Further Studies on the Formation of the Germinal Layers in Chelonia.

(Contributions to the Embryology of Reptilia III).

By

K. Mitsukuri, *Ph. D., Rigakuhakushi.*

Professor of Zoology, Science College, Imperial University, Tōkyō, Japan.

With Plates II.—IV.

Some years ago conjointly with Mr. Ishikawa, I published a paper *on the Formation of the Germinal Layers in Chelonia (No. 1)*. Our observations were then made solely on Trionyx japonicus, *Schlegel.* Recently I have had an opportunity of studying the earlier stages in the development of Clemmys japonica, *Gray.* The results of my observations in the latter species confirm our views on all essential points. In addition, I have been able to elucidate more fully the earlier phases in the formation of the mesoblast not only in Clemmys but also in Trionyx. These, in my opinion, lead to conclusions of considerable importance and deserve the attention of those who are engaged in the study of the vertebrate development.

The materials for the investigation were as before obtained from tortoises which breed freely in captivity. As each deposit of eggs was marked as it was laid, I had an unusual opportunity of obtaining a complete series of embryos. In some cases, I took eggs out of one deposit at several different times, in order to determine more accurately the succession of the changes that take place.

On the present occasion, I shall not touch on the formation of the mesoblast or mesenchyma at the periphery of the blastoderm an account of which, I reserve for a future paper.

Clemmys japonica, *Gray.*

The earliest stage in my possession was obtained from the eggs opened directly after they were deposited. The blastodermic region, owing to its lighter specific gravity, is always found at the upper pole, in whatever position an egg may happen to have been deposited. Surface views of the blastoderm are represented in Figs. 1 and 1a (Pl. II). It corresponds to that shown in Figs. 1a and 1b of the paper on Trionyx (No. 1) but is somewhat younger. The blastoderm shows a comparatively large pellucid area, in which the embryonic shield is placed excentrically, nearer its posterior edge, and is joined to this edge by irregular opaque patches. The most conspicuous feature in the dorsal view of the embryonic shield (Fig. 1) is the dorsal opening of the blastoporic passage. It is placed in the median line near the posterior end of the shield, and is a crescent-shaped, transverse slit, the concavity being to the front. The embryonic shield shows indistinctly two concentric zones which are most marked in front and at the sides. The innermost area is the most opaque part of the blastoderm. Near the front edge of this area, there is however a somewhat less opaque area which corresponds, it will be seen, on referring to the ventral view (Fig. 1a), to the ventral opening of the blastoporic passage. In the same view (Fig. 1a), the innermost and most opaque area is seen to be the thick part through which the blastoporic passage leads from the dorsal to the ventral opening.

The minute structure of this early stage will become clear from a study of Figs. 5 and 5a (Pl. III, a longitudinal section) and Figs. 6–10 (a series of transverse sections).

Fig. 5 is a longitudinal section of this stage, near the median line. The blastoporic passage is very conspicuous, beginning at the dorsal surface and leading obliquely forwards to the ventral surface. There is a marked difference of structure between the part of the blastoderm in front of the passage and that lying behind it. In front of the passage, there are only two layers: the epiblast and the primitive hypoblast. The epiblast is already a well defined sheet, extending beyond the area included in the figure—though how much beyond I have not ascertained. In the transparent area in front, it is a thin layer consisting of pavement cells; as we trace it posteriorly however, the cells become taller, changing gradually from the pavement to the columnar shape. In the region directly in front of the dorsal opening of the blastoporic passage, the nuclei are in several strata, although the epiblast seems to consist in reality of only a single layer of tall cells. As regards the lower layer or primitive hypoblast, it is, in the anterior transparent area, a loose layer of cells containing many fine yolk-granules (Compare Trionyx No. 1, Fig. 15). In front it passes into the bed of yolk in which nuclei are seen. Traced backwards, it becomes at first only two or three cells deep, and then suddenly quite thick as we reach the outer of the two concentric zones seen in the surface view of the embryonic shield. The cells here are arranged in a loose network, with large meshes. Further backwards, the lower layer is formed of columnar cells and assumes an appearance like the superjacent epiblast. It is thicker, however, and seems to be formed of several layers of cells. At the anterior dorsal lip of the blastoporic passage, the epiblast becomes continuous with the primitive hypoblast.

In that part of Fig. 5 (or Fig. 5a) showing the region behind the blastoporic passage, there is a different state of things. Beginning at the hindmost part, the epiblast is already formed as far back as is given in the figure. As we trace it forward, it is distinct up to the blastoporic passage, but here it becomes merged in a large thick mass of cells which stretches along the floor of that passage. The lower layer, which is continuous behind with the yolk-bed and which is distinguished by containing a larger quantity of yolk granules, is also joined to the large mass on the floor of the blastoporic passage by irregular reticulate strands of cells. Thus, behind the blastoporic passage the layers are fused together, and there can be no doubt that the thick mass of cells is the beginning of what Rabl calls the peristomal mesoblast.* Eventually it will stretch backward for the space of 180° like an open fan, between the epiblast and the definitive hypoblast.

Figs. 6–10 are a series of cross-sections from another embryo in the same stage, arranged from behind forward. They confirm what we have learned from the longitudinal section and give us some additional information.

In Fig. 6, taken from directly behind the blastoporic passage, the epiblast is marked by a groove at about the median line, and is at this point proliferating downwards cells which form a small mass continuous with the lower layer by loose strands of the cells. This proliferation is seen more or less distinctly in several consecutive sections until it joins in front the large mass on the floor of the blastoporic passage. This median line of proliferation I take to be the commencing primitive streak. This becomes broader and more conspicuous in later stages. The same thing was seen in Trionyx (*loc. cit.*, Figs. 6 and 8).

In Fig. 7, the epiblast is no longer distinguishable in the median region, which is occupied by the solid thick mass of cells already

* C. Rabl:—Theorie des Mesoderms. Morph. Jahrbuch, 1889.

noticed in the longitudinal section. There becomes established later on at this place the yolk plug, as reference to Fig. 22 will show, and as has been proved sufficiently, I think, in the case of Trionyx (*loc. cit.*).

In Fig. 8, the section passes through the blastoporic passage which is, however, still open above on the left. The floor of the passage is proliferating cells below.

In Fig. 9, through the front part of the ventral opening of the blastoporic passage, the cells of the lower layer are arranged in the median line in a regular columnar epithelium. This is what is usually known as the chorda-hypoblast, although, as I shall show in the sequel, it gives rise to something besides the chorda dorsalis. Laterally the chorda-hypoblast becomes continuous with loose irregular reticulate masses of lower-layer cells containing a large quantity of yolk granules. Still more laterally the lower layer is reduced to a very thin sheet corresponding to the transparent region of the surface view. Outside of this it becomes continuous with the bed of yolk-matter.

As we trace the series of sections forward, the part of the lower layer showing a regular columnar arrangement becomes smaller and smaller, the loose network-like part encroaching gradually on it. Columnar cells remain longest on the lowest stratum of the median part (Compare Trionyx, *loc. cit.*, Figs 13 and 14).

In Fig. 10, the whole lower layer in the region of the embryonic shield consists of a loose network of cells.

This embryo thus consists almost entirely of only two layers, the epiblast and the primitive hypoblast. The only part which we can distinguish as the commencing mesoblast is a median mass of cells found behind, and on the floor of, the blastoporic passage, continuous above and below with the two primary layers. In front of the blastoporic passage, the chorda-hypoblast is being gradually arranged in the median line in the form of a regular columnar epithelium from cells forming the loose network of the primitive hypoblast. The process proceeds from behind forward. This embryo thus closely agrees with the first stage of Trionyx, which we described in our former paper.

Formation of the Mesoblast.

How does the embryo described above change into one in which the three germinal layers are completely and definitely established? As stated in the account of the formation of the mesoblast given in our paper on Trionyx, the process is briefly this: as the forward continuations of the peristomal mesoblast whose commencement we saw in the above stage, a pair of mesoblastic bands become gradually established, one on each side of the median chorda-hypoblast, between it and the gut-hypoblast (=*Darm-Entoblast*). This is the gastral mesoblast of Rabl. From my study of Clemmys, I am now in a position to give more exact details in regard to the first stages in the formation of this gastral mesoblast, and as will now appear I have to qualify somewhat my views of these earliest stages. This is necessitated by the discovery I have made concerning the chorda-hypoblast. I find that in Chelonia at least, only the median part of what I have hitherto called the chorda-hypoblast gives rise to the chorda dorsalis, and that each side of it becomes eventually incorporated in the mesoblast. Thus, in Fig. 11, from an embryo somewhat older than that represented in Fig. 1, the median unshaded portion alone becomes the chorda dorsalis, while the shaded lateral parts form in the sequel part of the mesoblast.

As this is a very important point, I shall try to prove it before proceeding further, from observations which in my opinion make the above conclusion inevitable.

I wish to call attention first to Fig. 23 (Pl. IV.), from the head region of a somewhat old embryo given in Figs. 4 and 4*a*. Here the gut-hypoblast (or *Darm-Entoblast*), instead of joining the chorda-hypoblast, turns upwards and becomes continuous with the mesoblastic mass. The chorda-hypoblast in immediate continuation of the chorda also turns upwards and becomes merged in the mesoblastic mass. Between the chorda-, and gut-, hypoblast, a diverticulum of the archenteron enters into the mesoblastic mass on each side of the chorda. Now it seems to me that this section can have but one interpretation, and represents, as clearly as we can reasonably expect in a meroblastic egg, the process of the mesoblast development so well known in Amphioxus. Here, however, from the outer and upper walls of the diverticulum (I am speaking more especially of the left side of the section), a mass of cells stretches outward. That this mass has budded out from the walls of the diverticulum is evident from the karyokinetic figures which we see in it. This section ought therefore, strictly speaking, to be compared to such a section of Amphioxus as is given in Fig. 40 (Pl. IV.), in which the mesoblast has stretched more ventrally. To make the comparison yet closer, the gut would have to be still in open communication with the coelom in the section of Amphioxus. Now, when the chorda, the mesoblast and the gut-hypoblast finally separate from one another in Clemmys, at what point does this separation take place? In order to answer this question conclusively, I have introduced Figs. 33–36 from the head region of another embryo of about the same stage. Fig. 33 is essentially like Fig. 23. The right side of this section is more forward than the left side and is significant, for the gut-diverticulum is quite simple, and there has not yet been as much proliferation of mesoblast cells from its walls as on the left side or in Fig. 23. But the point that I want to be particularly noticed in the section is on the left side which, as I said before, is essentially like the corresponding side of Fig. 23. Two sections behind this (Fig. 34), the hypoblast has pushed towards the median line and obliterated the gut diverticulum, of which, however, its outer and inner walls are as yet distinguishable. Fig. 35 is two sections still further behind, where the parts are pressed closely together. Two or three sections yet further back, the chorda, the gut-hypoblast, and the mesoblast have entirely separated from one another (Fig. 36). This series shows conclusively that the part of the primitive hypoblast immediately adjoining the chorda becomes a part of the mesoblast.

Now turn to Fig. 12 (Pl. III), taken from the head region of the embryo shown in Figs. 3 and 3*a*, somewhat younger than that of Fig. 4, from which Fig. 23 is taken. In this section, between the chorda-*anlage* and the gut-hypoblast, there is a stretch of columnar epithelium slightly arched upwards. From the point where this epithelium joins the gut-hypoblast, there stretches outwards a mass of mesoblast cells between the epiblast and hypoblast. Now there can be no reasonable doubt that this section presents substantially the same structure as the left side of Fig. 23. If the epiblast did not press down so closely, and if the epithelium (lightly shaded in the figure) between the points marked with † and * arched upwards with a greater curvature, the section would be exactly like the left side of Fig. 23. We therefore come to the conclusion, that the epithelium (lightly shaded in Fig. 12), stretching between the chorda-*anlage* and the gut-hypoblast, and continuous with both, ought to be considered as the walls of the gut-diverticulum, and a slight bay between † and * as representing the gut-diverticulum itself. From the outer walls of this diverticulum, the mass of mesoblast cells (darkly shaded in the figure) are stretching outwards. This section again reminds us of the section of Amphioxus shown in Fig. 40.

I wish to call attention next to the embryo represented in Fig. 2. This is interesting, because the gastral mesoblast in extending itself forwards from the peristomal mesoblast has not yet reached the anterior end of the embryonic shield, where there is as yet no head-fold, and we are thus able to see in what manner this forward extension takes place. Figs. 13–22 are sections of this embryo. If we look at Fig. 16 from the anterior part, we at once recognize its similarity to Fig. 12. There is the epithelium stretching between the chorda-*anlage* and the gut-hypoblast, and representing the walls of the gut diverticulum. From the outer end of this epithelium there stretches outwards the mesoblastic mass (darkly shaded). We see here as well as in Fig. 12 a very clear indication, (1) that the mesoblastic mass is divided into two layers: the somatopleuric and the splanchnopleuric, and (2) that the former is continuous with the above mentioned epithelium and the latter with the gut-hypoblast. The latter fact necessitates the conclusion that the gut diverticulum in extending itself into the proliferated mesoblast mass does so from its outer end.

When the chorda, the mesoblast, and the gut-hypoblast become independent of one another, at what point does the separation take place? In tracing the series backwards, we pass suddenly from sections like Fig. 16 to others like Fig. 17. This point corresponds in the ventral view (Fig. 2) probably with the place where the two converging depressions come together and form the single median groove. The change is brought about by the gut-hypoblast extending itself close to the chorda-*anlage*, thus shutting off the gut diverticulum from the enteric cavity. In other words, the point marked * in Fig. 16 has moved itself to the point marked †. In Fig. 17 the splanchnic mesoblast is still continuous with the gut-hypoblast. This inward extension of the gut-hypoblast seems to be the signal for the separation of the three structures above mentioned, for very soon we come to sections like Fig. 18, in which the chorda-*anlage*, the mesoblast, and the gut-hypoblast have separated from one another. From this, it is evident that the epithelium lying between the chorda-anlage and the gut-hypoblast (the lightly shaded part in Fig. 16) and representing the walls of thegut-diverticulum, becomes a part of the mesoblast.—the same conclusion as was reached in the case of Fig. 23.

If we now trace the series forwards from Fig. 16 we find the proliferated mesoblast mass (darkly shaded in the figures) growing smaller and smaller (Figs. 15 and 14). A few sections forward, we come to the appearances depicted in Fig. 13. In this, we find laterally the gut-hypoblast consisting more or less clearly of a single layer of columnar cells. As we advance towards the median line, this epithelium suddenly stops at some distance from the chorda-*anlage*, and between it and the latter structure we find a stretch of epithelium with more than one layer of cells. This part I have shaded lightly in the figure. The increase of cell-strata in this part is possibly due to the fact that cells here are dividing rapidly to give rise to the mesoblast mass. The darkly shaded part (the proliferated mesoblast cells) in this figure ought therefore perhaps to have been extended further inwards.

Further forwards, the chorda-*anlage*, the mesoblast, and the gut-hypoblast are all as yet undifferentiated, and the lower layer consists of a single layer of columnar cells throughout.

It is evident from the foregoing description, that the lightly shaded part of Fig. 13 is equivalent to the similarly shaded part of Figs. 16 and 12, which in its turn is comparable to the walls of the gut-diverticulum in Fig. 23, and that this part becomes eventually incorporated in the mesoblast. This obliges us to come to the conclusion that the median part of Figs. 9 and 11, which shows a regular epithelial arrangement—the part which I have hitherto called the chorda-hypoblast—does not give rise to the chorda alone but has potentially the elements of the mesoblast in it. I have tried to show what I mean by shading in Fig. 11. The median unshaded part is the part that gives rise to the chorda ; the lightly shaded stretch, immediately adjacent to it on each side, which corresponds to the similarly shaded part of Figs. 13–17, gives rise to the gastral mesoblast and becomes finally incorporated in the mesoblast. The section represented in Fig. 11 is therefore strictly comparable to the well-known section of Amphioxus reproduced in Fig. 39. According to this view, the apparently insignificant features : the bulging downwards of the chorda-*anlage*, and the slight bay on each side of this median projection receive their explanation.

I think, I have now sufficiently demonstrated the proposition I started with, viz: In Chelonia, at least, only the median part of what has hitherto been called the chorda-hypoblast gives rise to the chorda dorsalis while the lateral parts of it become eventually incorporated in the mesoblast.

I will now rapidly go over different stages and give a brief but connected history of the above occurrences, as well as of those points not yet touched upon.

I have already described in full the stage represented in Figs. 1 and 1*a*. It will be remembered that this embryo consists almost entirely of two layers, except in the region behind the blastoporic passage, where the peristomal mesoblast is beginning to form. This mesoblast soon increases in bulk and spreads out behind in all directions like an open fan. From the two edges of this fan, the formation of the gastral mesoblast proceeds gradually forward (see Fig. 2 and its sections Figs. 13–22). It has sufficiently been shown above that a certain stretch of epithelium found on each side of the chorda-*anlage* in early embryos (like that given in Figs. 1 and 1*a*) becomes eventually a portion of the mesoblast. Only there is as yet nothing to distinguish it as such. While the remaining part (i.e. the gut-hypoblast) of the lower layer becomes gradually reduced to a single stratum of columnar cells, this stretch of epithelium distinguishes itself by proliferating cells which spread partly upwards, but mostly outwards between the epiblast and the gut-hypoblast (the embryo given in Fig. 2 and its sections Figs. 13–22). At this stage, therefore, the internal end of the gut-hypoblast on each side is at a considerable distance from the chorda-*anlage*. (Figs. 12 and 16). The gut-hypoblast, however, soon extends itself towards the chorda-*anlage*, shutting off the epithelial stretch in question from the enteric cavity (Fig. 17). This inward extension of the gut-hypoblast is probably the cause of the grooves converging posteriorly into the single median chorda groove seen in the surface views, Figs. 2 and 3*a*. This movement seems also to be the signal for the separation of the three structures: the chorda-*anlage*, the mesoblast, and the definitive or gut-hypoblast (Fig. 18). The separation is, however, complete only in the middle of the embryo, for, as we go backward and approach the blastoporic passage, the three structures are again united (Fig. 19 and 20). This is necessarily the case, I think, because the posterior part of the embryo is growing in length. In Fig. 22, we see that the yolk-plug has become very distinct. This has been brought about by the change of shape in the dorsal opening of the blastoporic passage. Whereas it is at first concave forwards as in Fig. 1, it becomes later horse-shoe shaped (see Figs. 3 and 4), with the concavity turned backwards.

Of an older embryo (Figs. 4 and 4*a*) with the head-fold and the commencing amnion, a section from the head (Fig. 23) has already been sufficiently described. As we pass backward, we see that the cavity of the gut-diverticulum is obliterated (Fig. 24). A little further behind, three structures: the chorda-*anlage*, the mesoblast, and the gut or definitive hypoblast become separated after the manner shown in Figs. 33–36. We then obtain a section such as Fig. 25. In the middle region of the body, the formation of the chorda dorsalis is completed (Figs. 26 and 27) exactly in the way described in our Trionyx paper (No. 1). Further backwards, the definitive hypoblast of two sides which is united across in Fig. 27, separates again from each other (Fig. 28) and still more posteriorly, the three structures above mentioned become united once more (Figs. 29 and 30). Figs. 31 and 32 are so exactly like Figs. 21 and 22, or similar figures in Trionyx, that they need no special description here.

Trionyx Japonicus. *Schlegel*

After making out the earlier phases in the formation of the gastral mesoblast in Clemmys, I thought it necessary to reexamine Trionyx. I succeeded in finding two embryos in which the gastral mesoblast had not yet reached the anterior end and was in process of formation. After cutting them into sections, I found that they present exactly the same structures as the Clemmys embryo given in Fig. 2. In order to prove this point, I have selected and figured two sections (Figs. 37 and 38) from one of the embryos. In Fig. 37, the gut-hypoblast is at some distance from the median line, and there is a stretch of epithelium between it and the chorda-*anlage*, which is continuous externally with the proliferated mesoblast mass. Several sections behind, in Fig. 38, the gut-hypoblast has shifted its inner end to the chorda-*anlage*, and the aforesaid stretch of epithelium has been cut off from the enteric cavity and incorporated with the mesoblast.

In a note published in the *Anatomischer Anzeiger*, No. 7, 1891 I called attention to the section given in Fig. 23 of the present paper, and Figs. 25 and 29 of our Trionyx paper, and pointed out that while both represented essentially the same structure, mesoblast cells in Trionyx were budded off as separate stellate cells, while those in Clemmys appeared as a more or less solid mass. I have now to qualify that statement. On referring to the original sections from which Figs. 25 and 29 of Trionyx were drawn, I find that they are very faithful representations. But in sections which I have obtained lately, I find that the cells appear aggregated in a loose mass, and are by no means so separate as in Figs. 25 and 29. This difference, I think, is due to difference in the fluid in which they were killed. The earlier embryos were treated with corrosive sublimate, while the later ones were all preserved in picro-sulphuric acid. I am now inclined to thinkt that, Trionyx and Clemmys are after all, not so unlike in this respect.

So far as I am aware, the facts described above in regard to the earlier phases in the formation of the gastral mesoblast are now brought out for the first time. It appears to me that they have considerable significance, for they make the process of the mesoblast formation in higher vertebrates agree with the same process in Amphioxus much more closely than we have hitherto been able to do. These facts have also, in my opinion, an important bearing in explaining the mesoblast formation in the chick. Fig. 100 in Balfour's *Comparative Embryology*, Vol. II. is substantially the same as Fig. 17 or 38 of the present paper. Balfour says :—" From what has just been said, it is clear that in the region* of the embryo the mesoblast originates as two

* I. e. in front of the primitive streak.

lateral plates split off from the hypoblast, and that the notochord originates as a median plate, simultaneously with the mesoblast, with which it may sometimes be at first continuous." (*loc. cit.* p. 157. edition of 1885). Now suppose that the earlier phases of the mesoblast formation in Chelonia are very rapidly gone through or altogether skipped over, and the state represented in Figs. 17 and 38 is reached *per saltum:* we shall then necessarily obtain appearances which would make us conclude that the lateral plates of the mesoblast, or at least the part of it immediately adjacent to the chorda, becomes split off from the hypoblast. My contention then is that the process of the mesoblast formation in the chick is an extremely abbreviated form of the process described above in Clemmys or Trionyx. The question naturally arises whether cells are added on to the mesoblast from the gut-hypoblast, outside of the stretch of epithelium representing the walls of the gut-diverticulum. I wish to leave this question open at present: there are appearances which lead me to think so, but they are by no means conclusive. Theoretically, there should be no cells added outside of the gut-diverticulum.

SCIENCE COLLEGE, JUNE, 1891.

Contributions to the Embryology of Reptilia.

(No. 1.) I. On the Formation of the Germinal Layers in Chelonia. Quart. Jour. Micr. Sc., Vol. 27. Also reprinted in Jour. Sc. College, Tōkyō, Vol. I, pt. 3,

(No. 2.) II. On the Foetal Membranes of Chelonia. Jour. Sc. College, Tōkyō. Vol. IV., pt. 1,

Explanation of Figures.

Plate II.

Fig. 1.—Dorsal view of a Clemmys embryo taken out 1 hour after its deposition. Zeiss aa × 2. (XXXV)

Fig. 1a.—Ventral view of the same. aa × 2.

Fig. 2.—Ventral view of a Clemmys embryo 3 days old. aa × 2. (XXXVII).

Fig. 3.—Dorsal view of a Clemmys embryo 3 days old. aa × 2. (XXXVII).

Fig. 3a.—Ventral view of the same. The head-fold marks definitely the anterior end of the embryo. aa × 2.

Fig. 4.—Dorsal view of a Clemmys embryo 3 days old. aa × 2. (XXXXVIII).

Fig. 4a.—Ventral view of a Clemmys embryo 3 days old, from the same deposit as Fig. 4. Note the two depressions, one on each side, immediately behind the head-fold and partly covered by it. These depressions are the diverticula of the enteric cavity seen in Fig. 23. aa × 2.

Plate III.

Fig. 5.—Longitudinal section of the Clemmys embryo represented in Figs 1 and 1*a* near the median ventral line. BB × 2.

Fig. 5a.—Part of Fig. 5 more highly magnified. DD × 2.

Fig. 6-10.—Selected transverse sections from a Clemmys embryo from the same deposit and of the same stage as Fig. 1. BB × 4. (XXXV*a*).

> *Fig. 6.*—From directly behind the dorsal opening of the blastoporic passage.
>
> *Fig. 7.*—Still nearer the dorsal opening of the blastoporic passage.
>
> *Fig. 8.*—Through the blastoporic passage.
>
> *Fig. 9.*—Anterior to the ventral opening of the blastoporic passage.
>
> *Fig. 10.*—Through the anterior part of the embryonic shield.

Fig. 11.—Transverse section somewhat in front of the ventral opening of the blastoporic passage. From a Clemmys embryo a little more advanced than Fig. 1. This is introduced to show the destiny of different parts of the lower layer (or primitive hypoblast): the median unshaded part becomes eventually the notochord, the lightly shaded part on each side immediately outside the

chorda-*anlage* gets incorporated in the mesoblast, and the parts external to these shaded portions become the gut or definitive hypoblast. This should be compared with the section of Amphioxus reproduced in Fig. 39. BB × 4.

Fig. 12. Transverse section from the anterior part of the Clemmys embryo represented in Figs. 3 and 4*a*. DD × 2.

Figs. 13-22. Selected transverse sections from the Clemmys embryo given in Fig. 2. DD × 2.

Figs. 13-16.—From the anterior part, show the manner in which the gastral mesoblast extends itself forwards. The gut-hypoblast is at a considerable distance from the chorda-*anlage*. The lightly shaded part is the part of the original primitive hypoblast that becomes incorporated in the mesoblast. The darkly shaded part represents the mesoblast mass which has been budded out from the lightly shaded part.

Fig. 17.—The gut-hypoblast has extended itself inwards to the chorda-*anlage*.

Fig. 18.—A little behind Fig. 17. The chorda, the mesoblast and the gut-hypoblast have separated from one another.

Fig. 19.—Further behind. The three structures have again united.

Fig. 20.—Directly in front of the ventral opening of the blastoporic passage.

Fig. 21.—Through the blastoporic passage.

Fig. 22.—Through the yolk-plug.

Plate IV.

Figs. 23-32.—Selected transverse sections from the Clemmys embryo given in Figs. 4 and 4*a*. DD × 2.

Fig. 23.—Through the head region and the gut-diverticula. See the ventral surface view, Fig. 4*a*.

Fig. 24.—Third section behind.

Figs. 25-28.—From the middle region of the body, showing the formation of the notochord.

Figs. 29 & 30.—In front of the ventral opening of the blastoporic passage The chorda-*anlage*, the mesoblast, and the gut-hypoblast have again united with one another.

Fig. 31.—Through the blastoporic passage.

Fig. 32.—Directly behind the dosal opening of the blastoporic passage.

Figs. 33-36.—Selected transverse sections from the anterior part of a Clemmys embryo very similar to Fig. 4 and from the same deposit. Introduced to show the manner of separation of the three structures: the notochord, the mesoblast and the gut-hypoblast. Fig. 33, BB × 4, Figs. 34-36, DD × 2. (XXXXVIII).

Fig. 33.—Through the head-fold.

Fig. 34.—Second section behind.

Fig. 35.—Second section behind.

Fig. 36.—Fifth section behind.

Figs. 37-38.—Two transverse sections from a Trionyx embryo 1½ days old. DD × 2. (127).

Fig. 37.—Is more forward. The gut-hypoblast is at some distance from the chorda-*anlage* and the median line.

Fig. 38.—The gut-hypoblast has extended itself inwards to the chorda-*anlage*.

Figs. 39-40.—Sections of Amphioxus. From Hatschek, after Hertwig.

Fig. 1

Fig. 4

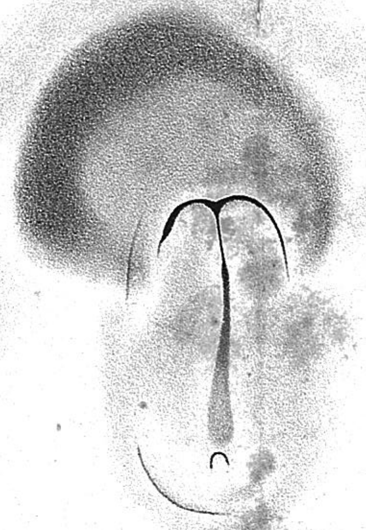


Fig. 2

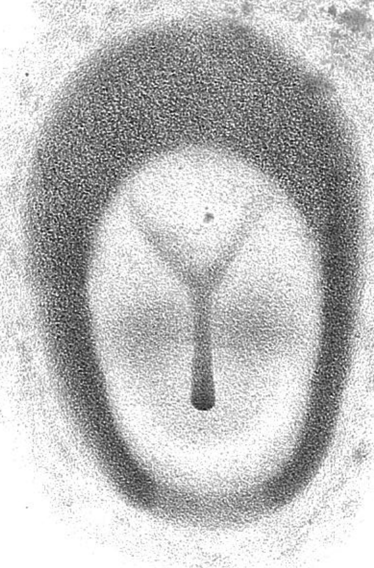


Fig. 1a

Fig. 3

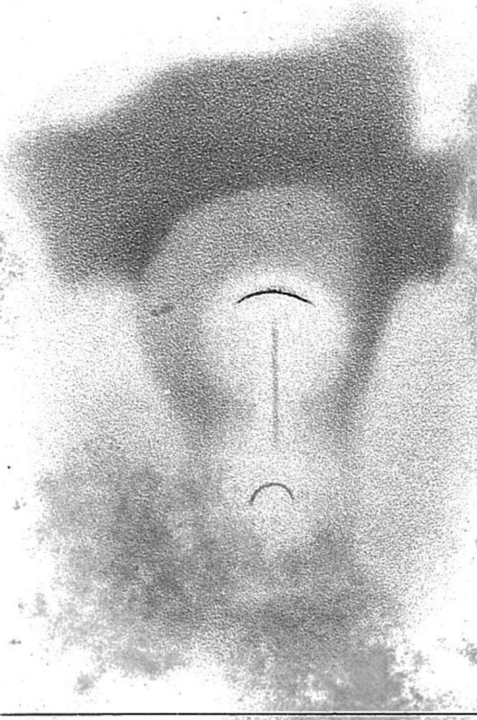


Fig. 3a

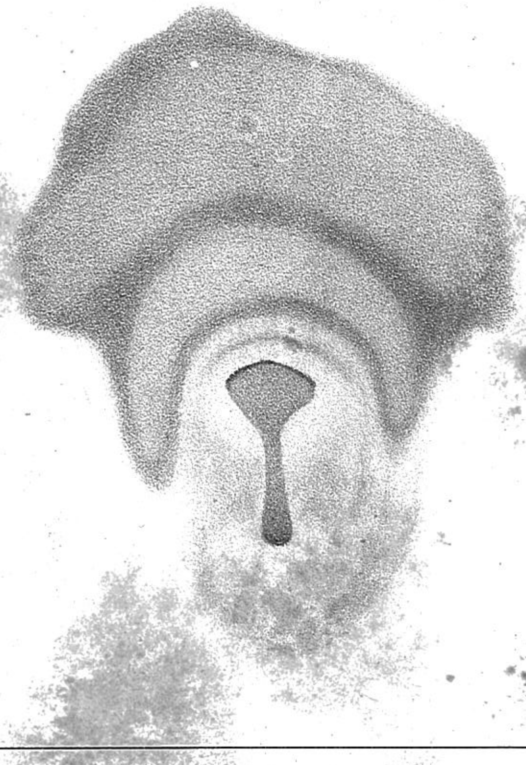
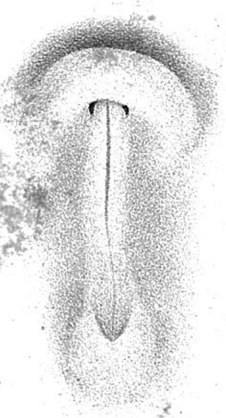
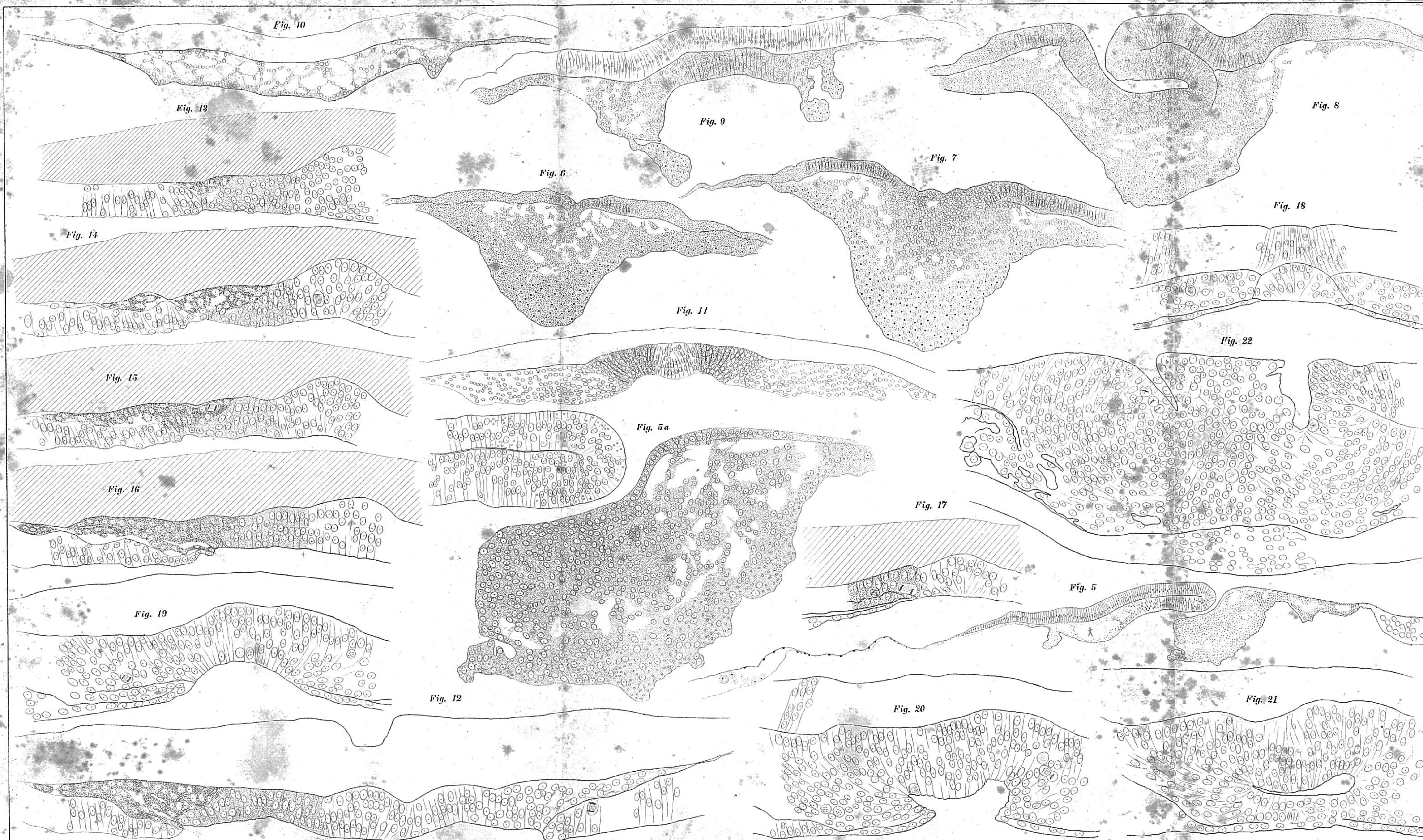


Fig. 4a



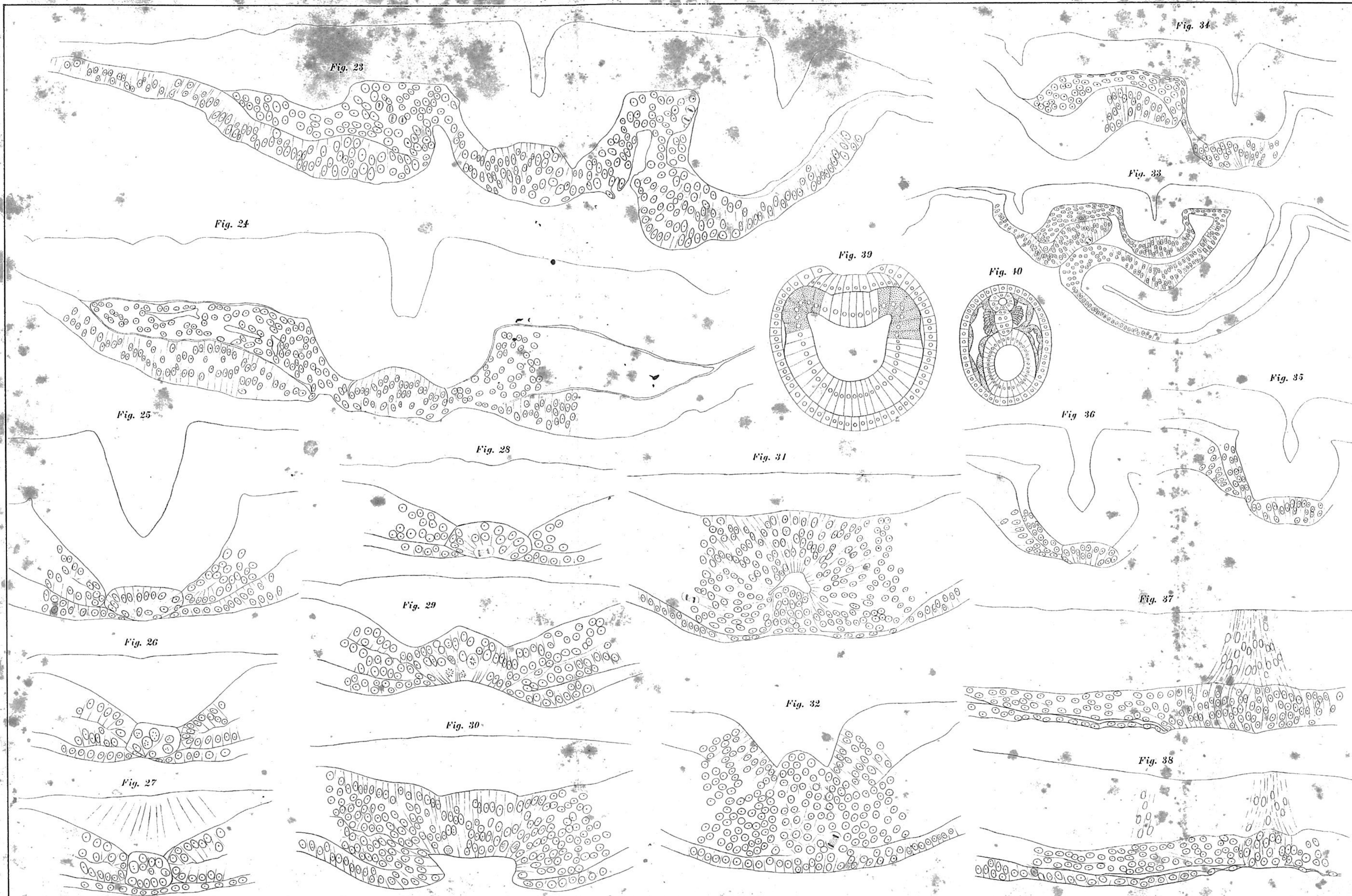
K. Mitsukuri & K. Nagahara del.

Lith. & Imp. The. Seishi-bunsha.



K. Mitsukuri & K. Nagahara del.

Lith. & Imp. The Seishi-Bunsha.



K. Mitsukuri & K. Nagahara del.

Lith. & Imp. The. Seishi-bunsha.