On the Formation of the Germinal Layers in Petromyzon.

By

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With Plates XIII. and XIV.

In spite of the fact that several eminent investigators have been engaged in studying the embryology of the Cyclostomata, the development of Petromyzon presents as yet many points which remain obscure or which are stated in contradictory terms by different writers. The present investigation was undertaken to throw light on some of these points. It was carried on during the academic year of 1890–91 in the Zoological Laboratory of the Science College, under the supervision of Profs. K. Mitsukuri and I. Ijima. I may, in this place, be permitted to express my sincere thanks for the valuable advice which they have given during my work, and the kind manner in which they have forwarded my researches.

The species which I have studied is probably either *Petromyzon planeri* or a variety of it. The larger part of the materials used was collected and preserved by Prof. Mitsukuri during the month of February, 1890, in fresh-water streamlets between paddy-fields near the town of Gifu, and my special thanks are due to him for placing them at my disposal. The other part of the materials was obtained by myself at the same place during the spring of the present year (1891). We wish to express our deep obligations to Mr. Nawa, the enthusiastic naturalist of Gifu, without whose aid we could not have succeeded in obtaining the objects of our search, and to the authorities of the Middle, and of the Normal, Schools of that town for affording us laboratory accommodation.

The eggs and larvæ were hardened partly in Kleinenberg's picro-sulphuric acid, and partly in corrossive sublimate. A few larvæ were also killed in Flemming's solution. The sublimate specimens gave the best results, although good sections were obtained in all cases. Acids, on the whole, had the effect of making yolk-granules highly refractive, and thus greatly increased the difficulties of observation.

As to the staining fluids, picro-carmine gave by far the most satisfactory results. This, being the nuclear stain and not affecting yolk granules, made observation comparatively easy. Other colouring fluids like borax-carmine or haematoxylin (of Kleinenberg as well as of Böhmer) stained diffusely both the protoplasm and the nuclei, and above all, were most unsuited for my purpose from the fact that they stained yolk granules the deepest.

The age of the eggs and of the larvæ was not determined by the actual time elapsed, but by their appearances as opaque objects and by their structure as shown by sections.

Care was taken to have sections of each stage made in several planes so as to enable us to obtain as accurate an idea as possible of it.

I shall give the results of my investigation under the following heads :—

1. The Gastrulation.
2. The Formation of the Mesoblast.

I. The Gastrulation.

My observations on segmenting eggs are too incomplete to enable me to make any definite statement in regard to the process. The earliest stage on which I wish to offer remarks is that of the oldest morula. When this is viewed as an opaque object, the animal pole and the vegetative pole can be externally distinguished: the former is whitish and somewhat translucent both in fresh and in hardened specimens, while the latter is opaque and of a pale yellow colour. A slight depression goes round the egg marking the boundary between these two portions.

In sections (fig. 1) the contrast of the two portions is well brought out. A spacious segmentation cavity is placed excentrically in the egg and occupies the whole interior of the animal pole. The cells forming the roof of this cavity are arranged in 2–4 layers and, compared with the cells of the opposite pole, are smaller, comparatively free from yolk granules, and have smaller nuclei which stain on the whole deeper. The cells forming the floor of the segmentation cavity are 7–8 deep, full of yolk-granules with other characters as indicated above. All the cells are polygonal in shape, evidently through mutual pressure.

The next step of development consists in the gradual thinning out of the roof of the segmentation cavity. Opinions differ in regard to the extent to which this process is carried before gastrulation sets in. According to Scott[1] and Shipley,[2] the roof of the cavity becomes one cell layered before the gastrulation begins. On the other hand,

1. W. B. Scott:—Beiträge zur Entwicklungsgeschichte der Petromyzon. Morph. Jahrb. Bd. VII.

2. A. E. Shipley:—On some points in the Development of *Petromyzon fluviatilis*. Quart. Journ. Micr. Sci., Vol. 27.

Kupffer[1] maintains that the process of invagination takes place, at the same time as the differentiation of the epiblast. He states further: "*Ehe der Vorgang der Blastodermbildung die Region der grössten Zellen an Gegenpol erreicht hat, erschein bereits der Blastoporus. Das Blastulastadium is hier also verkürzt, die Bildung der Blastosphäre coincidirt mit dem Beginn der Gastrulation.*"

Although I can not be positive about this point, my observations tend to confirm the views of Kupffer. Among a number of sections belonging to these stages, I can not find a single one which has the roof of the segmentation cavity consisting of a single layer of cells. I have, however, one series in which I think the process of invagination is just ready to begin. I have figured a section of this series in fig. 2. Pl. XIII. It will be seen that in this egg a portion of the side wall of the segmentation cavity is thinned out into one layer of cells which have assumed a regular columnar shape. This is probably the point where invagination begins, and possibly corresponds with the differentiated region in fig. 10 of Kupffer's paper. The rest of the roof of the segmentation cavity is still two to three cells thick. I have also some eggs, in which, while the gastrulation is going on, the roof of the segmentation cavity is in some places one cell and in other places two cells thick. In such cases, wherever the roof consists of a single layer, the cells assume a columnar shape, different from the irregular polygonal shapes of earlier stages.

As to the manner in which the roof of the segmentation cavity thins out, previous writers seem to consider that it is brought about by the inner cells of the roof passing round to the sides and to the floor of the segmentation cavity, while the outermost layer of cells becomes the definitive epiblast. If the upper cells passed really to the sides and to the floor of the segmentation cavity, they ought to be

1. C. Kupffer:—Die Entwicklung von Petromyzon Planeri. Arch. f. Mikros. Anat. Bd. 35.

easily recognized in these parts, especially on the floor, as they are so markedly different from the cells of the vegetative pole. Any trace of such migration is however not to be found. I am strongly inclined to believe that the thinning out is brought about by the cells of different layers in the roof of the segmentation cavity wedging themselves in between one another, thus gradually reducing the number of layers. As this process of wedging in necessarily increases the superficial extension the cells are able to spread themselves to the invaginated tube as well as over the cells of the vegetative pole by epibole during the gastrulation. Hence the thinning out is continued and carried on at the time that the invagination is taking place. According to this view, the definitive epiblast is not formed necessarily by the cells which formed the outermost layer of the animal pole in earlier stages, but by those which were left after other cells have been invaginated.

The gastrulation now commences. Eggs undergoing this process show several marked changes even when viewed as opaque objects. In the first place they acquire the appearance of being solid and firm, no doubt in consequence of the obliteration of the segmentation cavity. They also decrease in their absolute size, as can be easily perceived by the naked eye. This must necessarily be the case when we remember that a large portion of the cells is invaginated into the inside of the egg. The shape of the egg also undergoes change. Instead of being almost spherical as hithertofore, they have become more or less elongated in the longitudinal axis of the future embryo. The anterior end can now be told from the posterior, as the former is rounded, while the latter is pointed. The dorsal surface can likewise be distinguished from the ventral, as the former shows a faint median ridge. Thus the egg assumes a distinct bilateral symmetry.

The internal changes accompanying these alterations in outward appearance can be gathered from figs. 2, 3, and 4, Pl. XIII. As already stated, I am inclined to believe that fig. 2 shows the stage in which the invagination is just commencing. The cells where this process begins (*bp.* fig. 2) assume first of all a columnar shape, while the rest still remain materially unchanged. Once begun, the invagination progresses rapidly, the roof of the invaginated tube being formed by the cells coming from the animal pole, while its floor is furnished by the yolk cells. According to Shipley *(loc. cit.)* and Kupffer *(loc. cit.)*, the epiblast extends itself over the vegetative half of the egg by the conversion of the outermost row of yolk-cells into small columnar cells. This appears to me very doubtful. I think it more probable that the whole yolk-cells are bodily invaginated into the interior, *i. e.*, the point marked *x* in fig. 2 moves itself constantly towards the blastopore (*bp.*), so that the whole mass of yolk-cells is really pushed inside. According to this view, what was at first the external surface of the yolk-cell mass, becomes the floor of the invaginated tube. The segmentation-cavity is pushed forwards and early obliterated. This process is very much like that described by Hertwig[1] in Triton.

Fig. 4 represents a median sagittal section through an egg in which the invagination is almost completed. Fig. 3 is a transverse section of an egg a little younger. From these two sections some idea of the character and disposition of the cells forming the different layers can be easily formed. The roof of the mesenteron consists from the first of a single layer of columnar cells appressed against the under surface of the epiblast. I could not find throughout the entire extent of the invaginated tube, either in longitudinal or transverse series, any trace of cells interposed between the epiblast and hypoblast. I can not therefore accept Scott's invaginated mesoblast *(loc. cit.)*, nor can I find any trace of Kupffer's teloblast *(loc. cit.)*. The epiblast cells are also columnar

1. O. Hertwig:—Die Entwicklung des mittleren Keimblattes der Wirbelthiere. Jena, 1883.

but smaller and less tall than the hypoblast cells. Their cell-limits are also more distinct. The floor of the invaginated tube is formed by the upper row of yolk-cells, which have assumed a more or less columnar shape.

I wish to emphasize here the fact that the invaginated tube is not the same as the cavity of the future mesenteron. As the subsequent history shows, the whole mass of yolk-cells is absorbed with the exception of the outermost row lying immediately inside the epiblast. This latter becomes the definitive hypoblast (fig. 27 and fig. 19). Substantially the same thing has been shown by Scott (*l. c.* pp. 121–122), except that he regards the outermost row as destined to form the mesoblast, and the row next inside as forming the definitive hypoblast. When once we remember the above fact, one can easily perceive that what Kupffer believes to be the liver, in his figs. 27 and 28 (e), could not be such. This organ can not arise among the cells which are afterwards absorbed. I am inclined to think that what he calls the liver in his fig. 28 is the slit which arises between the main body of yolk-cells and its outermost row (cf. fig. 27).

II. The Formation of the Mesoblast.

At about the time when the gastrulation is almost completed, the egg undergoes one of the most important changes. To the two primary layers already dealt with, a third layer, the mesoblast, is added. The formation of this layer forms one of the most difficult subjects in the study of the embryology of *Petromyzon*. The alterations at first met with are altogether in the internal structure and can therefore be made out only in sections.

For the sake of convenience, I shall treat of the mesoblast under the following heads :—

A. The mesoblast in the neighbourhood of the blastopore (or the peristomal mesoblast of Rabl)

B. The mesoblast on each side of the dorsal axial organs (or the gastral mesoblast of Rabl)

C. The lateral and ventral portions of the mesoblast.

D. The metameric segmentation of the mesoblast.

A. *The mesoblast in the neighbourhood of the blastopore*, (or the *Peristomal Mesoblast* of Rabl) has never yet been described in Petromyzon by previous writers. I have, however, obtained sections from which it appears to me safe to conclude that mesoblast cells are budded out from all around the lip of the blastopore except the dorsal median point where the epiblast turns round to join the hypoblast. Fig. 5 represents the median sagittal section through an egg in which the gastrulation is already completed. In this figure, we find the floor of the invaginated tube formed, for a short distance from the blastoporic opening, of specially small cells. In continuation with this stretch of small cells, there is seen a wedge-shaped aggregation of similar small cells (*mes. p.*) extending ventrally between yolk-cells and the epiblast. It is marked off from the epiblast by a slit, while it lies in contact with yolk-cells and is marked off from these only by the small size of its constituent elements. In this mass, as well as in the stretch of small cells forming the floor of the invaginated tube, many karyokinetic figures are seen and testify to the fact that cells are very rapidly multiplying in this region. This mass is in my opinion the *peristomal mesoblast* (of Rabl).

Fig. 6 is a section from the same series passing through the lateral lip of the blastopore. Here we find a mass of similar small cells, intervening between yolk cells and the epiblast, and stretching not only ventrally but also dorsally.

In embryos a little more advanced, these relations are much more clearly brought out. Fig. 7 is the median sagittal section of such an embryo. We again find the mass of small cells budded out from cells forming the floor of the invaginated tube for a short distance from the entrance.

Figs. 8–11 are a series of frontal sections from an embryo of about the same stage. Fig. 11 is the most posterior represented. It passes through the line *ab* in fig. 12, *i.e.*, just through the ventral lip of the blastopore. Here the mesoblastic mass which is distinguished by the small size of its cells and the intensive staining of their nuclei, is not paired but is continuous across, being interposed between the epiblast and the yolk-cells. The median portion of it is continuous with the epiblast and reminds us strongly of a section through the primitive streak of higher vertebrates, with which it is no doubt homologous. Fig. 10 shows the section passing through the line *cd* in fig. 12. The mesoblastic mass is on the whole paired but still continuous across the median line, as the section is not yet out of the region where mesoblast cells are being budded out from the floor of the invaginated tube. Fig. 9 is through the line *ef* in fig. 12. The mesoblastic mass is distinctly paired in this section, and passes in front into the gastral mesoblast in fig. 8. These facts are sufficient, I think, to justify my conclusion stated above, viz: that mesoblast cells are budded out from all around the lip of the blastopore except the dorsal median point.

B. *The gastral Mesoblast.* In the region of the body anterior to the blastoporic opening, the mesoblast arises as paired masses, one on each side of the chorda-*anlage*. The manner in which this mass is formed, is fundamentally the same throughout every portion where it is found ; but in different regions, it presents some modifications. In the cephalo-cervical region, the process has retained best its primitive characteristics, while in the region of the trunk, it has become altered to a great degree, owing no doubt to the presence of the yolk-cells. On this account, we shall begin with the description of the mesoblast formation in the first named region, although in point of time it is formed latest in this part, as the formation of the gastral mesoblast proceeds from behind forwards.

As has been shown by Scott, (*l. c.*, Taf. VIII, fig. 10 b) the head is formed by the blind end of the invaginated tube protruding itself above the general surface of the egg. The head when first formed is thus a double-walled tube—the wall of the invaginated tube being enclosed by the epiblast. Fig. 19 is a section from near the blind end of the invaginated tube at the time when the head is ready to protrude. The lumen of the invaginated tube is here more expanded than further behind. Its roof is formed by a regular columnar epithelium in contact with the epiblast. Adapting itself to the slightly thickened medullary plate, the columnar epithelium is slightly bulged out downwards in the median line. This median part becomes afterwards the *chorda dorsalis* while the part immediately adjacent to it on each side forms the gastral mesoblast. Laterally, in continuation with the epithelium of the invaginated tube, the outermost row of the yolk-cells is arranging themselves into a regular epithelium. This, as the subsequent history shows, becomes the wall of the definitive gut, all the yolk-cells enclosed within this layer being eventually entirely absorbed (cf. fig. 27). This, in my opinion, corresponds with the layer marked *z* in fig. 11b. or that marked *Dm* in figs. 11a and 14 in Taf. VIII of Scott's paper (*loc. cit.*), although this writer regards the layer as giving rise to what he calls the "*Dotter-mesoderm.*"

Fig. 20 shows a transverse or rather oblique section through the head-region of an embryo a little more advanced. The neural cord has made a considerable progress and by its downward growth has pressed down the median portion of the gut-roof, *i. e.* the chorda-*anlage*. The latter is formed of a regular row of rather high columnar cells. Its lateral continuation on each side is reflected upwards and forms a deep diverticulum of the enteric cavity. This forcibly reminds us of the well known section of *Amphioxus* and the sequel shows that the resemblance is of a deep significance.

In fig. 21 taken from a somewhat advanced embryo, the development has advanced considerably. The chorda has now been completely isolated from its mother-layer, and lies firmly appressed with its upper margin against the neural cord, in which it causes a slight indentation. Owing to this isolation, the part of the gut-wall which was before situated immediately lateral to the chorda-*anlage*, has now slipped itself below the chorda, shutting the latter from the enteric cavity. Hypochordal cells (*hch*) are derived from this part. On each side of the median line, a conspicuous ridge or fold (*x*) of the outer wall of the enteric cavity has raised and pushed itself towards the median chorda-prominence, thus reducing the previously wide gut-diverticulum to a mere slit and by that process separating off a triangular mass of cells, the mesoblast (*mes. f.*).

In fig. 22, which represents a still more advanced stage, the mesoblast mass on each side has been entirely cut off from the wall of the enteric cavity. The constriction is solely brought about by the inward growth of the prominent ridge or fold of the outer wall of the enteric cavity previously referred to. The inner end of the ridge strikes itself against the mesoblastic layer opposite it, surrounding the chorda. It does not however coalesce with the same, but slips further downwards beneath the chorda and the hypochordal cells. At the same time, the two limbs of the prominent fold become separated ; the dorsal limb or the part that originally formed the wall of the gut-diverticulum becomes incorporated in the mesoblastic mass; the ventral half which was originally turned towards the definitive gut-cavity, loosens itself from the dorsal half and passes towards the median line beneath the chorda and hypochordal cells, until it meets and fuses with its fellow of the opposite side to form the definitive roof of the enteric cavity.

The process of mesoblast formation in the cephalo-cervical region as given above, is exactly like what we know to go on in *Amphioxus*. Calberla, Scott, and Shipley do not make any mention of such a primitive mode of the mesoblast formation in *Petromyzon*. Kupffer's description of the process in the head region accords well with my own observations, however with this great difference that according to him the chorda is cut off and completed long before the mesoblast is formed. That such is not the case, I have no doubt in my own mind; my sections speak too plainly for any doubt. My account also agrees exactly with the results recently made known by Mitsukuri[1] in Chelonia. If one compares my fig. 19 with his fig. 11, one will be easily convinced that a certain stretch of epithelium on each side of the median chorda-*anlage* becomes eventually incorporated in the mesoblast.

Let us now pass to the trunk and observe the mode of mesoblast formation in that region. Fig. 13 shows a transverse section from the dorsal region of an embryo in which no cephalic protuberance is seen as yet. In the median line the epiblast shows a thickened medullary plate (*n. p.*) formed of high columnar cells. Immediately beneath, but distinctly separate from, this plate, there is the chorda-hypoblast (*ch. h.*) forming the entire roof of the invaginated tube. Its

1. Further studies on the formation of the Germinal Layers in Chelonia: The Journal of the College of Science, Imp. Univ., Japan. Vol. V. p. I.

constituent elements have acquired the tall columnar shape by repeated longitudinal division. The hypoblastic cells immediately outside the chorda-hypoblast have an elongated shape and are in active multiplication as shown by many karyokinetic figures seen in this part. The products of the divisions are accumulated as a triangular mass between the epiblast and the yolk. This is the *mesoblast* (*mes.*). It is clearly distinguished from the yolk mass not only by a distinct slit but also by the characters of its constituent elements which are smaller, have their nuclei stained deeper, and are less full of yolk granules than yolk-cells. Also karyokinetic figures are often visible in this mass, while they are of rare occurrence in the yolk-cells. Thus, the mesoblast arises here as paired masses budded out from a few hypoblast cells placed immediately outside the chorda-hypoblast. This account agrees substantially with that given by Calberla.[1] The mesoblast cells facing the epiblast acquire a columnar shape, arrange themselves more or less regularly, and become the parietal layer of the mesoblast. In fig. 13, this layer has been more or less formed and has become continuous with the external extremity of the chorda-hypoblast. The cells lying below the parietal layer are irregularly arranged and are still having new cells added from the hypoblast cells of the aforesaid part.

Figs. 14 and 15 are from the dorsal region of slightly older embryos. Fig. 14 is essentially like fig. 13. In fig. 15, which represents a section more posterior than fig. 14, the mesoblast mass of one side is isolated, while that of the other side is still continuous with the hypoblast. In fig. 16, the mesoblast masses of both sides are entirely cut off from the hypoblast and have assumed a triangular shape, of which the inner and outer sides are formed by the parietal, while the ventral side

1. E. Calberla:—Ueber die Entwicklung d. Medullarrohres u. d. chorda dorsalis d. Teleostier u. d. Petromyzonten. Morph. Jahrb. Bd. III.

is occupied by the visceral layer. There are a few cells lying between the two layers which ought perhaps to be looked upon as belonging to the visceral layer. The chorda-*anlage* is now distinct and separated from the adjacent hypoblast by peculiar spindle-shaped hypochordal cells which are probably derived from cells originally lying between the chorda-hypoblast and the origin of the mesoblast. In fig. 17 from a still older embryo, the formation of the chorda is now completed. The hypochordal cells surround it as before. The gut-hypoblast has slipped itself under the chorda and the hypochordal cells, and forms the definitive roof of the enteric cavity. Fig. 18 shows the stage in which the medullary cord, the chorda, the hypochordal cells and the mesoblast masses have been completely differentiated.

It need scarcely be pointed out that the process of the mesoblast formation in the trunk is an abbreviated form of that observed in the cephalo-cervical region. One form does not, of course, suddenly pass into the other, and in the anterior part of the trunk (at just about the place where the head-protuberance is joined to the more posterior globular part) we can see the phases of gradual transition.

Fig. 23 is from the more anterior part of this region. *X* is the fold that starts from the outer wall of the enteric cavity to push itself towards the median line. The gut-diverticulum is decidedly shallower than in fig. 20. Cells seem to be budding forth from the outer extremity of its wall. Figs. 24 and 25, from an older embryo, are sections taken more posteriorly. In fig. 24 the more anterior of the two, the mesoblast mass is being budded out from each side of the chorda-*anlage* but there is now no trace of the alimentary diverticulum. The hypochordal cells have slipped under the chorda. The cells of the definitive gut-hypoblast are still outside the strip where the mesoblast cells have been budded out. In fig. 25, they are pushing themselves below the chorda and hypochordal cells to form the permanent median roof of the enteric cavity. My observations lead me to conclude that the process of the mesoblast formation begins in the region round the blastopore and proceeds forwards as the gastral mesoblast. This is contrary to the conclusion of Kupffer and of Shipley.

C. *The lateral and ventral portions of the mesoblast.* In the cephalo-cervical region which is free from yolk-cells, the lateral and ventral portions of the mesoblast are formed by the simple downward growth of the distal or outer extremity of the mesoblastic fold. In the region of the trunk, the process is somewhat modified on account of the presence of yolk-cells. The gastral mesoblast in this region does not extend itself laterally and ventrally as a solid sheet of cells. The distal border of the gastral mesoblast gives rise to free and separate cells, which penetrating between the yolk-cells and the epiblast establish in time a complete layer of a single row of cells.

This has been sufficiently demonstrated by Shipley (*loc. cit.*) At the same time, the peristomal mesoblast becomes free from the lips of the blastopore, and as separate scattered cells push their way between the epiblast and the yolk-cells and contribute to the formation of the mesoblast in the part ventral to the blastopore. This circumstance may be the reason why the previous investigators have overlooked the peristomal mesoblast. Thus, at about the stage given in fig. 18, the whole mesoblast has no connection with any part of the primary layers. The subsequent growth of the layer is solely effected by cell-multiplication within the layer itself. The posterior growth of the mesoblast is brought about not by any fresh addition from the hypoblast or any other structure but only by the extension of its own territory.

D. *The metameric segmentation of the mesoblast.* The metameric segmentation of the mesoblast begins first in the dorsal region and proceeds both backwards and forwards. When the segmentation has been completed the first somite lies just behind the auditory vesicle (*auv.* fig. 26).

According to Scott, (*l. c.*, p. 160), the segmentation into metameres precedes in the Selachians the separation of the protovertebrae from the lateral plate, but both processes occur simultaneously in *Petromyzon*. I learn, however, from my sections that *Petromyzon* is exactly like the Selachians in this respect.

The formation of the germinal layers in *Petromyzon* as given above accords well with the general plan of the development of these layers in the Vertebrata, as shown in *Amphioxus* by Hatchek, in *Triton* and *Rana* by O. Hertwig, and in *Trionyx* and *Clemmys* by Mitsukuri. The points of agreement and disagreement with previous writers[1] on *Petromyzon* as Calberla, Scott, Shipley, and Kupffer have been incidentally touched upon, in the course of the present paper and need not again be entered into in detail. In the main, my results agree best with those of Calberla.

Zoölogical Laboratory, Science College, Dec. 1891.

1. I regret that I have not seen the work by Max Schultze.

Explanation of Figures.

Complete list of reference letters.

au. v.	auditory vesicle.
bp.	blastopore.
ch.	chorda.
ch. h.	chorda-hypoblast.
ep.	epiblast.
ent.	enteric cavity.
hb.	hind brain.
hch.	hypochordal cells.
hp.	hypoblast.
it.	invaginated tube.
lp.	lateral plate (of mesoblast).
mes.	mesoblast.
mes. f.	mesoblastic fold.
mes. p.	peristomal mesoblast.
mes. s.	mesoblastic somite.
n.	neural cord.
np.	neural or medullary plate.
p. p. c.	pleuro-peritoneal cavity.
p. mes.	parietal mesoblast.
v. mes.	visceral mesoblast.
s.	segmentation cavity.
sm.	somatic mesoblast.
yc.	yolk-cells.

Plate XIII.

Fig. 1. Vertical section through an egg of the morula-stage. BB × 2 *(Zeiss)*.

Fig. 2. Sagittal section through an egg of the oldest morula-stage. BB × 2.

Fig. 3. Transverse section through the dorsal region of an embryo in which the gastrulation is going on. BB × 2.

Fig. 4. Sagittal section of an egg after the completion of gastrulation. BB × 2.

Figs. 5 and 6. Sagittal sections of an embryo in which the peristomal mesoblast is first seen. BB × 2. Fig. 5, a section through the median line; fig. 6 a section through a plane slightly lateral to the median line.

Fig. 7. Median sagittal section through the hind end of an embryo far older than that of the last two figures. BB × 4.

Figs. 8-11. Series of selected transverse sections from an embryo of about the same stage as that of fig. 7. BB × 4. Fig. 11 has been cut through the line *a b*, fig. 10 through the line *c d*, fig. 9 through the line *e f*, and fig. 8 through the line *g h*, in the embryo shown in fig. 12.

Fig. 12. Diagram of a median sagittal section through an embryo of about the same age as that of fig. 5, to show the directions of sections represented in figs. 8-11.

Plate XIV.

Fig. 13. Transverse section through the dorsal region of an embryo a little older than that represented in fig. 7.

Figs. 14 and 15. Transverse sections from the dorsal regions of a little more advanced embryo; fig. 15 is more posterior than fig. 14.

Fig. 16. Transverse section from the dorsal region of a still more advanced embryo.

Fig. 17. Transverse section from the dorsal region of a still more advanced embryo.

Fig. 18. Transverse section from the dorsal region of a much further advanced embryo.

Fig. 19. Transverse section through the anterior part of an embryo in which the head-prominence is ready to protrude.

Figs. 20-22. Obliquely transverse sections through the cephalo-cervical region. BB × 4.

Fig. 20. Transverse section of an embryo of a little more advanced stage than that of fig. 19.

Fig. 21. Transverse section from a still older egg.

Fig. 22. Transverse section from a still further advanced embryo.

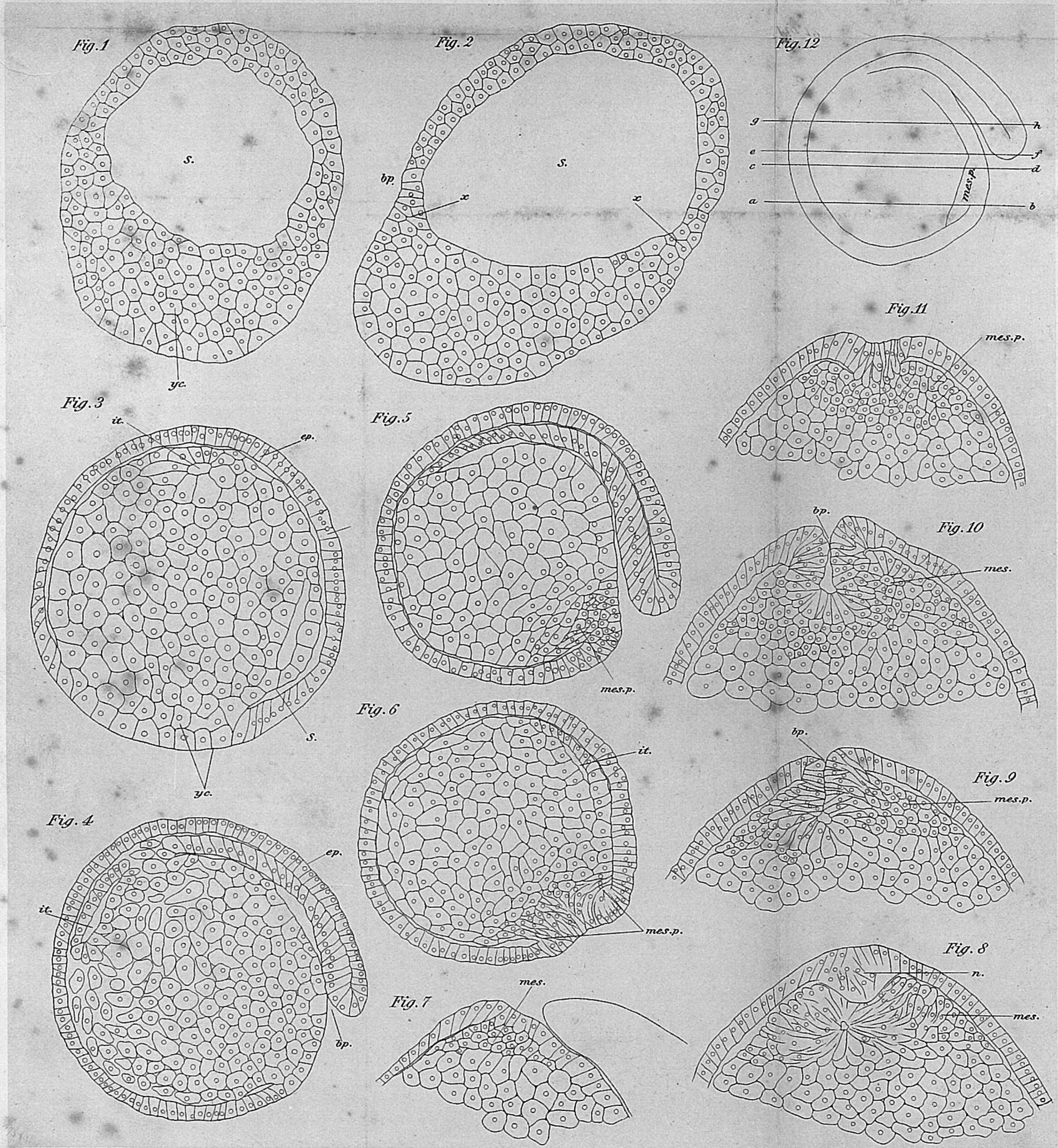
Figs. 23-26 represent obliquely transverse sections through the plane by which the cephalic protuberance starts up. ~~BB × 4.~~

Fig. 23. Transverse section of a little older embryo than that of fig. 20. DD × 2

Figs. 24 and 25. Transverse sections from a still older embryo, fig. 24 being a more anterior one than fig. 25. BB × 4

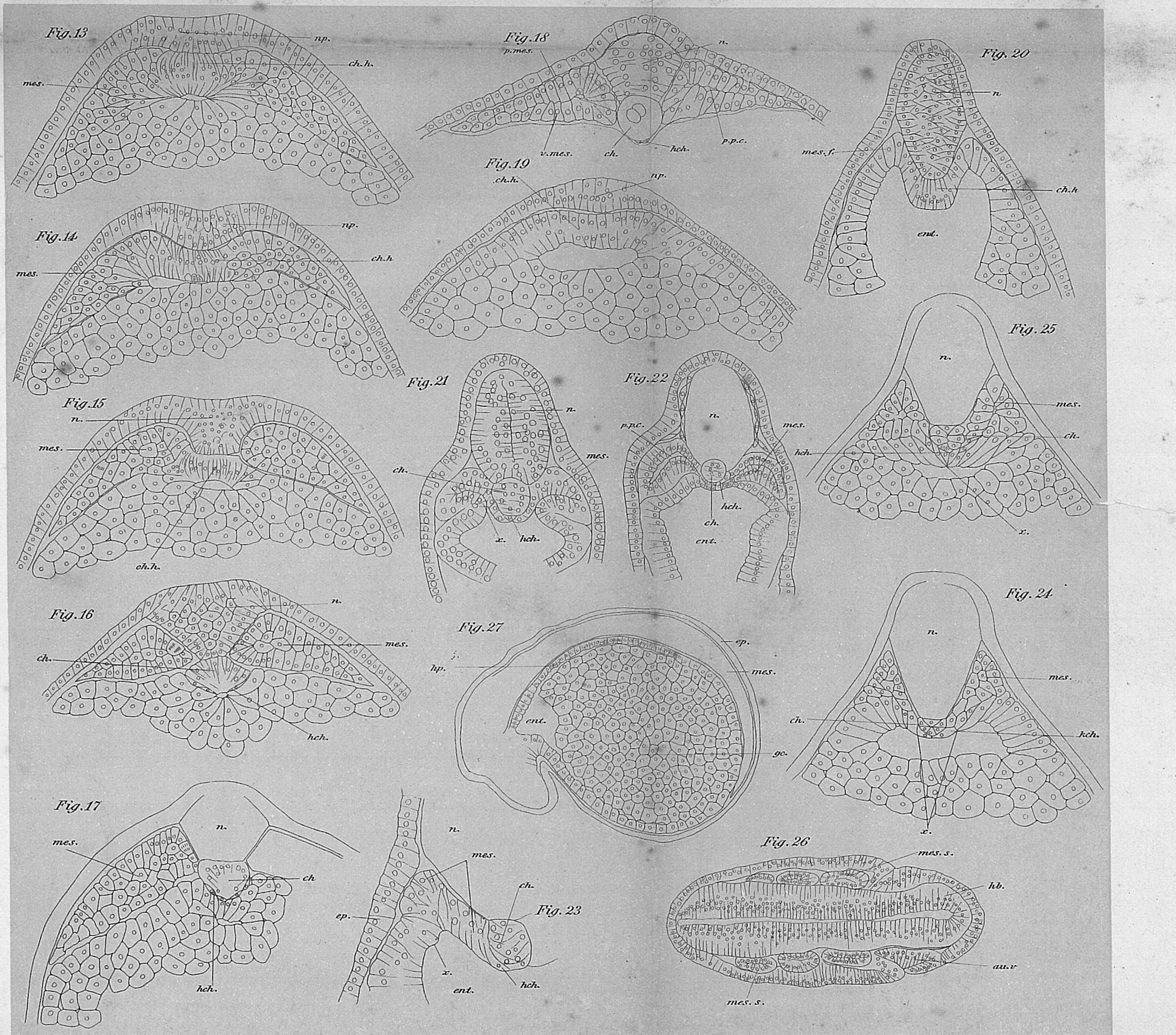
Fig. 26. Frontal section through the cephalo-cervical region of a much further advanced embryo. DD × 2

Fig. 27. Sagittal section through an embryo of an advanced stage. A × 4



S. Hatta, del.

Litho. at Seishi-bunsha.



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