion extending out from that region forming a portion of the intermediary layer.
Fig. 12. Result of first segmentation, the "tails" on either side the rudimentary "bourrelet périphérique."
Fig. 13. Preparations for second segmentation.
Fig. 14. Resulting four segments with the temporary furrows of the yolk.
Fig. 15. Diagram of amoeboid motions in blastoderm of eight cells.
Fig. 16. Blastoderm of eight cells showing a common irregularity.
Fig. 17. Amoeboid appearance of two cells.
Fig. 18. Preparation for dividing from eight to sixteen cells, showing irregularity in time of division.
Fig. 19. Blastoderm of 16 cells.
Fig. 20. Blastoderm of 24 cells.
Fig. 21. Optical section through the edge of a blastoderm in a late stage of segmentation.
Fig. 22. Beginning, and fig. 23, later stage of invagination of hypoblast. The upper shaded portion representing the epiblast and lower layer cells; *s*, the segmentation cavity, and the dark narrow line just beneath, the intermediary layer; the broad shaded portion shows the outline of the lateral invagination. The black line beneath *h*, the hypoblast, and *e. a.*, embryonic area is possibly the segmentation cavity of Ryder.
Fig. 24. Blastoderm of fig. 23 from above, showing the outline of the segmentation cavity, the line across shows the plane of the preceding section.

PLATE XV.

Fig. 24*. Cells from the margin of blastoderm; and beneath, free cell formation in peripheral cushion; amphiaster *x*, and an aster *b*, are shown and also a stage in the formation of cell walls.

Figs. 25, 26 27. Three stages in formation of notochord shown in (optical) section.

Fig. 28. Same at later stage.

Fig. 29. Side view of embryo, etc., showing the blastoderm with (faintly) its thickened margin.

Fig. 30. Oblique view of tail end of fig. 29, showing the neural groove, the tail swelling and the thickened margin of the blastoderm, the slight notch in the margin at *t s* may possibly represent the neurenteric canal.

Fig. 32. Formation of optic lobes and protovertebrae; the apparent distortion of the figure is due to an attempt to represent the embryo in perspective.

Fig. 33. Stage a little later than 32 showing optic lobe and mesenteron and a few of the anterior protovertebrae.

Fig. 34. Still later with fore-, mid- and hind-brain differentiated and pericardial region forming.

Fig. 35. Splitting of mesoblast into somatopleure and splanchnopleure.

Fig. 36. Dorsal view of head a little later than fig. 34 with formation of the lens of the eye and the appearance of the lumen in the brain and optic vesicles.

Figs. 37, 38, 39. Successive changes in appearance of notochord.

PLATE XVI.

Fig. 40. Heart and blood vessels in stage nearly corresponding with fig. 51, or shortly before hatching.

Fig. 41. Relations of notochord, aorta, urogenital canal, mesenteron and proctodeum in hatched fish.

Fig. 42. Lumen in mesenteron in embryo about like fig. 43.

Fig. 43. Embryo with the lens of the eye, auditory vesicle and nasal pits well advanced. The blastoderm has closed and the tail has begun to grow out. Kupffer's vesicle was present but is omitted in the drawing.

Fig. 44. Anal region in hatched fish.

Fig. 45. Details of head in fig. 43.

Figs. 46 and 47. Out-growth of liver in its earliest stage from the side and from beneath.

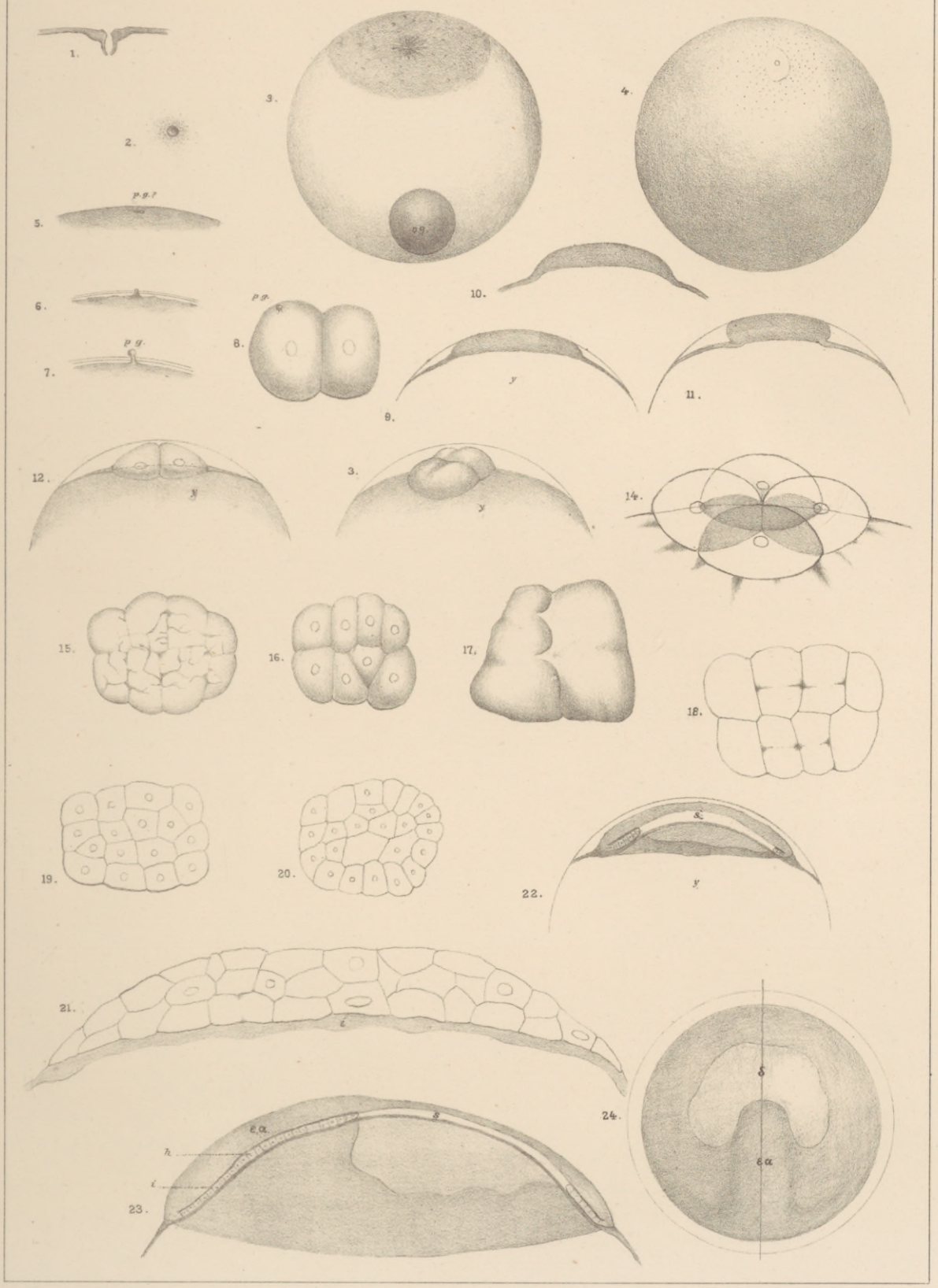
Fig. 48. Anterior portion of fish two days old from beneath, showing anterior end of urogenital canal. The stomodeum and mesenteron have not yet met. H. W. Conn, del.

Figs. 49 and 50. Fish of about four days. H. W. Conn, del.

Fig. 51. Embryo of unknown teleost just before hatching.

Fig. 52 and 54. Two stages in formation of Kupffer's vesicle, *u*, seen in optical section.

Fig. 53. Fish between four and five days old.



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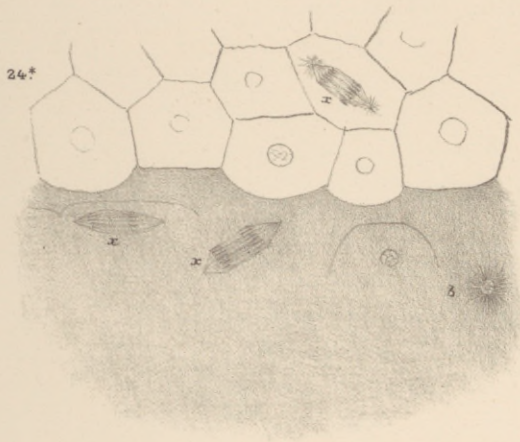

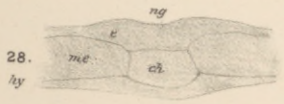

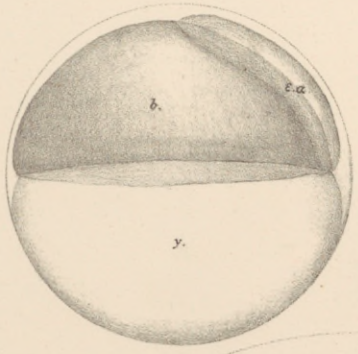

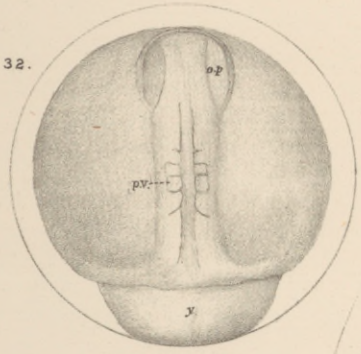

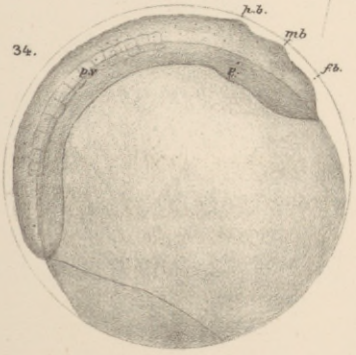

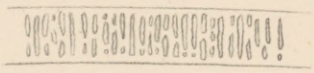

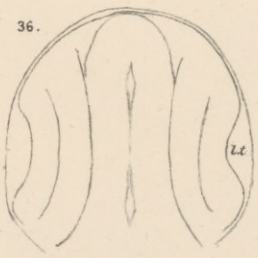

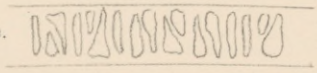

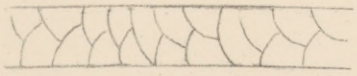

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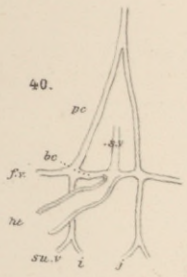

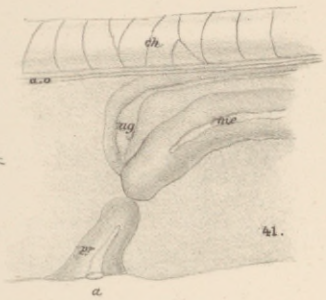

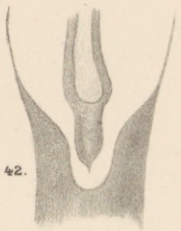

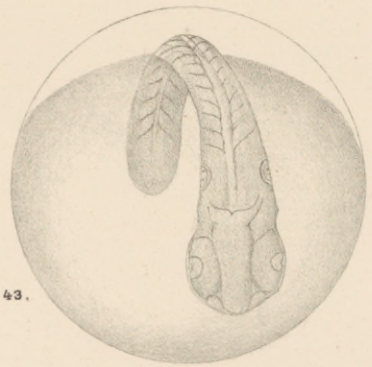

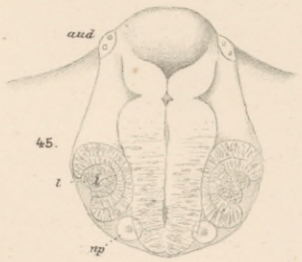

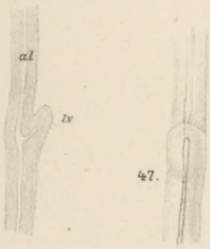

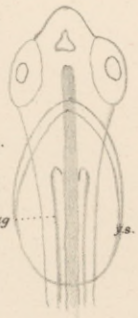

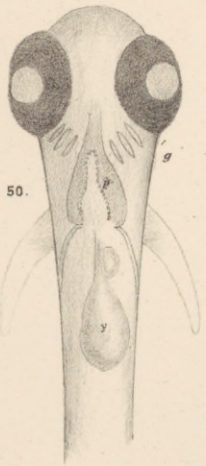

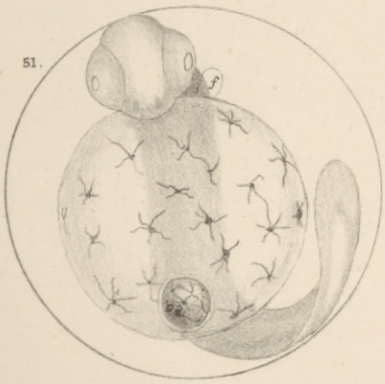

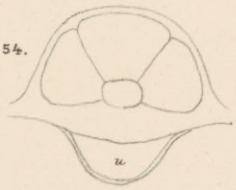

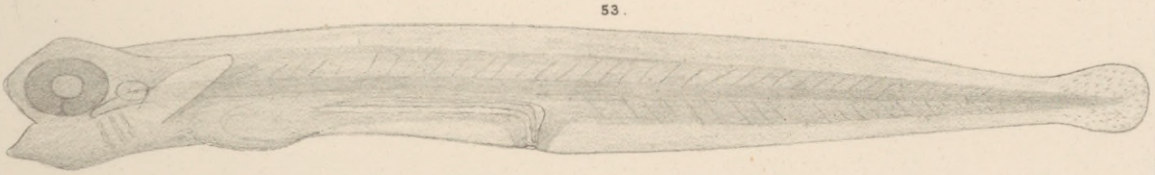

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