JOURNAL OF THE COLLEGE OF SCIENCE, IMPERIAL UNIVERSITY,
TŌKYŌ, JAPAN.
VOL. XXI., ARTICLE 11.

On the Gastrulation in Petromyzon.

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With Plates I.–III.

In a former paper,[1] I dealt with the gastrulation of the ovum in Petromyzon, but owing chiefly to the want, at that time, of the living material at my hand, my sketch was necessarily incomplete. Since that time, I have had several opportunities to renew the observation; especially during the last spring, an unusually abundant supply of the material enabled me to carry on my work on this subject more satisfactorily than it had ever been possible. Unlike Tokyo, my former residence, there is in Sapporo a number of bodies of water in which the animal lives; it runs up in crowds rivers and brooks in and about the city to spawn in the sandy beds of these streams and also of springs. This circumstance induced me to try the artificial fertilization of ovum repeatedly, and I am glad to say the experiment was always attended with success. The material obtained in this way was sufficient for following the steps of changes undergone by the

1) On the Formation of the Germinal Layers in Petromyzon. Jour. Coll. Sc., Imp. Univ., Tokyo, Japan. Vol. V., Pt. I., 1891.

ova during gastrulation; and these steps are, so far as it seems to me, very peculiar but none the less interesting. I will describe, in the following pages, the chief points obtained.

Before passing to the explanation of the processes, it should specially be mentioned here that the ova in the earlier stages, from the blastula to the gastrula inclusive, are very liable to suffer artificial injuries caused by the effects of the killing reagents employed. At any rate, some unequal shrinkage is, in the stages in question, almost unavoidable: the animal hemisphere which is not only formed of a thin layer, but contains the hollow segmentation cavity, contracts necessarily more severely than the solid vegetative hemisphere, thereby causing not a little alteration in the shape of the ova. It is, therefore, advisable that the living material should be employed, at least, for the study of the superficial changes; and it follows that great care should also be taken in examination of sections for the preparation of which hardened ova are of course necessary. I have endeavoured to show such shrinkage in some of the figures given (*Figs.* 17 and 21), in which the ovum itself had considerably contracted causing strong wrinkles in the non-contractile chorion. It is probably from the same cause that hardened ova often deviate in configuration from the living.

For the sake of simplicity, I will start with what I have come to consider as the old morula stage given in *Fig.* 1. The ovum at this stage presents the shape of a sphere, the lower third of which shows a solid opaque appearance and represents the so-called vegetative or yolk hemisphere; while the upper larger part, the animal hemisphere, is more or less translucent containing the hollow segmentation cavity within. By stages, the ovum not only increases in bulk, but assumes the outline of a tall ellipse

(*Fig.* 2), the long axis of which passes through from pole to pole. The superficial area of the translucent hemisphere has been greatly added to since the last stage, while the opaque part has still further diminished in extent; it now occupies the lower fifth of the ovum, as will be seen from *Fig.* 2. To simplify description, the translucent hemisphere may be called the upper, and the opaque the lower, hemisphere.

This peculiar stage has been, so far as I am aware, entirely overlooked by all the previous authors. Max Schultze is the only author[1] who figured correctly (*Fig.* 15, Taf. I.) the ovum of the stage in question, but he seems to have paid no special attention to this peculiar shape of the ovum and gives no account of it. The same omission was committed by the present author; *Fig.* 1, *Pl. I*, given by him in the above cited paper shows an oblique section through a little further advanced ovum in which the gastrula invagination is about to take place.

Now at a certain point in the line of junction[2] of the translucent hemisphere with the opaque, a shallow depression transverse to the long axis appears (*Figs.* 3*a* and 3*b*, *bg.*); this gradually extends itself to the right and left along the junction line, and at the same time it deepens, so that the depression, or as it may now be called the groove, is deepest in the part that appeared first and is shallowed out towards both its extremities. The deepest part in the middle represents, as further history will show, the dorsal side of the forthcoming creature.

The groove in question is not difficult to make out; the chorion which in the foregoing stages had been closely applied

1) Max Schultze: Die Entwickelungsgeschichte von Petromyzon Planeri. Haarlem, 1856.

2) This line will be called, in the following pages, the junction-line, denoting simply the boundary between the translucent and opaque parts of the ovum.

to the surface of the ovum, now stands apart at some distance from the latter. Seen from the lateral side, the ovum appears deeply constricted in the direction of the groove. For the sake of convenience this groove will be called the boundary groove from its position.

One might suppose that the groove just referred to represents an early trace of the blastopore, as was probably so assumed by Max Schultze.[1] The present author came previously to the same erroneous assumption. The blastpore, however, appears later, as will become clear from the following description.

At a time when the arms of the groove nearly reach the opposite point of the ovum, a second depression (*Figs.* 5*a* and 5*b*, *bp.*) appears in the same meridional zone as, and a little below, the first one; *this constitutes the first rudiment of the blastopore.* The blastoporic depression is likewise transverse in position (*Figs.* 5*a*, 5*b*, 6*b*, and 6*c*, *bp.*); it is, however, not hollowed out evenly on the bottom in the shape of a pulley's groove, as in the case of the boundary groove (*Fig.* 3*a*, *bg.*), but is pushed forwards, so that in section the bottom presents an acute angle in the deepest part of the depression; thus producing dorsally a steep ridge overhanging the depression itself, while ventrally it is shallowed and loses itself on the surface of the opaque hemisphere. The whole of this crescentic groove appears like a nail-mark left on a soft body such as dough.

In the above statements I have set aside an important point which is, however, full of significance. Shortly after the appearance of the boundary groove, it is seen that the rounded base of the ovum, on which this rests in the position given in *Figs.* 1, 2, &c., shows a more or less flattening of the surface near the

1) *loc. cit.* p. 13.

grooved part marked with ⁂ (*Figs.* 4*a* and 4*b*), so that the ovum appears as if it were truncated at this part, as may be well seen in a lateral view. The field that lies between the groove and the flattened area of the base is gradually produced into an eminence which is soon converted into a cone with rounded tip (*Fig.* 4*a*, *c. em.*). The cone is directed not merely outwards, but also forwards, in extreme cases overhanging the groove in front like a beak, while laterally it slopes downwards to the right and left like a pair of wings (*Fig.* 4*b*).

Corresponding to the changes which have just been mentioned, the view from the lower pole of the ovum necessarily undergoes notable alterations. It is no longer circular in outline, but is pear-shaped being gradually narrowed towards the protruded (dorsal) part and coming to a point in that part as if it had been compressed from side to side, while the remaining (ventral) part is still rounded as before. The ventral rounded part still retains a spherical form, while the compressed part is, as just noticed, flattened. This flattened part of the basal surface remains, however, not long in this condition, but is gradually transformed into a slit-like depression (*Figs.* 5*a* and 5*b*, *bp.*); it is this depression which was spoken above as an early trace of the blastopore. When the latter becomes obvious, the conical eminence grows for a time still more prominent (*Fig.* 5*a*), but subsequently it becomes depressed assuming a flattened out appearance (*Fig.* 6*a*).

According to my view, this series of occurrences is nothing else than the beginning of the gastrula invagination. As the lower opaque hemisphere begins to invaginate within the upper hemisphere, *i. e.*, to push on toward the opposite pole, it at first flattens instead of remaining convex as heretofore. The pushing inward is, however, hindered by the mass lying within, and the inward pressure is thus converted into one directed against the part lying in front of and dorsal to it, the part thus pressed showing itself as the conical eminence (*Fig.* 5*a*, *c. em.*). When finally, the resistance to the inward movement is overcome, the flattened area moves further inwards towards the segmentation cavity as a continuation of the process of invagination and thus the blastoporic depression is finally established. When this is done, the conical eminence is at liberty to subside, becoming lower and flattened out.

It is further evident that the boundary groove is a product of the same process, though passive in origin: along the junction-line between the translucent and opaque halves, the thin wall of the translucent hemisphere is connected with the upper edge of the opaque half; and when the conical eminence is protruded, it is only the opaque part which takes part in this protruding. Thus, as the part below the junction-line protrudes while the part above it is kept distended by its liquid contents, and as the junction-line remains in its original position, there is necessarily produced a constriction along this line between the two parts; and this constriction is nothing but the boundary groove. Accordingly, the demarkation of the groove against the opaque part, which is at first faint, becomes sharp and distinct with growing elevation of the eminence. I have observed that at that time the groove appears to be brought nearer to the base (*Figs.* 4*a* and 5*a*, *bg.*) than before,—a phenomenon denoting the progressing invagination of the opaque hemisphere. The ventral view of the ovum remains as yet unaltered.

The appearance and subsequent disappearance of the conical eminence were doubtless observed by Max Schultze[1],

1) *loc. cit.*, p. 7.

Calberla[1] and A. Goette.[2] Goette regarded the eminence as the equivalent of the "Randwulst" of the Teleostean ovum and of the "Randzone" of the Amphibian ovum, while it seems not to have been understood by the two other authors at any rate. To a discussion on this point, I will return further on.

Let us now turn to follow the further fate of the two depressions above described, by which way the superficial changes of the gastrulation can be best learned.

In the first place, the history of the blastoporic depression will be dealt with. The limbs of the dorsal lip which overhangs the depression extend themselves further and ventrad along a level somewhat below the boundary groove above referred to, describing a large arc (*Figs.* 7*a* and 7*c*, *bp.*). The part of the ridge thus added likewise overhangs the depression at every part, but it becomes gradually lower towards both its distal extremities, finally to become lost on the general surface of the ovum.

This addition of the blastoporic lip in its extent is, however, brought about not by lateral prolongation of the blastoporic depression in its slit-like form, as might be supposed, but is produced by the opaque hemisphere generally sinking down in its proximal largest part. To do this, a small field of the hemisphere which lies immediately behind (ventral to) the slit is at first flattened (*Fig.* 6*c*, *bp.*); then, while the flattening is further extended into the ventral part, the flattened area is gradually changed into a large shallow depression which is deeper towards the median and the steepest part of the dorsal lip (*Fig.* 7*c*, *bp*). In other words, the slit-like blastopore is now converted into the

1) E. Calberla: Zur Entwickelung des Medullarrohrs und der Chorda dorsalis der Teleostier und Petromyzonten. Morphol. Jahrb., Bd. 3, 1877.

2) A. Goette: Entwickelungsgeschichte des Flussneunauges (Petromyzon fluviatilis). Hamburg und Leipzig. 1890.

the form of a widely gaping funnel, the dorsal part of which is wanting. The funnel formed would be entire if the opaque field were pressed down in the direction of the long axis of the ovum (cf. *Fig.* 17); but instead of that it is pushed dorsad and forwards, that is, in an oblique direction making an angle of some 60° with the axis (*Fig.* 17, *bp.*), so that a half-funnel is brought about. This sinking down of the opaque half is nothing but a part of an extended invagination of the lower hemisphere of the ovum.

The arc of the blastoporic lip attains its maximum extent in the stage when it describes about a semicircle (*Fig.* 7*c*). At this time the blastoporic funnel is represented by a large hollow excavation; the hind part of the opaque hemisphere which is sickle-shaped in outline still shows a convex surface. From this stage on, both the limbs of the arc are brought nearer to each other; *i. e.*, the angle contained in it grows less (*Fig.* 8*c*, *bp.*). And at the median deepest point of the funnel the blastoporic passage is converted into a circular pore. The convergence of the arc-limbs, however, goes on only until they assume the shape given in *Fig.* 8*c*, and stops long before the rest of the opaque hemisphere has all been changed into a concave surface; and this shape of the blastopore is kept up until the entire withdrawal of the opaque part within the ovum. When this is nearly accomplished the blastopore shows a feature similar to that in earlier stages and is reduced into a small oval depression still wanting the ventral lip (*Fig.* 10*a*). The depression is then gradually converted into a circular pore (*Fig.* 11*b*, *bp.*) which constitutes the definitive blastopore.

This transformation of the oval depression into the circular pore is, as is very well seen in *Fig.* 11, brought about by the upheaval of the hitherto non-existant ventral margin of the blastopore. The ridge thus formed is nothing else than the ventrally prolonged arms of the arched lips which had heretofore bounded the pore on the dorsal and lateral sides: the limbs have now become united at the median point of the ventral junction-line. In this way, the blastopore is for the first time surrounded all around by a lip. It should, however, be specially mentioned that the lip is in the ventral part very slight in elevation so that the general surface of the ovum still passes over into the floor of the invaginated pocket at a wide angle.

Now the gastrulation is completed; no trace of the opaque area can be detected on the external surface of the ovum. The ovum is much reduced in bulk, so that it is in size about two-thirds of that of the stage of *Fig.* 2 and shows a solid, instead of a hollow, consistence.

It is a remarkable fact that the part of the opaque area which answers to the yolk-plug in the Amphibian ova is not represented here at any stage, as will be seen from the above accounts. A yolk-plug is present only when the invagination of the yolk field is delayed to a late stage in which the ventral lip of the blastopore is completely established, as is best seen in the Amphibian ova.

The process mentioned above was observed by Max Schultze, A. Shipley and A. Goette. Shipley states: "The invagination at first has a wide-arched slit-like opening, but this soon narrows into a small circular pore"[1], but he says nothing further on this important part of the history; the sinking of the opaque hemisphere in the shape of a hollow funnel did not, it seems to me, attract his attention. Max Schultze[2] knew some of the changes

1) A. Shipley: On some Points in the Development of Petromyzon fluviatilis. Quart. Journ. Microsc. Sc., 1887, p. 5.

2) *loc. cit.* p. 13.

taking place in the opaque hemisphere; he gives, though briefly, an account of the depression of the lower hemisphere. Six of his figures (*Figs.* 4–5*a*, Taf. II.) show a close resemblance to those of the earlier stages of mine and at least attest his correct observation.

The accounts given by Goette are, on the other hand, more accurate; he says: "Diese (the first indication of the archenteron) besteht in einer queren, halbmondförmigen Furche, welche aufwärts gegen den Vorsprung oder die künftigen Rückenseite des Embryo durch eine wulstige Lippe begrenzt, deren seitliche Fortsetzungen aber nach unten gegen die spätere Bauchseite unmerklich verstreichen. Dies hängt zusammen, dass die Furche, indem sie sich taschenförmig vertieft, dabei die Richtung konzentrisch zu der oben gezeichneten Rückenfläche des Embryo einschlägt, also ventralwärts flach abläuft. Diese spaltförmige Tasche ist die Urdarmhöhle, ihre äussere Oeffnung das Prostoma"[1]. These lines are, it is obvious, to some extent in accordance with the statements given above by me concerning early stages. To my great regret, he gives no account of the further changes of the semilunar furrow which "ventralwärts flach abläuft," notwithstanding the fact that these changes represent, in my opinion, one of the most important points of the gastrulation, by which the *Petromyzon* ovum is put in sharp contrast to other ova of unequal-holoblastic segmentation, for instance, of the Anuran. First of all, the flattened yolk-hemisphere in *Petromyzon* is converted, towards later stages, into a deep and hollow depression, so that it is, as shown above, never brought into the form of a yolk-plug throughout the whole phase of gastrulation. This fact evidently shows a great activity of the opaque hemisphere itself, by which activity alone the invagi-

1) *loc. cit.*, p. 2.

nation is carried on. In the Anura, on the other hand, the yolk-hemisphere shows no indication of a depression on its surface at all and is exposed on the surface of the ovum down to a late stage until the gastrulation is completed; this exposed part of the yolk-field is what is called the yolk-plug. It is therefore plain that the invagination of the yolk-hemisphere is, in this case, not carried on by its own power alone, but is to a large extent effected by the sinking sickle-furrow, that is, by the infolding animal layer: in short, its own activity is almost *nil.* To this point, I will return further on.

In the second place, no less striking are the changes undergone by the boundary groove during the gastrulation. It was mentioned above that this groove becomes obvious for the first time at a certain point on the junction-line and gradually embraces the ovum (*Figs.* 3*a*–4*b*, *bg.*), constricting it into two unequal portions: the larger translucent and the smaller opaque. For a certain interval of time it does not, however, surround the ovum completely (*Figs.* 5*a*–6*b*); that part of the ovum just opposite the point of its first appearance remaining free from the constriction, although this part becomes likewise involved eventually (*Fig.* 7*a*). With the increasing growth of the conical eminence which, it will be remembered, appears below the boundary groove, the latter not only cuts in deeper, but is pushed forwards by the eminence to cause in its middle portion a curvature with the convexity turned toward the front: in other words, the boundary groove retains no longer its original transverse position. Towards the ventral part, the groove becomes shallow passing over into the junction-line which in this part still remains in the original condition (*Figs.* 4*a* and 4*b*, *bg.*). As the visible blastopore appears and consequently the conical eminence decreases in height, the groove grows further in curvature, for it is pushed forwards on the dorsal surface of the ovum by the eminence now flattened in consequence of withdrawal of the cells composing it. As the invagination goes further, the elevation increases in its antero-posterior extent, pushing the groove still further forwards. The eminence may now be called the embryonic shield (*Figs.* 6*a*, and 6*b*, *em.s.*). By the time when the invagination has extended over the larger part of the vegetative field (*Fig.* 7*c.*), the ovum is completely surrounded by the groove, although still shallow in the ventral part just constricted (*Fig.* 7*a*, *bg.*).

The translucent part of the ovum, against which the remaining parts are marked off by the groove, is in the course of development, continually diminishing in extent, and at a stage like that represented in *Figs.* 8*a* and 8*b*, it is reduced into a small swelling, while the embryonic shield has extended considerably. This reduction of one part and the augmentation of the other are, in early stages, evidently the effects of one and the same process, *i. e.*, of the invagination, by which the segmentation cavity is partially obliterated; but in later stages, the matter is much complicated, as will be explained in the following lines.

I will here call attention to the change of the position of the groove in the stages spoken of. A comparison of the groove in *Fig.* 7*a* with that in *Fig.* 8*a* shows an apparent forward shifting of it, *i. e.*, an extension of the embryonic shield (*em.s.*). If this shifting of the groove were done, as in early stages, merely by the sinking down of the opaque hemisphere from the surface, it ought necessarily follow that the junction-line at the ventral median line (indicated with ⁑) would have much approached the dorsal blastoporic lip, since in order to add such a considerable extent to the embryonic shield, a great deal of the opaque hemisphere must invaginate within the ovum. But as seen in the figures referred to, this hemisphere in fact suffers but little change of extent, except that it is more or less depressed on the surface. We are thus led to conclude that the marked translocation of the groove is mainly brought about by the extension of the embryonic shield produced by the *backward shifting of the translucent animal layer.* The opaque hemisphere has only little, if at all, to do with the invagination or with the reduction of the segmentation cavity. Thus, it is obvious that the marked translocation of the groove is an apparent one: its real shifting due to the actual invagination of the opaque hemisphere is probably very little. As will be shown further on, this translucent layer, shifting backwards, partly contributes to the growth of the blastoporic lip and is partly turned inwards.

In this way, the translucent part containing the segmentation cavity, is, in a still further advanced stage, reduced into a small vesicle and comes to be situated somewhat in the ventral aspect of the ovum (*Figs.* 9*a*); the boundary groove around it is accordingly seen also on the ventral aspect. Finally with the thorough obliteration of the segmentation cavity, the groove is, of course, lost from sight (*Fig.* 10a). Now the ovum assumes a new outline; it is swollen in the anterior part and decreases in bulk posteriorly, while, in a little younger stage, it is rather bigger in the posterior part with the ventral part of the yolk-hemisphere still bulging out (*Fig.* 9*a*). The ovum is now pear-shaped (*Fig.* 10*a*).

Standing thus in intimate connection with the gastrula invagination, the boundary groove marks precisely the extent in which the process goes on: every part of it is pronounced on the external surface of the ovum.

The previous authors give no account at all of the groove just dealt with. Max Schultze only touches on it[1]; however, he ascribes to it, as above referred to, quite another meaning, regarding it as the earliest indication of the blastopore. Prof. Ch. Ishikawa discovered, independently from myself, the groove in the Japanese Giant Salamander (*Megalobatrachus maximus*), of which an account of the early development was published last summer.[2]

I will finally give a brief explanation of the changes of the conical eminence which has been touched upon incidentally. The conical eminence is at first brought about by the commencing invagination of the opaque hemisphere, and in the course of the process, it is extended and flattened into the embryonic shield which is anteriorly and laterally bounded by the boundary groove (*Figs.* 5*a*–8*b*). The embryonic shield comes later to be produced in front into a conical knob from the upper end of the ovum, when the translucent swelling containing a small remnant of the segmentation cavity has been driven into the ventral aspect (*Figs.* 9*a* and 9*b*). When this swelling at last disappears from the ovum and the ovum assumes a pear-shape, a prominent elevation of an oval outline becomes obvious on the dorsal surface of it; it is rounded and broad in the anterior part and is gradually narrowed towards the blastopore to become lost in the neighbourhood of the lateral blastoporic lips (*Fig.* 10, *m.p.*). This elevation is doubtless the medullary plate which answers

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1) *loc. cit.* p. 13.

2) Ch. Ishikawa: Gastrulation of the Japanese Giant Salamander (*Megalobatrachus maximus*). Zool. Magaz., Vol. XVII, 1905.

to the corresponding structure, for instance, in Amphibian ova. A narrow but sharply defined groove (*Fig.* 10 *b*, *r.r.*) running lengthwise along the median line of this oval field represents the "Rückenrinne." It is broader and deeper towards the blastopore and communicates with it (*Figs.* 10 *b* and 10 *c*, *r.r.*), while anteriorly it fades away.

The gastrulation in the present condition is not yet closed, but has yet to envelope the opaque hemisphere which is still exposed between the blastopore and the boundary groove that has just disappeared. This exposed part is brought within to some extent, doubtless by the active invagination of the opaque field itself, which has been uninterruptedly taking place from the first: it is flattened and depressed in the like manner as in the preceding stages. An active growth of the dorsal blastoporic lip is, however, indisputable from the fact that the medullary plate is obviously added by stages in its antero-posterior length. This addition in the plate is indeed not less than one-third of the original length, by the time a prominent pair of the medullary ridges becomes visible (compare *Figs.* 10 *a* and 10 *b* with *Figs.* 11 *a* and 11 *b*). Such an enormous growth of the blastoporic lip denotes nothing else than its overgrowth on the opaque field, which accomplishes, under co-operation of the invagination of the opaque field itself, the process of gastrulation. Now the blastopore assumes a circular outline; the precise mode by which the pore is brought into the definitive form has already been stated in foregoing pages.

I will now turn to follow the internal changes corresponding to the superficial occurrences described in the preceding pages. Let us start again from a young blastula stage. *Fig.* 12 shows a vertical section through the two poles of the blastula which is still spherical in outline. The segmentation cavity (*s.c.*) which is semilunar in shape occupies the upper half of the ovum; the cavity is roofed by a dome-like layer, while its floor is formed by a thick mass of cells. The cupola part (*a.h.*) is the animal hemisphere which looks translucent, when observed as an opaque object, because it is thin, consisting of a layer only 2–3 cells in thickness; these cells are smaller than those which compose the remaining part of the ovum, the vegetative hemisphere (*v.h.*). The smaller cells, the micromeres or animal cells as they are called, can further be distinguished from the larger cells by their smaller nuclei and by the smaller quantity of yolk-granules which they contain. The macromeres, which contain larger nuclei, are loaded with an enormous quantity of yolk.[1)]

The segmentation cavity is not bordered by a sharp limit; the cells in both the animal and vegetative hemispheres project, or even are set free, into the cavity: in this stage, the cells of both kinds are evidently still undergoing segmentation.

In the vertical section (*Fig.* 13) through the axial plane of the ellipsoidal ovum (cf. *Fig* 2), the segmentation cavity (*s.c.*) shows conspicuous changes; it is no longer semilunar, but circular in outline; and it now occupies the upper four-fifths of the ovum. The micromeric wall (*micm.*) forming the animal hemisphere is still represented by a layer which is 2–3 cells thick; the vegetative hemisphere is, on the other hand, formed as before of

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1) B. Lwoff (Ueber die Bildung der primären Keimblätter etc. bei den Wirbeltieren. Bull. Soc. Natural. Moscou, 1894) informs that the yolk-granules contained in the macromeres are coarser than those in the micromeres, a distinction which I can not make out in spite of careful observations.

a thick crowd of macromeres (*macm.*), although this has much decreased in thickness as compared with the corresponding part in the foregoing stage. Moreover, the inner zone of the layer is no longer loose in structure, but is very compact. The same is also true of the micromeric layer. Consequently, the demarkation against the segmentation cavity is no longer an irregular line, but is even and sharp. We may therefore say that here the segmentation is over and that the ovum is fully grown in the axial diameter by multiplication of the component cells.

It should be further noticed that the floor of the segmentation cavity is not flat, but is excavated, so that the massive layer of the floor and the thinner cupola layer pass over gradually, not abruptly, into each other. The animal hemisphere is fully distended; the distension seems to be maintained by a more or less viscous fluid of albuminous nature, which fills up the segmentation cavity and which coagulates when hardened and is affected, though faintly, by staining reagents.

A median sagittal section through an ovum, in which the boundary groove is just manifest, is represented in *Fig* 14. A change which draws attention first of all is a shallow indentation formed at the junction of the micromeric layer with the macromeric (*b.g.*); this is the boundary groove cut through. No less conspicuous is another change met with in a small area of the micromeric wall (*mic. ep.*), which forms the upper half of the groove. Here it is thinned out, being reduced into a layer one cell deep, while the remaining parts are still in the same condition as before; so that the transition of these parts is no longer gradual but abrupt, the micromeric layer one cell thick passing at once into the multicellular macromeric wall (*Figs.* 14 and 23). The micromeric layer under notice is not an aggregation of indifferent cells as in a younger ovum, as will be seen by a comparison of this part with the corresponding part in the ovum last described (*Fig.* 13), but it constitutes a typical columnar epithelium which stands in direct connection with the indifferent mass of yolk-cells. While the micromeric layer is in this way undergoing histological differentiation, the macromeric part also does not remain unchanged; it is produced by cell-multiplication outwards into a rounded elevation (*c. em.*), which represents the forerunner of what I have called above the conical eminence.

The origin of the boundary groove has already been explained and is not difficult to understand. Notwithstanding the eminence protruded, the junction line, *i. e.*, the connection of the epithelium with the solid mass of yolk-cells, retains its original position, while the thin-walled animal hemisphere above this line is kept distended by pressure of liquid contents within it. Consequently, a constriction or a groove is necessarily brought about along the junction line as a passive result; this is the boundary groove (*Fig.* 14, *b. g.*)

The boundary groove is very striking in the median sagittal section through a little further advanced ovum (*Fig.* 15, *b. g.*), in which a dorsal small part (***) of the vegetative hemisphere is flattened. In this section it is very well seen that the groove looks striking, not because it had sunk deeper, but merely because of the further protrusion of the eminence (*c.em.*), which now shows a conical configuration when observed as an opaque object (see *Figs.* 4*a* and 4*b*). In sections of the same stage (*Fig.* 15) it is seen that the cells composing the eminence and the flattened part are much smaller and more thickly crowded than those of the remaining part of the macromeric hemisphere (this point is unfortunately not well shown in the figure). Further, a part of the macromeric mass lying close to the boundary groove has slipped a little into the segmentation cavity, as may be gathered by comparing the points marked with ⁂ in *Figs.* 14 and 15. From these facts it is not difficult to infer that the component cells in this part, especially in the inner zone, have been undergoing repeated division. This increase in bulk of the inner mass must be one of the causes of the upheaval of the conical eminence.

Notwithstanding that there is detected as yet no trace of an indentation which represents the commencing depression of the blastopore, the flattening of the macromeric hemisphere can safely be regarded as the first step of gastrula invagination (p. 3).

The relations of these parts are made still clearer in the section through the median sagittal plane of a little older ovum (*Fig.* 16) in which the blastopore had come into view in the form of a nail-mark (compare with *Figs.* *5a* and *5b*). The flattened area of the vegetative hemisphere is converted into a large notch (*bp.*); this notch represents evidently the earliest trace of the visible blastopore. I wish to explain in the following lines the mode by which the notch is brought about, the position where it appears and the effects which are imparted by the formation of it to other parts. In the first place there is little room for doubt that the notch is formed not passively by downward pushing of the conical eminence (*c.em.*), but by a gradual infolding of the flattened field, which infolding is carried on by its own activity and is the continuation of the process that caused the flattening. The pushing in of the macromeric mass of this part forms a large fold raised into the segmentation cavity; and the component cells which in younger stages formed the superficial row of the flat field now take a radial arrangement.

It follows, in the second place, that the blastoporic notch appears *not at the junction line, i. e.,* not at the boundary between the micromeric epithelium and the macromeric cell-mass, *but in the macromeric part itself.* In younger stages (*Fig.* 14), the upper limit of the conical eminence is represented by the boundary groove which there coincides with the junction line. When the curvature of the boundary groove takes place (*p.* 11), the groove in the dorsal part shifts a little further forwards; nevertheless, the conical eminence is formed almost entirely of the macromeric part (*Fig.* 15). Therefore, decidedly macromeric must be the flat field which forms the lower slope of the eminence; and it is this field which is converted into the blastoporic notch (*Fig.* 16). The junction-line corresponds, in the stage spoken of (*Fig.* 16), to the free margin of the dorsal blastoporic lip (*), as it may now be called; this line is, however, by no means distinct nor sharply defined.

Lastly, as an effect of the actual (visible) invagination of the macromeric part, the segmentation cavity is obliterated to a certain extent and consequently assumes a new outline (*Fig.* 16. *s. c.*). The conical eminence suffers a greater alteration: it is changed into a less conspicuous elevation which lies between the blastopore and the boundary groove, its original conspicuous part having been pushed in, forming the walls of the invagination. The boundary groove (*b.g.*) which has become, as stated above, manifest by the growing prominence of the eminence, becomes likewise inconspicuous. It is no doubt these changes which caused Goette to state: "Sie (the eminence) verstreicht aber sehr bald, nachdem gerade unter ihr, also zwischen dem Rande der Keimhöhle und dem Gegenpol, die erste Andeutung des Urdarmes sichtbar geworden ist." [1] I can not but wonder at his keen

1) *loc. cit.*, p. 2.

insight which is expressed in this short account; indeed, he correctly observed the position at which the blastopore becomes first visible. According to him, it is found "zwischen dem Rande der Keimhöhle und dem Gegenpol," while all other investigators maintain that the pore occurs at the boundary between the micromeric and the macromeric part, *i. e.*, at the junction-line itself. This part—the part of the first appearance of the blastopore—is, however, not regarded by Goette, as I do, as a macromeric part, but as a micromeric; for, according to him, the conical eminence is brought about by "Anhäufung der dort scheinbar entstehenden und hinabrückenden Mikromeren,"[1] and the blastoporic cavity is nothing else than a fissure appearing in this cell-mass. Starting from this standpoint, he assumes that the whole process of gastrulation consists in two processes: *a*) the backward shifting of the micromeric layer roofing the segmentation cavity and the forward pushing of the layer forming the archenteric roof, which both represent his so-called "dorsale Einstülpung," and *b*) "Umwachsung der Makromeren durch die Mikromeren." The process which brings forth the "Anhäufung" of the micromeres represents, according to Goette, this backward shifting of the micromeric roof of the segmentation cavity. This assumption is, I believe, not in accordance with the facts, and the point of dispute lies after all in the origin of the cells giving rise to the "Anhäufung" or the conical eminence.

There are several facts which make us infer their being macromeric in origin. First of all, these cells are loaded with thickly-crowded yolk-granules and contain large nuclei, so that they can not be distinguished, in these respects, from those in

1) *loc. cit.*, p. 4.

other macromeric parts occupying the largest ventral portion of the ovum (see *Fig.* 23). While these characteristics disappear rather abruptly in the micromeres forming the columnar epithelium, there is ventrally, in this respect, never any distinction between this mass of cells and the remaining macromeric part. In addition to this, there occur active cell multiplication, —a fact which shows that the cells are not derived from other parts, but are formed here. Compare *Fig.* 14 with *Fig.* 15. Notwithstanding the fact that the eminence has grown much more conspicuously in the latter section than in the former, there can be detected no indication of downward shifting of any cellular elements which may contribute to the growth of the eminence. After all, it is plain that the cells forming the conical eminence are the macromeres which have here multiplied *in situ*, not "scheinbar" but in reality.

Having made out the early steps of the gastrula invagination and the relations to the parts with which it stands in connection, I will turn to the explanation of further changes going on in different parts: the changes in reference to *a*) the differentiation of the micromeric elements forming the roof of the segmentation cavity, *b*) the invagination of the macromeric and micromeric parts, and *c*) the translocation of the boundary groove and the obliteration of the segmentation cavity in consequence of the progressing invagination.

a) The epithelial structure of the micromeric layer, which is in early stages confined to a small area, is by stages extended farther and farther (*Figs.* 16–22). Already at a stage when the segmentation cavity is reduced into a small vesicle (*Figs.* 8*a* and 8*b*), the differentiation almost reaches, at least on the dorsal median part, the entire roof of the segmentation cavity (*Fig.* 19).

In an ovum with ventrally shifted segmentation cavity (*Figs.* 9*a* and 9*b*), the median sagittal section of which is represented in *Fig.* 20, the micromeric layer assumes epithelial character throughout and is at its ventral limit (⁑) sharply marked off against the macromeric part, just like its dorsal limit at the stage when the invagination was about to take place (*Figs.* 14 and 23).

Laterally the differentiation proceeds in a similar way as in the median zone just stated, as will very well be seen in the three sections represented in *Figs.* 25, 26 and 27. Of these three figures, the last (*Fig.* 27) is a transverse section through an ovum a little younger than that shown in *Fig.* 8*a* or *Fig.* 19; the lower depressed part (*bp.*) shows a part of the blastoporic funnel; on either side of the macromeric cell-mass, we see very well its connection with the micromeric layer which presents the typical character of a columnar epithelium. The two other figures (*Figs.* 25 and 26) were drawn from a series of horizontal longitudinal sections through an ovum of about the same age as that shown in *Figs.* 7*a* and 7*b* or in *Fig.* 18. In the section taken from the dorsal part (*Fig.* 25), the differentiation of the animal layer already reaches the junction-line, while in the other section (*Fig.* 26) from the ventral part, the layer is still many cells in thickness.

Great care was taken to make out in every possible way the actual mode by which the aggregated elements of the micromeric layer are brought into the epithelial structure; but all my efforts have been fruitless after all. Although wanting positive evidence, we can make some surmises from another point of view. There can be only two possible modes: either the outermost cell-row of the indifferent animal layer comes to be converted *in situ* into the definitive epithelium, or the indifferent cells wedge in between other cells so as to produce an epithelial layer one cell thick. If the former mode were the case, the cells of the inner rows must either fall into the segmentation cavity, or shift downwards along the inner surface of the outermost row, becoming heaped up, as it were, at the foot of the animal layer. In reality, there are never and nowhere detected such indications. On the contrary, in the part where differentiation is going on, the cells of the outer row and those of the inner rows are found pushing in between one another, and the layer of such condition passes over gradually into the part which has already become a true epithelium (*Figs.* 14–19 and *Fig.* 23). The latter supposition seems, therefore, to be in accordance with facts. And this is reasonable, since the animal layer has to extend exceedingly in order to invest completely the macromeric hemisphere, *i. e.*, to accomplish gastrulation, as will be shown in future pages.

b) As regards the invagination, a part of the flattened macromeric field which is still seen projecting from the surface below the blastopore (*Fig.* 16, †) comes first under notice. In a little further advanced ovum (*Fig.* 17), this part is lost from sight; the invagination, on the other hand, goes deeper. The invaginating pocket is not turned towards the center of the segmentation cavity as in younger ova (*Fig.* 16), but pushes its way dorsad and forwards under withdrawal of the protruded part which at first is in the floor of the pocket but must sooner or later lie in the roof (*Fig.* 17). The dorsal wall of the blastoporic pocket is consequently brought to involve the macromeric field, and here the archenteron (*ar. en.*) is formed for the first time. It is therefore plain that this additional invagination (*Fig.* 17) is made entirely by the cells which formed the projected part. Now it follows that the cells composing the roof of the archenteric pocket first formed are those which formerly gave rise to the conical eminence, while its floor is represented by the macromeric mass which had lain ventral to the eminence.

The layer constituting the archenteric roof shows an epithelial character (*Figs.* 17 and 18, *mac. ep.*); it is, however, not difficult to distinguish from the regular cylindrical epithelium of the outer layer. It looks irregular because it is composed of tall cells which are variable in shape and length and are thickly loaded with yolk-granules; further it is distinguished by the nuclei of the cells being larger than those of the outer layer cells. The outer regular and the inner irregular epithelium pass over into each other at the dorsal lip of the blastopore. On the other hand, the inner layer is to be traced uninterruptedly into the outermost row of the macromeres, which is partly invaginated but is in the greater part still exposed. The irregular epithelium arises without doubt by delamination, so to speak, from the subjacent cells. The row of cells forming the floor of the segmentation cavity has nothing to do with the epithelial layer in question; it always shows its original sharp contour. On the contrary, in front of the anterior end of the invaginating pocket, there is always seen some disturbance of cells, and some detached cells are seen projecting into the segmentation cavity (*Figs.* 17–19, ⁂ and *Figs.* 25 and 26, ⁂). This appearance is due, it seems to me, merely to the fact that here the outermost row of macromeres is being split off from the underlying crowd of cells, to be turned into the archenteric roof.

Passing now to a little further advanced ovum (*Fig.* 19), the roof of the archenteron is no longer formed of the irregular epithelium of macromeric origin (*mac. ep.*) alone, but a regular columnar epithelium (*i. mic. ep.*) is added to form the posterior section of it. The latter part is not only similar in every histological respect to the outer epithelium (*Fig.* 24 *e. mic. ep.*), but is directly continuous with it at the dorsal margin of the blastoporic aperture. No doubt both have the same micromeric origin. The anterior limit of the inner micromeric epithelium (*Fig.* 19, *) against the macromeric epithelium is by no means distinct, but both the layers gradually pass over from one to the other. Traced towards the lateral part, the micromeric epithelium forming the archenteric roof gradually decreases in its antero-posterior extent, finally to get lost at the blastoporic margin still represented by the junction line. Therefore, the micromeric roof is at present posteriorly limited by a crescentic outline.

The contribution of the micromeric layer to the formation of the archenteric roof has already been pointed out by Balfour[1]; but it was more clearly made known by Goette[2] and Lwoff.[3] Goette's view differs, however, from the accounts given above by me in so far as concern the origin and the extent of the layer. Goette's assumption of the gastrulation, which Lwoff confirms, attributes micromeric origin to the archenteric roof in its whole extent, while according to the results of my present work, the anterior part of the roof is represented by macromeric layer. At any rate, so long as the blastoporic lip extends in the form of a large arc (*Figs.* 5*b*, 6*c*, and 7*c*), the archenteric roof is formed entirely of the irregular macromeric epithelium

1) Balfour: Comparative Embryology, vol. II, p. 85. A. Shipley gives a brief account of the same fact (*loc. cit.*, p. 5).

2) *loc. cit.*

3) *loc. cit.*

alone (*Figs.* 16–18, *mac. ep.*); it is probably this part of the epithelium, which misled Goette and Lwoff to assume the archenteric roof as being, in its entire extent, formed of an irregular epithelium 1–2 cells in thickness. As soon as the limbs of the arc commence to come nearer each other (*Fig.* 8*c*), the micromeric epithelium appears inside in the archenteric roof (*Fig.* 19); it is thus supposable that the approximation of the arc-limbs, *i. e.*, of the lateral blastoporic lips, stands in an intimate connection with the contribution of the micromeric epithelium to the formation of the archenteric roof, as the following consideration will show.

With the commencing infolding of the micromeric epithelium, the blastoporic lip is strongly raised in its median part (*Fig.* 8*c*), and the slit-like passage of the blastopore is converted at this part into a circular pore. This circumstance is brought about by the complete withdrawal within of the macromeres in this part and by the subsequent lifting of the blastoporic lip formed of the micromeric epithelium which is now being invaginated. For illustration of this point, the three figures, *Figs.* 27–29, are instructive; they were drawn from a series of sections through an ovum a little younger than that shown in *Fig.* 8*a* or *Fig.* 19 and represent cross-sections passing respectively across the ventral part (*Fig.* 27), the blastoporic passage (*Fig.* 28) and the dorsal lip of the blastopore (*Fig.* 29). In the first figure (*Fig.* 27), the micromeric layer (*e. mic. ep.*) is not yet turned inwards, though the invagination of the macromeres has proceeded to a great extent (*bp.*). In the next figure (*Fig.* 28), the macromeric field is no longer exposed on the external surface of the ovum, but has retired far inside; and the blastoporic passage is bordered, from right and left, by the lateral walls formed of the infolding micromeric epithelium (*e. mic. ep.*). In the third figure (*Fig.* 29), the already infolded epithelium forms the median part of the archenteric roof (*i. mic. ep.*) which is obliquely cut through. It is therefore obvious that so long as the macromeric field is exposed on the external surface, the blastoporic lips on the lateral sides, *i. e.*, on the limbs of the arc, stand apart from each other; when the macromeric half is entirely withdrawn inside and the infolding of the micromeric epithelium commences its work, the lips are brought nearer: hence results the change of shape in the blastoporic arc. I should here add that neither the micromeric nor the macromeric part is passive in the process, but both are in active state. The infolding of the former is, of course, carried on by its own activity, *i. e.*, by extension of the layer in general, which is brought about by both the cell-multiplication of the epithelial components and the dovetail-insertion of the micromeres forming the cupola of the segmentation cavity. The invagination of the macromeres is likewise actively taking place; this is in fact the same active process as that which more early caused the blastoporic depression.

At the stage of *Fig.* 19 there is already comparatively little cell disturbance in front of the archenteron (*Fig.* 19, **). This shows that the delamination of the outermost cell-layer of the macromeric mass to form the archenteric roof is of a short duration and of a limited extent. On the other hand, there is a strong reason, as briefly stated on p. 13, for believing that the backward shifting of the outer micromeric layer (*Fig.* 19, *e. mic. ep.*), at the cost of which the formation of the archenteric roof is accomplished, is of a greater importance. This shifting seems to be more active in later stages (*Figs.* 20, 21, and 24, *i. mic.*

ep.), in which the larger part of the archenteric roof is already occupied by the micromeric layer. In the stage of an ovum represented in *Fig.* 21, the anterior end of the archenteron has pushed its way across the segmentation cavity and has struck against the ventral part of the peristomal mesoderm which is brought forth from the ventral junction line destined to be transformed into the ventral lip of the blastopore.

The mitosis met with very frequently in the outer micromeric layer (*Figs.* 19–21 and *Figs.* 27–30) has naturally no other significance than an active growth of the layer, by which not only the outer layer itself but also the archenteric roof is extended.

At the stage shown in *Fig.* 21, however, a large part of the macromeric hemisphere is still exposed. This part invaginates, as before, by its own activity as shown by the depression on the surface; but in order to bring forth the complete gastrula, this part is doubtless overgrown to a great extent by the dorsal and lateral blastoporic lips now formed of the micromeric epithelium. The overgrowth is, however, certainly brought about, not by a free growth of the lips' rim as it were, but by the outer micromeric epithelium itself which is shifting backwards uninterruptedly from earlier stages and is extended by the active superficial growth mentioned above. This layer of course migrates inwards about the blastoporic lips, but, at the same time, it narrows the blastoporic aperture, causing the lips to grow on in such a manner that the blastopore is ultimately converted into the definitive circular form (*Fig.* 22).

From the above observations, we see that the micromeric epithelium is brought into the archenteric roof by its infolding and that it overgrows a large part of the macromeric hemisphere by its backward shifting and superficial extension. In consequence of these processes, the large arched blastopore suffers changes in its shape and ultimately acquires the definitive form. After all, the whole occurrences are nothing else than those which answer to the overgrowth of the animal layer over the large blastopore in *Amphioxus* in which it takes place only after complete invagination of the vegetative hemisphere, while here in *Petromyzon* earlier phases of the overgrowth overlap to some extent later phases of the invagination. At any rate, it is true that some later phases of the gastrulation in *Petromyzon* as well as in *Amphioxus*, consist entirely in overgrowth of the micromeric epithelium.

The invaginated micromeric epithelium is not of a great breadth from side to side; however, it occupies completely the roof of the archenteron which presents, on cross-sections, a small lentiform shape, while the lateral walls and the floor of the cavity are formed by the macromeric cell-group (*Figs.* 24 and 29). It is plain that this relation of the micromeric epithelium to other parts has been brought about in such a way that the macromeric epithelium (folded at the blastoporic lip) partly migrated inwards and partly caused the lip to grow farther backwards, while the depressed macromeric surface was deeply withdrawn leaving a concavity on the surface. The archenteric cavity is accordingly represented by the depressed or grooved surface of the macromeric hemisphere itself (compare *Fig.* 29 with *Fig.* 28). It is this part of the micromeric epithelium which is called by Lwoff[1] the "Dorsalplatte." It must be remarked here that the boundary between the micromeric "dorsal plate" and the macromeric lateral walls and floor however does not correspond to the

1) *loc. cit.*

junction line: a number of the macromeric elements at this part on either side is pressed out of the archenteric border, and a new connection is established between the micromeric epithelium and the macromeric part. Such a particular process is perhaps indispensable, because a broad surface of the macromeric hemisphere is converted into a narrow passage of the blastopore. The cells thus pushed out assume a looser epithelial arrangement which stands on both sides in connection with the "dorsal plate," as if they were its lateral continuations (*Figs.* 27–30, *l. mac. ep.*). It is this structure which much attracted the attention of observers and misled especially W. Scott[1] to assume it as the mesoderm split off from the main mass of the macromeric elements. For the covenience of reference, I will call it the lateral irregular epithelium, although it is by no means to be regarded as a definite structure.

Anteriorly the "dorsal plate" is more or less broad and passes over into an irregular epithelium of the archenteric wall brought about by early invagination of the macromeric elements. At its posterior extremity, the "dorsal plate" is divided at every stage after its appearance into two arms which embrace the blastopore from the dorsal side forming its lateral lips (*Fig.* 28). In the complete gastrula, the lateral lips represented by the folding of micromeric epithelium are extended to the ventral median part, the two arms meeting with each other at the ventral blastoporic lip; but the folding in is here still very slight (*Fig.* 22). After all, the oval depression of the blastopore is for the first time converted into a circular pore which constitutes the definitive blastopore.

1) W. Scott: Beiträge zur Entwickelungsgeschichte der Petromyzonten. Morphol. Jahrb., Bd. VII, 1882.

It is a remarkable fact that a part of the macromeric cell-mass, known as the yolk-plug in Amphibian ova, does not come into view at all. As before noted (p. 11), the yolk-plug is, in fact, a part of the vegetative hemisphere, which is still exposed after having already been surrounded by the furrow of the sickle-groove brought about by infolding of the animal layer. In the *Petromyzon* ovum, there is never an occasion for such a temporary phenomenon to manifest itself, because the definitive ventral lip of the blastopore is wanting in the younger stages and is found only in the complete gastrula.

A wedge-shaped structure composed of small cells and found immediately inside the ventral blastoporic lip in the gastrula (*Fig.* 22, *p.m.*), represents the ventral part of the peristomal mesoderm, which when traced anteriorly, is continuous to the gastral part of the mesoderm already formed in this stage on both sides of the "dorsal plate." It is proliferated out of the micromeric epithelium forming the blastoporic lips. Earliest traces of the proliferation is seen already in a little younger ova (*Fig.* 23, *p. m.*).

We have therefore before us archenteric walls, in which the "dorsal plate" makes up the larger posterior section of the roof of the cavity and in which the anterior part of the roof is formed of the macromeric epithelium, while the lateral walls and the floor are represented by indifferent macromeres. The "dorsal plate" gives rise, in its median part, to the gastral mesoderm (*Fig.* 24, *g. m.*), and in the blastoporic lips, to the peristomal mesoderm. The irregular macromeric epithelium forming the anterior archenteric wall loses gradually its epithelial structure and together with the lateral irregular epithelium, becomes indistinguishable from other macromeres; consequently the archenteric cavity in this part, though visible for some time here and there as irregular spaces, is finally lost from sight (*Fig.* 22).

c) Passing now to the translocation of the boundary groove and obliteration of the segmentation cavity, I have left the history of the boundary groove at a stage when the visible blastopore appears for the first time (*Fig.* 16). Thenceforth the groove shifts farther and farther forwards, as the archenteric pocket is added in length, to some extent by inward migration of the micromeric epithelium, but largely by invagination of the macromeric hemisphere, which process reduces the segmentation cavity (*Figs.* 17–19). It is thus plain that the forward shifting of the groove indicates the extent into which the segmentation cavity is obliterated. It must however be borne in mind that the ovum soon arrives at a stage, in which side by side with the active invagination of the macromeric hemisphere there is going on another process, viz., the backward shifting of the outer micromeric epithelium. Henceforth the translocation of the groove is, as stated on p. 13, only apparent. In other words, the obliteration of the segmenatation cavity by the invagination of the macromeric hemisphere, is very little when compared with the reduction of it by the shifting backwards of the micromeric epithelium. In this way the segmentation cavity is at length entirely obliterated long before accomplishment of the gastrulation (*Fig.* 21); and consequently the boundary groove is also lost from sight.

The invagination of the macromeric hemisphere and the backward shifting of the outer micromeric layer represent, however, by no means the sole cause for the obliteration of segmentation cavity. The macromeres which form a part of the hemisphere in the neighbourhood of the cavity lose their mutual union, thus disturbing the sharp contour of the cavity; and furthermore, some scattered cells come to fill up the last remnant of the cavity, which is thus entirely obliterated (*Figs.* 21 and 30).

The loosening of the cellular union of the macromeres, which is doubtless a very remarkable occurrence, is not confined to the neighbourhood of the segmentation cavity where it first takes place, but eventually extends over a wider extent and especially to the ventral part. Even the whole mass of the cells may be more or less influenced by the process (*Fig.* 21 and 22). The main mass of the hemisphere formed of macromeric elements has hitherto been in firm union, so as to give them a polyhedral shape by mutual pressure and to cause the sharp contour against the segmentation cavity. This close union has been, I believe, kept up owing to the pressure exerted by the liquid contents of the segmentation cavity upon the cell-mass. The pressure is probably intensified by stages with growing reduction of the cavity. Upon this pressure, as before stated, depends also the external form of the boundary groove during invagination, it having the effect of distending the animal layer. The liquid is at length forced to escape in some way as the cavity is by stages reduced; it is probably pressed out through the outer micromeric layer when the pressure attains the maximum. As soon as this happens, the pressure is suddenly much weakened, and the loosening of the cellular union should set in. The segmentation cavity is thus obliterated.

In the gastrula thus brought forth, the invaginated parts of the ovum, including the micromeric and macromeric parts, will be called the entoderm, while the micromeric epithelium which remains as the external coat of the ovum merits the name of the ectoderm.

Having described above the main course of the gastrulation, I may be permitted to give some more considerations on a few points:

1. I have stated before that I have come to consider the stage given in *Fig.* 1 as an old morula stage, and not as a blastula. This is due to the following consideration. It seems to me correct that under the term "blastula" is understood a stage in which blastomeres are no longer in a merely aggregated state, but are converted into the form of an epithelium, since this histologically differentiated condition ought certainly to be distinguished from the stages that consist of indifferent cells only, as is very obvious in *Amphioxus*. Applying this view to the present case, the stage given in *Fig.* 1 is not really gone further than an old morula. In this case the blastulation, as indicated by differentiation of the blastomeres into an epithelium, should be looked upon as being much delayed; it is still being carried on during the whole period of the gastrulation and is finished only a little earlier than the latter process. In other words, the two processess, blastulation and gastrulation, overlap each other to a great extent in the period of their occurrence. The prime cause of this belated mode of development is indisputably due to delay of segmentation on account of an enormous accumulation of yolk within the ovum.

2. I have not been able to detect in any stage what might be considered as "concrescence." It is true that at a much later stage when the embryo begins to grow in its long axis,

there is detected, in a small extent of the hind region, a process which resembles concrescence. But that seems to me to be of a significance different from the process which embryologists are wont to call by the name just mentioned; the process observed by me is probably identical with that which was pointed out in the Amphibia by Eycleshymer.[1]

3. I have stated that the macromeric hemisphere has an activity of its own. This is an important factor in bringing about the gastrulation in *Petromyzon*. That such is the case in the *Petromyzon* ovum which contains much larger quantity of yolk than the frog's ovum and that there is no yolk-plug in the former are very striking facts. I can find no other way of explaining this peculiarity than by assuming the frog's ovum to be secondarily holoblastic as was maintained by Professor Mitsukuri seven years ago.[2] I intend to deal with this question more fully at another place.

I wish to express my deepest thanks to Professor Mitsukuri for his kindness in looking through the manuscript.

Nov. 26, 1905.

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1) A. C. Eycleshymer: The Formation of the Embryo of Necturus, with Remarks on the Theory of Concrescence. Anat. Anz., Bd. XXI, 1902.

2) K. Mitsukuri: On the Fate of the Blastopore, the Relations of the Primitive Streak, and the Formation of the Posterior End of the Embryo in Chelonia, together with Remarks on the Nature of Meloblastic Ova in Vertebrates. Journ. Coll. Sc., Imp. Univ., Tokyo, vol. X, 1896.

Explanations of Plates.

List of Abbreviations.

a.h..........Animal hemisphere.
ar.en.......Archenteron.
b.g..........Boundary groove.
bp...........Blastopore.
bp.l. ....Blastoporic lip.
c.em.........Conical eminence.
ch...........Chorion.
em.s.........Embryonic shield.
g.m.........Gastral mesoderm.
i. mic. ep...Inner micromeric epithelium.
l. mac. ep..Lateral irregular epithelium.
macm......Macromeres (macromeric layer).
mac. ep....Macromeric epithelium.
micm.......Micromeres (micromeric layer).
mic.ep. ...Micromeric epithelium.
m.g. ......Medullary groove.
p.m. ......Peristomal mesoderm.
s.c. ....Segmentation cavity.
v.h..........Vegetative hemisphere.
vl.bp. ......Ventral lip of blastopore.
\+ ....Boundary between micromeric and macromeric epithelia in archenteric roof.
* ....Anterior blind end of archenteron.
** ....Disturbed part of macromeric cell-mass.
⁑ ....Lower limit of micromeres.
⁂ ....Flattened part of vegetative hemisphere.

Plate I.

Surface views of the ova undergoing the process of gastrulation. The figures were all drawn by the author from living specimens. The magnification is about ×30 for all the figures.

Fig. 1—An old morula. The upper translucent part (*a.h.*) is the anima hemisphere; the lower opaque part (*v.h.*) is the vegetative hemisphere.

Fig. 2—A fully grown morula. The animal hemisphere (*a.h.*) has become much larger than that in the stage of *Fig.* 1, while the vegetative hemisphere (*v.h.*) is rather reduced in extent.

Fig. 3*a*—Side view of a little further advanced ovum. The conical eminence (*c.em.*) appears as a rounded elevation. The boundary groove (*b.g.*) has come into view.

Fig. 3*b*—Dorsal view of the same ovum. The proportion of the extent of the animal and vegetative hemispheres is well seen; the latter is about $\frac{1}{4}$ of the former in bulk.

Fig. 4*a*—Side view of a little further developed ovum. The conical eminence (*c.em.*) has become prominent, and a part of the vegetative hemisphere below the eminence has flattened (⁂). The boundary groove (*b.g.*) has become deeper and is extended farther ventrally.

Fig. 4*b*—Dorsal view of the same ovum. A comparison of this with *Fig.* 3*b* shows that the part giving rise to the conical eminence is strongly compressed from side to side.

Fig. 5*a*—Side view of an ovum in which the gastrula invagination has just begun (*bp.*); the boundary groove (*b.g.*) has extended itself farther ventrally.

Fig. 5*b*—Dorsal view of the same ovum. The blastopore (*bp.*) is seen like a nail-mark below the conical eminence (*c.em.*).

Fig. 6*a*—Side view of an ovum in which the invagination has gone a little further.

Fig. 6*b*—Dorsal view of the same ovum. The conical eminence is con verted into a flattened and broad elevation (*em.s.*), the embryonic shield, and consequently the boundary groove (*b.g.*) is curved anteriorly.

Fig. 6*c*—The same ovum from the basal surface. The crescentic blastopore (*bp.*) has increased in length of both its limbs.

Fig. 7*a*—Side view of a further advanced ovum. The constriction of the boundary groove (*b.g.*) is not only striking, but now surrounds the ovum completely. While the invagination is further carried on, the basal surface is depressed and flattened further ventrally.

Fig. 7*b*—Dorsal view of the same ovum. The embryonic shield (*em.s.*) has become broader, and the boundary groove (*b.g.*) has shifted anteriorly in consequence of the advancing invagination.

Fig. 7*c*—Basal view of the same ovum. The invaginating groove (*bp.*) is much extended, describing an almost semicircular arc. It is bounded outside by the blastoporic lip (*bp.l.*) of the same form and which is most prominent in the middle part. The field contained within the arc is depressed a great deal, and the depression is deeper towards the steepest middle part of the blastoporic lip, thus presenting a wide-gaped blastopore of a funnel-like shape.

Fig. 8*a*—Side view of an ovum in which the translucent part is reduced, in consequence of the progressing invagination, into a small vesicle at the anterior end of the ovum and is separated from the opaque embryonic shield by the strongly curved boundary groove (*b.g.*).

Fig. 8*b*—Dorsal view of the same ovum. The larger part of the ovum is occupied by the opaque solid part, the embryonic shield (*em. s.*); capping this is seen the small translucent vesicle at the anterior end.

Fig. 8*c*—Basal view of the same ovum. Both limbs of the arc described by the blastoporic lip (*bp.l.*) are brought nearer to the median line and to each other, while the blastoporic depression has become deeper than before.

Fig. 9*a*—Side view of a further advanced ovum. The whole extent of the dorsal surface is occupied by the embryonic shield (*em.s.*). The translucent vesicle is driven into the ventral side of the anterior part and is seen as a slight swelling. A great deal of the vegetative hemisphere remains still exposed; accordingly, the ovum is somewht conical in shape, the posterior part being bulged out.

Fig. 9*b*—Dorsal view of the same ovum. The translucent vesicle can not be seen, while the boundary groove is perceived as two slight indentations on both sides of the anterior end (*b.g.*).

Fig. 9*c*—Basal view of the same ovum. The blastoporic depression (*bp.*) has become deeper and narrower, but ventrally it passes over gradually into the vegetative field which is still spherical in shape, wanting as yet the ventral blastoporic lip.

Fig. 10*a*—Side view of a further advanced ovum. There is no longer seen any trace of the translucent vesicle. The segmentation cavity is thoroughly obliterated. The ovum now assumes a pear-shape, being larger in the anterior part and lessening in bulk posteriorly.

Fig. 10*b*—Dorsal view of the same ovum. The dorsal surface is no longer plain as before, but there is expressed an oval elevation (*m.p.*), the medullary plate, along the median line of which is seen the "Rückenrinne" running lengthwise. This is deeper towards the posterior end and communicates with the blastopore, while anteriorly it is shallower and fades away at length.

Fig. 11*a*—Side view of a much advanced ovum. The medullary plate is converted into a pair of prominent medullary ridges (*m.r.*). The ventral lip of the blastopore (*vl.bp.*) is now established.

Fig. 11*b*—The same ovum seen from the dorsal and posterior sides. The blastopore shows its definitive circular form by the establishment of the ventral lip (*vl.bp.*)

Plate II.

Fig. 12—A section through both the animal and vegetative poles of an ovum a little younger than that represented in *Fig.* 1. The segmentation of the blastomeres are being actively carried on. The macro- and micro-meres project into the semilunar segmentation cavity, and some of them are even detached and nearly free in that cavity. ×64.

Fig. 13—A section through both the poles of an ovum, the same in development as the surface view shown in *Fig.* 2. The ovum is ellipsoidal in shape; accordingly, the segmentation cavity is no longer semilunar in outline, but is circular. Segmentation is over. The blastomeric wall (*micrm.* and *macrm.*) shows not only a sharp contour against the segmentation cavity, but is reduced in thickness. ×64.

Fig. 14—A median sagittal section through an ovum, nearly the same in development as the stage shown in *Fig.* 3*a*. The relation of the conical eminence (*c.em.*) to the boundary groove (*b.g.*) is very well seen. The micromeric part is transformed into a columnar epithelium (*mic.ep.*) at a small area in the groove. × 64.

Fig. 15—A median sagittal section through an ovum, about the same in development as the surface view represented in *Fig.* 4*a*. The conical eminence (*c.em.*) is distinct; accordingly, the boundary groove (*b.g.*) is deep, and a small field of the macromeric hemisphere immediately below the eminence is flattened (⁂). × 64.

Fig. 16—A median sagittal section through an ovum of about the same stage as that shown in *Fig.* 5*a*. The commencing gastrula invagination (*bp.*) is well seen. The conical eminence and the boundary groove are conspicuous. The floor of the segmentation cavity opposite the invaginating blastopore (*bp.*) is a little raised into that cavity. × 64.

Fig. 17—A median sagittal section through an ovum a little older than the surface view given in *Fig.* 6*a*. The boundary groove has somewhat shifted anteriorly, being pressed by the invagination of the macromeres which formerly formed the conical eminence and which now take an epithelial arrangement (*mac.ep.*). The archenteron has appeared for the first time. The differentiation of the micromeres into the columnar epithelium (*mic.ep.*) has extended over an increased area. The wrinkled chorion (*ch.*) is to be seen. × 64.

Fig. 18—A median sagittal section through an ovum of about the same stage as the surface view shown in *Fig.* 7*a*. The archenteron (*ar.en.*) and the macromeric epithelium (*mac.ep.*) roofing the cavity have increased in extent. The boundary groove (*b.g.*) has shifted farther anteriorly, and the segmentation cavity (*s.c.*) is much reduced. Some macromeres outside the anterior blind end of the archenteron are in a disturbed state (**). × 80.

Fig. 19—A median sagittal section through an ovum as much advanced as the stage represented in *Fig.* 8*a*. Not only the archenteron (*ar.en.*) and the macromeric roof of it (*mac.ep.*) have increased in extent, but in the posterior section of the roof there have come into view the micromeric epithelium (*i.mic.ep.*), which may be distinguished from the macromeric part lying anteriorly to the point marked with + by the regular arrangement of cells as well as by their smaller nuclei. The boundary groove (*b.g.*) has shifted far anteriorly, and the segmentation cavity (*s.c.*) is very much reduced. Some disturbed macromeres (**) are seen outside the blind end of the archenteric canal. ×80.

Plate III.

Fig. 20—A median sagittal section through an ovum a little further advanced than that shown in surface view in *Fig.* 9*a*. The archenteron (*ar.en.*) has further extended, this extension being made by further invagination of the macromeres as well as by new addition of micromeric epithelium. The segmentation cavity (*s.c.*) is reduced into a narrow space; the boundary groove (*b.g.*) is marked by a slight depression on the ventral surface of the last remnant of the segmemtation cavity, which is about to be obliterated by the falling in of the macromeres loosened from mutual union. ×80.

Fig. 21—A median sagittal section through an ovum of about the same development as the surface view given in *Fig.* 10*a*. The archenteron (*ar.en.*) is much increased in extent; its anterior part strikes against the peristomal part of the mesoderm (*p.m.*) now formed, thus entirely obliterating the segmentation cavity. The micromeric section of the archenteric roof has greatly enlarged so that nearly two-thirds of the latter is taken up by the micromeric epithelium (*i.mic.ep.*); a small part of the macromeric field still remains uninvaginated. The strongly wrinkled chorion (*ch.*) is shown. ×80.

Fig. 22—A median sagittal section of a much advanced ovum which is, however, a little younger than the ovum figured in *Fig.* 11*a*. The process of gastrulation is finished. There is no trace of the macromeric field outside. The outer (*e.mic.ep.*) as well as the inner (*i.mic.ep.*) micromeric epithelia show a compact texture. The anterior part of the archenteron is lost from sight: the cells which formerly formed the wall at this part are now indistinguishable from other macromeric elements which have lost their mutual union. The peristomal mesoderm (*p.m.*) is well established. ×80.

Fig. 23—A piece of a sagittal section through an ovum a little younger than that represented in *Fig.* 14. The left side is the outer surface, and the right represents the surface turned towards the segmentation cavity. I have endeavoured to show here the transitional part of the micromeric to the macromeric layer and the mode in which the micromeric layer of many cells in thickness (*micm.*) passes over into the columnar epithelium (*mic. ep.*). Besides the difference in size of cells the nuclei contained in the micromeres (upper part of the figure) are much smaller than those in the macromeres (lower part); this contrast is, in this section, especially sharply expressed. The lighter colour of the micromeric part is due to the smaller quantity of yolk-granules contained. The epithelially differentiated part passes over, on one hand, abruptly into the macromeric part formed of a crowd of larger cells and, on the other hand, into the micromeric layer which is not yet well established but is formed of cells wedged in between one another in a dovetail-like manner. ×180.

Fig. 24—A transverse section through the dorsal part of an ovum about as far developed as that shown in *Fig.* 10*a*. The distinctive characteristics of the two kinds of cells are the same as given in the explanation of *Fig.* 23. Between the outer (*e.mic.ep.*) and the inner (*i.mic.ep.*) micromeric epithelium no difference in histological nature can be detected. The cells of the inner epithelium forming the roof of the archenteron proliferate on either side of the layer and give rise to cells of the same kind (*g.m.*), and which are destined to establish the gastral part of the mesoderm. The concave surface of the archenteric floor is nothing else than the depression or funnel-groove of the exposed macromeric field. ×180.

Fig. 25—A frontal section through the blastopore of an ovum of about the same stage as that represented in *Fig.* 7*a*. A large notch (*bp.*) at the lower end shows the blastoporic depression on the macromeric field. The macromeric surface is almost entirely withdrawn, and the lateral lips on either side of the notch are covered with the micromeric epithelium. A shallow depression on either side (*b.g.*) represents the boundary groove. As the section passes through the dorsal part, the segmentation cavity (*s.c.*) is comparatively small; on the contrary, the disturbed part of the macromeres (**) is met with in its whole breadth. The micromeric layer (*mic.ep.*) presents the structure of a columnar epithelium throughout its whole extent. ×80.

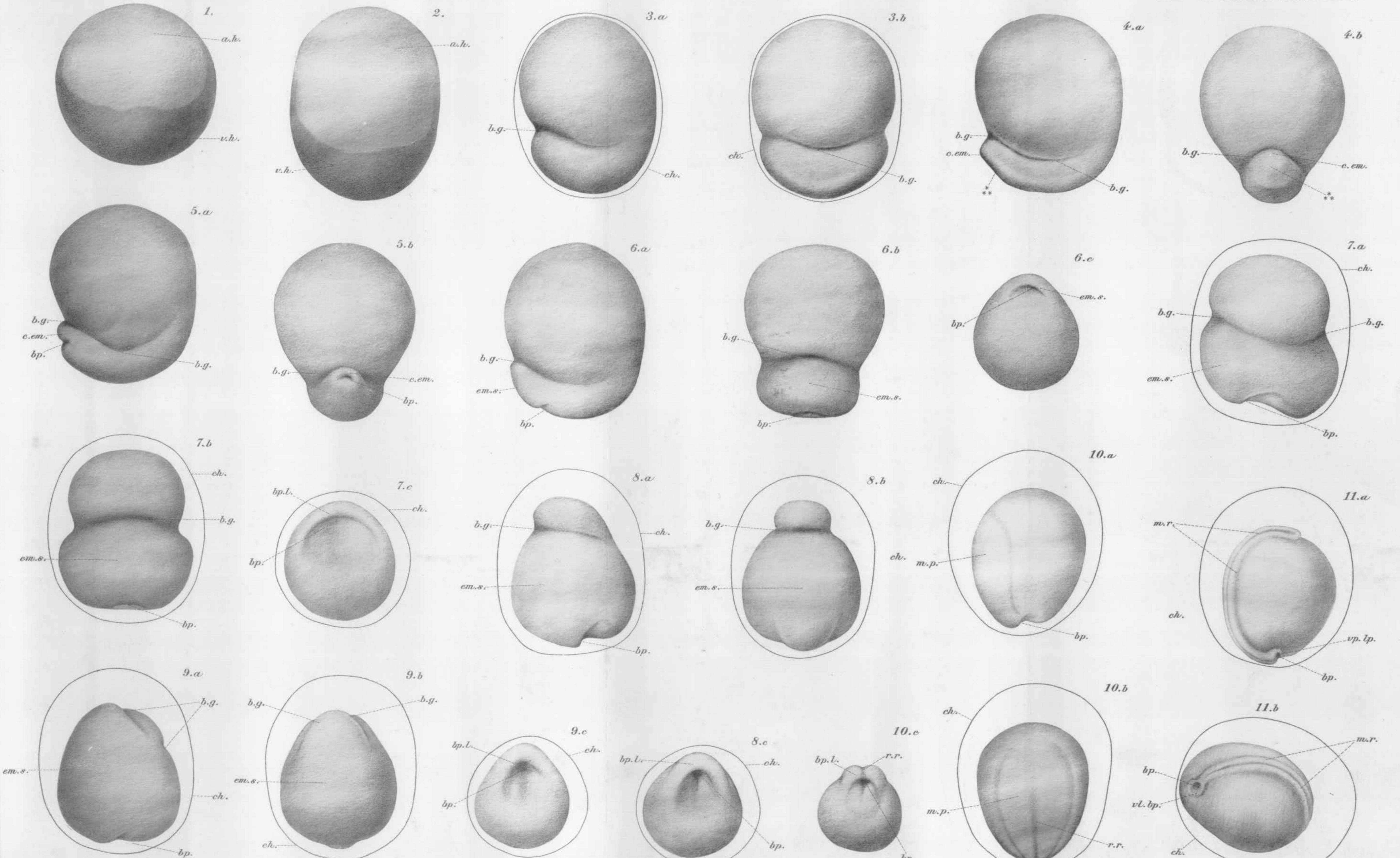
Fig. 26—A section from the ventral part of the same series as that shown in *Fig.* 25. The micromeric layer is nowhere differentiated at all. On the left side, a mere trace of the boundary groove (*b.g.*) is seen. As the plane of the section is inclined toward that side, the groove is not met with on the right side. The macromeric field shows no tendency to invaginate. The disturbed part of the cell-mass is found in this section also. ×80.

Fig. 27—An oblique transverse section through the blastoporic depression of an ovum of a stage intermediate between those represented in *Fig.* 8*a* and *Fig.* 7*a*. A shallow depression on the macromeric margin (*bp.*) shows the ventral part of the blastoporic funnel cut through. The archenteron (*ar.en.*) is roofed by the irregular macromeric epithelium (*mac.ep.*). An irregular row of macromeres on either side of and apparently continuous with the roof, (*l.mac.ep.*) represents the lateral irregular epithelium. ×80.

Fip. 28—A more dorsal section from the same series as that just described. The blastopore is represented by a deep notch (*bp.*). Lateral lips formed by the micromeric layer border the notch, this condition being brought about in consequence of complete withdrawal of the macromeres. ×80.

Fig. 29—A still more dorsal section from the same series, passing through the dorsal lip of the blastopore. The micromeric epithelium (*i.mic.ep.*) is reflected to form the archenteric roof, while the depressed macromeric field which has been withdrawn represents the archenteric floor. As the section is an oblique one, the anterior part of the archenteron is cut through lengthwise to some extent (the upper part of the figure). ×80.

Fig. 30—A horizontal section through an ovum a little younger than the stage represented in *Fig.* 10*a*. In the anterior part (the left part in the figure), the section passes through the dorsal blastoporic lip. The archenteric roof (*i.mic.ep.*) is cut through obliquely, while the posterior part (the right part in the figure) shows in section the ventral part beyond the anterior end of the archenteron. The lateral irregular epithelium (*l. mac. ep.*) is seen as a continuous layer. ×80.



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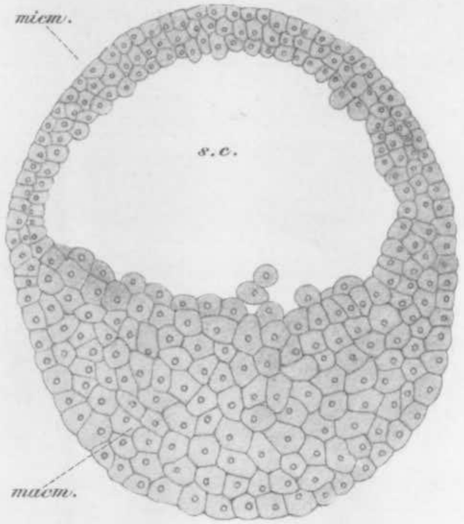

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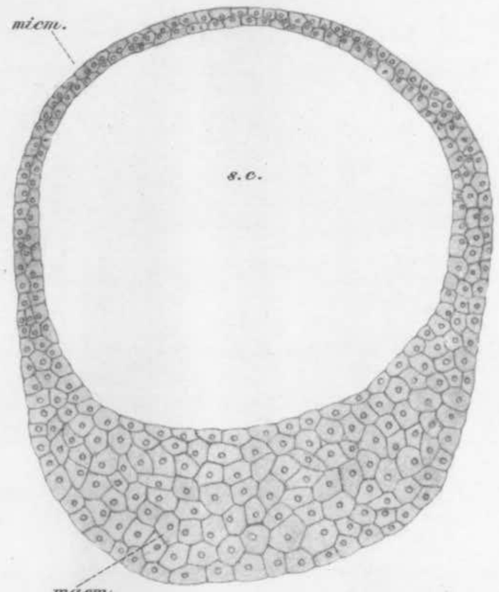

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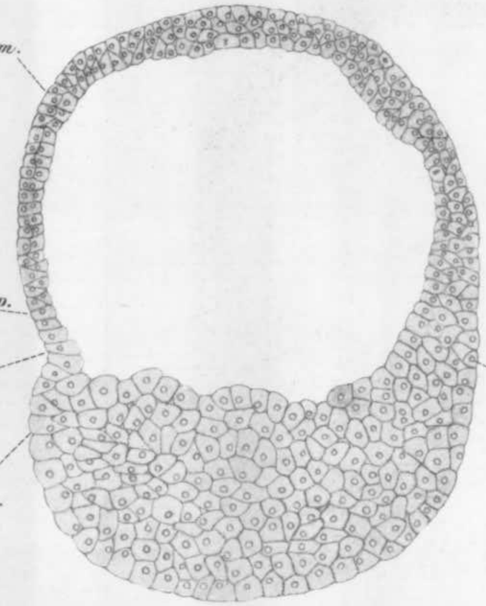

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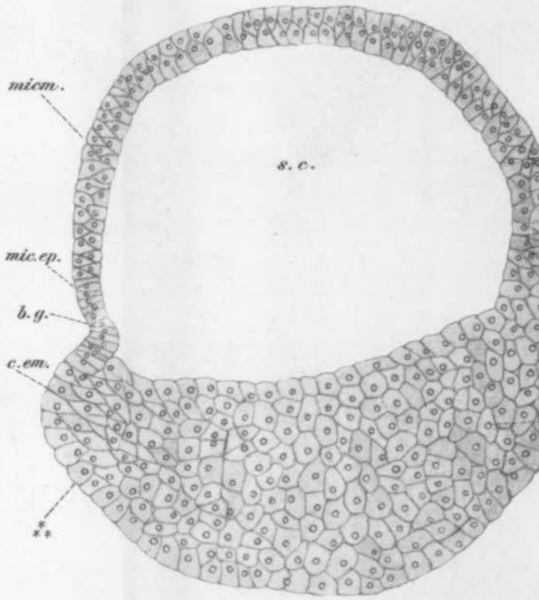

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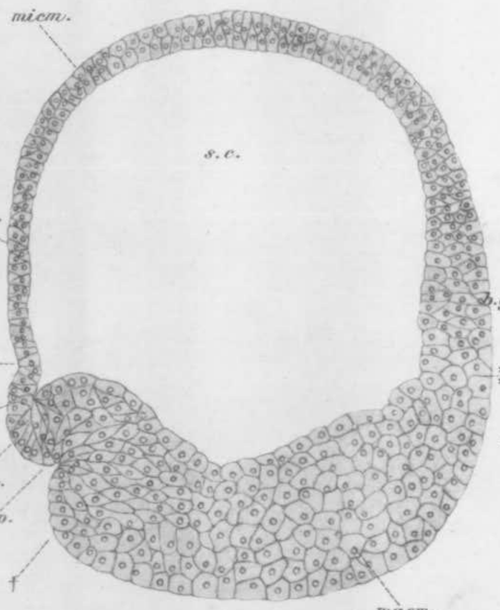

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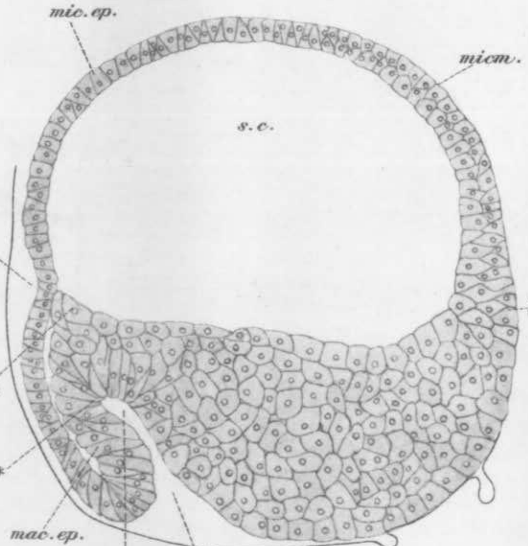

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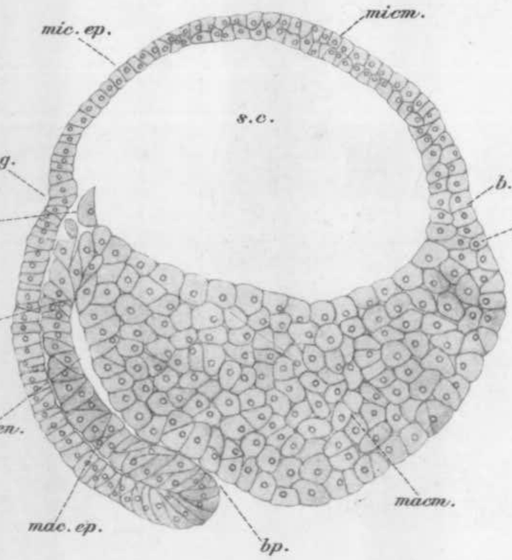

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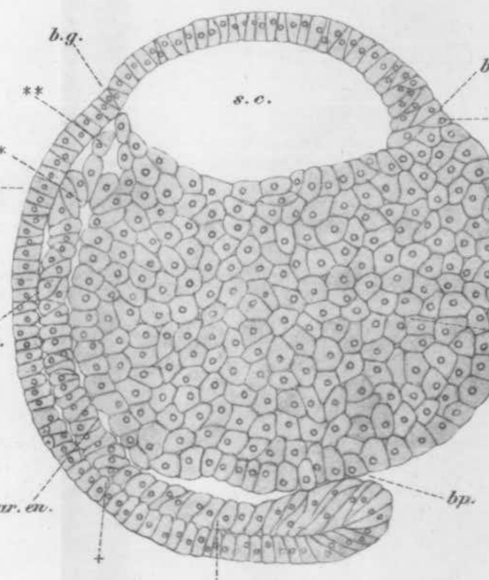

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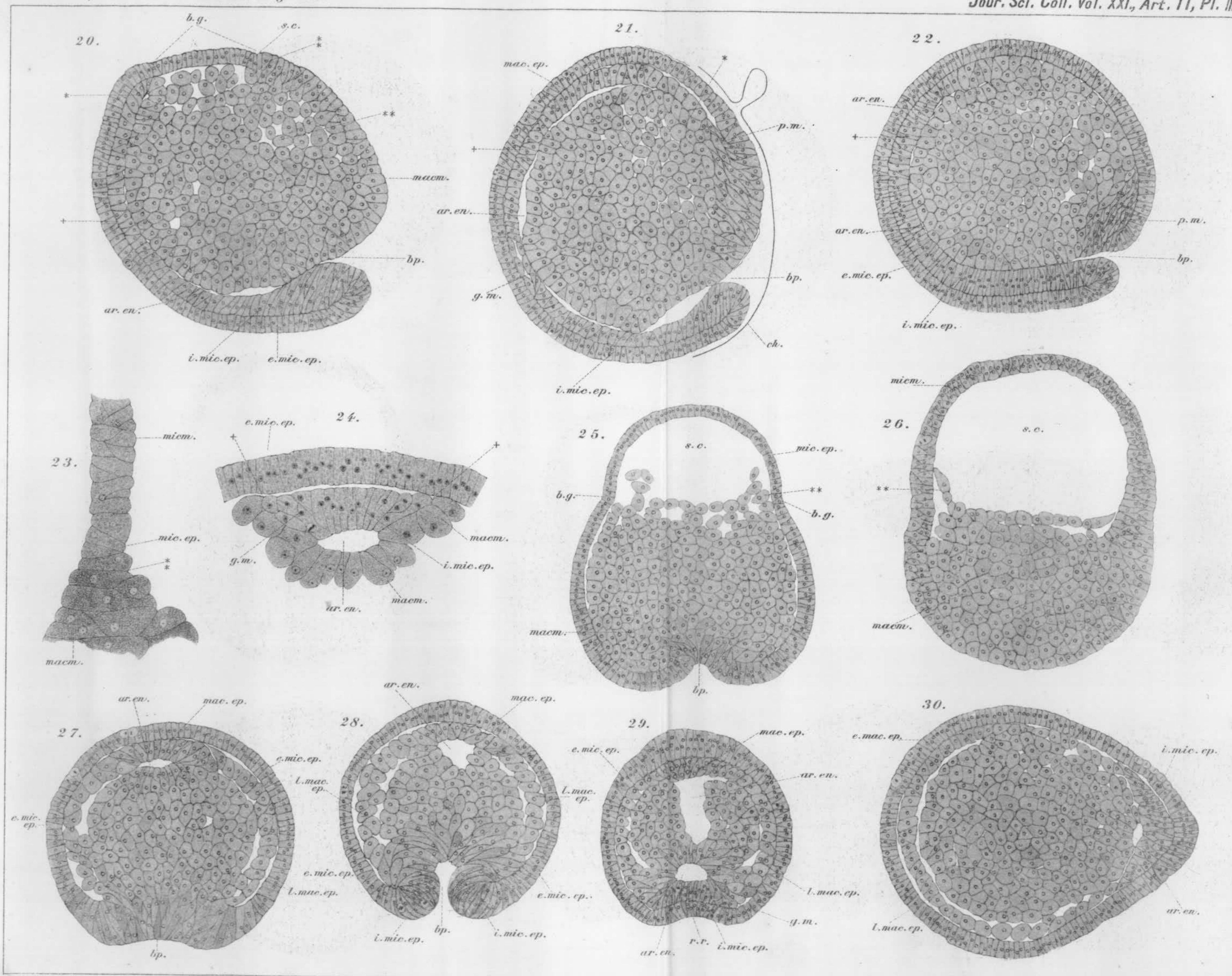
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