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Studies on the Development of Larval Nephridia. Part II. Polygordius

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IV. *Studies on the Development of Larval Nephridia.* Part II.—Polygordius.*

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[PLATES 25–28.]

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1. *Introduction.*

THE nephridia of the *Polygordius* larva have been made the subject of numerous investigations. Among the earlier authors, the work of SCHNEIDER (19), HATSCHEK (8), and FRAIPONT (6) is the most important, while in recent years the papers of GOODRICH (7), MEYER (17), and WOLTERECK (28 and 29) have made valuable additions to the literature. In the youngest larva figured by HATSCHEK (8), FRAIPONT (6), and MEYER (17), the head-kidney is already well formed and functional, and the important work of these investigators deals only with the period of development intervening between the youngest Trochophore stage and the formation of the worm. Of the stages antecedent to this, the pre-larval or pre-trochophoral period, nothing was known when the present work was undertaken. FRAIPONT (6) had made a few observations on the fertilised egg, while WILSON (26 and 25) had published a few notes and figures on the segmentation. It was important that this early period of development should be investigated, and especially the stages at which the larval excretory organs make their appearance. My main object in undertaking the present work has been, therefore, the investigation of these stages; the first appearance of the larval nephridia, and the origin of their flame-cells or *solenocytes*.

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* The first of the present series of papers, which deals with larval organs of *Phoronis*, appeared in the 'Mittheilungen a. d. Zoologischen Station zu Neapel,' vol. 17, 1906, p. 487.

At the time* I was unaware that WOLTERECK (29) was then making a detailed study of the early development and cell-lineage, so the publication of the present paper after his recent contribution necessarily renders it, in part, a confirmation of his results. He has, however, but briefly considered the origin of the nephridia, and the development of the solenocytes has received no attention. Both these points in the present paper I have investigated in more detail.†

The task has not proved an easy one, and technical difficulties have greatly hindered the progress of the work, and are partially accountable for this delay in its appearance. The differentiation of the nephridia takes place early, at almost the close of segmentation; much earlier, therefore, than I had expected from an examination of the youngest stages found in the tow-net. From some of these, I was led to believe that the head-kidneys would be found arising at a stage slightly earlier than the free-swimming period. The first year, for this reason, I neglected to obtain stages sufficiently early. I soon found, moreover, that the extreme minuteness of the embryo during the pre-larval period of development greatly added to the difficulty of the investigation. While the full-grown larva is sufficiently large to render orientation and manipulation easy, it is quite a different matter with the minute pre-larval embryo. These frequently measure, in the early stages, from 60–80 μ in diameter. To clearly make out their histological structure, it is necessary to employ an oil-immersion lens, and this is a very laborious and tedious process on larvæ so minute and active. To obtain side views necessitates rolling the flat larvæ on edge, a difficult procedure, and is especially so under an oil-immersion lens. It is most satisfactorily accomplished by compressing the larvæ slightly under a cover-glass supported at its corners by wax feet. While this answers very well for round embryos that readily roll, with flat larvæ like these it requires considerable time and patience to manipulate them into the required position; this is no sooner done than they immediately resume their former position.

The aim of the present paper, I have said, is to complete, as far as possible, our knowledge of the development and differentiation of the larval nephridia. In *Polygordius*, the question is of unusual interest. HATSCHEK (8), and more especially MEYER (17), has shown that the nephridia form a continuous series, that the second larval nephridium possesses the same structure as the first, that the third is in every way comparable to the second, that the head-kidneys and the permanent nephridia form one homologous series. This disposes of the belief that the head-kidney, and so the larval type of nephridium, is not a true nephridium, and therefore not to be compared with the permanent nephridia. The possible grounds for such a conclusion are, that the head-kidneys possess branched canals or heads, that they lie free in the

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* In the early part of 1902.

† Since this paper has been read, I have been informed by WOLTERECK that he has carefully studied the development of the head-kidneys and observed the development of the solenocytes. He finds, as I do that these structures arise as direct outgrowths of the internal ends of the kidneys. My statement therefore, that their origin has not been traced so far does not hold true at the present date.—July, 1907

blastocœl and not in relation with the cœlom, and that they are closed and possess flame-cells. All these conditions, I have pointed out in my first paper, hold also for the larval organs of *Actinotrocha*.

MEYER (17), in *Polymnia*, has shown that the nephridia of the trunk segments develop from large cells close under the ectoderm, one between each of the mesodermic segments, with which they have no connection at this stage. These cells divide into a number of cells, which form cell-strings which effect a junction with cellular outgrowths of the peritoneal epithelium of the cœlom sacs, becoming later the nephridia. In the anterior segments, the nephridia form in a similar manner, with this difference, however, that the internal ends are wanting, terminating blindly in conspicuous flame-cells. In other words, the head-kidney, like nephridia of the first few segments, still retains the primitive relationships of the Platyhelminth nephridia. In *Polymnia*, much the same condition holds as in *Polygordius*, where the larval and permanent nephridia follow one another in a series, the nephridium of the first segment never getting beyond the larval or protonephridial stage.

An argument that has been advanced against the homology of larval with the permanent nephridia, and especially in the case of the head-kidneys, is that these structures are usually intra-cellular canals, while the permanent nephridia are usually inter-cellular. This argument is, however, deprived of much of its force when it can be shown that the head-kidney of the *Polygordius* and Trochophore larva are really composed of many cells fused together without definite cell boundaries. Moreover, in the larva of *Arenicola*, according to LILLIE (13), the larval nephridium is at first intra-cellular, as in *Polygordius*, but as the lumen of the organ increases it becomes subdivided into a number of cells and is then inter-cellular. The lumen of the original intra-cellular canal becomes "bordered by a well-defined layer of cubical excretory cells whose number in cross-section is, in general, directly proportional to the measure of the circumference of the lumen" (p. 389). In advanced stages, the organs are composed of a large number of cells. No great importance can be attached, therefore, to this difference of intra- and inter-cellular structure.

In *Polygordius*, it can hardly be doubted that the nephridia of the first segment, the head-kidneys, are the oldest and most primitive, retaining the same relationships as do the protonephridia of Platyhelminths; like these, they probably date from a time in the history of the larva when the cœlom, as we know it in higher forms, had not yet arisen as a space other than that of the genital follicles.

Moreover, GOODRICH (7) has shown that the nephridia are not necessarily a part of the cœlom. And this is borne out, as I have shown in the first of the present series of papers, in the case of the nephridia of the *Actinotrocha* larva, which exist alongside of the cœlom, quite free and independent of it, only coming into relation with it in adult life, when, in addition to their renal excretory function, they act as genital ducts. In Annelids, MEYER (17) and GOODRICH (7) have shown that this junction of the nephridium with the cœlom may be effected in two ways: by means of a direct nephridial opening, as is probably the case in *Polygordius*, and by the establishment of a connection with the genital conduits or cœlomoducts, the nephridium opening into the cœlom through a funnel or modified cœlomoduct. In proof of this, we have, in *Capitella*, cases where (*Dasybranchus*) these two structures have joined one another, but still retain their own separate openings into the cœlom, while all possible combinations of these two structures are to be met with in different Oligochæts and Polychæts.

I have stated, in the introduction to the present series of papers, that the main object I have held in view in undertaking them has been the hope that it might be possible to establish, on embryological evidence, a common basis for the development of the flame-cells type of nephridium; the possibility of establishing some definite homology, on embryological grounds, of this type of nephridium, as found in the higher forms, with the protonephridia of lower forms. In the case of *Phoronis*, I have shown, there is nothing to hinder our comparing their larval nephridia with protonephridia. In the case of *Polygordius*, the answer is equally direct. The nephridia are essentially protonephridia and undoubtedly true nephridia, and so not modified portions of the cœlom or cœlomoducts.

2. *Historical.*

The peculiar larva of *Polygordius* was first described by the Swedish naturalist, Lovén, in 1840, who observed it swimming on the surface of the sea amongst quantities of small Entomostraca, off the coast of Sweden, during the month of August. It was considered by him to be the larva of some Annelid allied to *Phyllodoce*, and, although he obtained numerous stages, he formed but a very incorrect notion of its true organisation. It is hard to account for the universal interest the discovery of this larva at that time created. Its description immediately gave rise to innumerable controversies and speculations regarding its relationships and affinity, and almost immediately it became widely known under the name of "Lovén's larva."

Some considerable confusion has arisen, as Woltereck (28, p. 9) has pointed out, in the use of this term. The larva described by Lovén is different in many of its main features from the larva found by Hatschek, at Trieste, and which his classical researches have rendered so familiar. In Lovén's larva, the adult worm develops by a complicated series of folds within the larval cuticle and not by polar outgrowth, as in the species described by Hatschek. It is incorrect, therefore, to speak of this last as Lovén's larva. Yet Agassiz (1), Schneider (19), Fewkes (5), and Hatschek (8) indiscriminately designate it by this name. Woltereck proposes to distinguish between them by calling one the North Sea type and the other the Mediterranean, but this is also open to objection, for there are at least two different types in the Mediterranean, and the North Sea larva may not be limited to the North Sea region alone. In a recent paper Woltereck (32) has proposed the names of "*endolarva*" and "*exolarva*" respectively for these two types.

In Lovén's larva, the nephridia consist of two parts, one alone of which answers to the head-kidneys of Hatschek's larva. Within the larva, along the border of the folds of the developing trunk region, are two canals, one on each side, composed of many cells and ending in two heads; attached irregularly over the surface of the canals are numerous solenocytes. Apart from these organs are two excretory bodies on the inner ventral wall of the larva, attached by canals to the body wall, while their heads, bearing the solenocytes, lie free in the blastocœl. These last structures seem alone comparable to the head-kidneys of the species studied by Hatschek. The observations of the present papers are confined to the nephridia of *P. neapolitanus*, which is identical, as Fraipont (6) has pointed out, with Hatschek's larva. I have had no opportunity of examining the North Sea larva. As far as I can determine, I can find no great difference between the larva of *P. neapolitanus* and that found on the eastern coast of North America, and described by Fewkes (5) and Agassiz (1). The granules of pigment in the ciliated ring of the American species are a little larger and more abundant, otherwise they seem quite the same.

The presence of excretory ducts in the *Polygordius* larva were briefly mentioned by Schneider (19) in 1868, while a few years later they were again noticed by Metschnikoff (16), who clearly understood their structure. He describes them as two tubes or provisional excretory organs, one on either side of the gut, at the growing pole of the animal. Some eight years later, Hatschek (8) was the first to accurately describe them under the heading of head-kidneys, a term since widely adopted in text-books. In many ways his account remains a model of accuracy and careful observation, although he was mistaken in thinking their ducts in free communication with the blastocœl in which the head-kidneys lie. He compared the distal funnel-like ends of the kidneys to the corolla of a flower, being formed of a thin web-like membrane stretching between peculiar stiff processes. He described the protoplasm of the tubes as being granular and as containing a number of nuclei, usually two for the funnel portion, four for the canal portion; the whole organ he therefore thought to be represented by a similar number of cells, although no cell boundaries were to be distinguished. Internally the canals were ciliated, and from their relation to the mesodermic tissues he thought them also of mesodermic origin. He noticed how the primitive single tube developed a new branch at right angles to the first at the inner end, so that even in the young larva the organ soon comes to consist of a Y-shaped tube with two or more funnel heads, opening on to the exterior through the body wall at the anterior end of the mesoderm bands. Finally, in late larval stages, two funnel heads are developed on the first arm, while three heads are formed on the second arm, which runs at right angles and dorsalwards to the first. Thus the fully-formed larval organ consists of a Y-shaped tube opening into the blastocœl by five funnel-like heads. According to Hatschek, a small ciliated canal, running in the somatic layer of the mesoderm, branches off from each head-kidney, where the two arms of the Y meet. The nephridia of the succeeding segments are derived from this canal, one for each segment. While the head-kidney degenerates, they reach their full development, becoming the permanent nephridia, and acquiring openings into the cœlom. The presence, however, of this canal has been strongly contested by both Meyer and Fraipont, and it appears that it has in reality no existence. In longitudinal sections of fairly advanced larvæ, a dark line is seen running between ectoderm and mesoderm, from the region where the head-kidneys rest against the mesoderm bands to the posterior portion of the worm rudiment; this line is formed by the sharp demarcation that exists between mesoderm and ectoderm, and looks in some sections not unlike a delicate canal. It may have been this that Hatschek mistook for a longitudinal canal. It is certain that a longitudinal canal does not exist, and that the nephridia are independently developed from each segment, the nephridia of the anterior segments resembling the first or head-kidney nephridium. This resemblance decreases as the segments proceed backwards progressively towards the anal end of the larva.

Fraipont (6), in his monograph of *Polygordius*, carefully investigated the larval nephridia. He confirmed Hatschek's description in most points, with the important exception that he could find no opening of the nephridia into the blastocœl, the bottoms of the funnels being closed by thin membranes. The stiff processes supporting the web-like funnel were hollow, being delicate canals, closed at their distal ends, while at the other they were in communication with the lumen of the main nephridial canal. In the head of the nephridium, at the base of the funnel, was one large conspicuous nucleus, while scattered in the protoplasm of the tube were usually several more, but no cell walls were to be distinguished. In 1900, Goodrich (7) examined the head-kidneys of some larvæ obtained in Ceylon. He was able to determine that the stiff processes which Hatschek had first described supporting the membraneous funnel on the internal ends of the nephridia, and which Fraipont had shown to be hollow tubes, were comparable in all respects to the excretory flame-cells or solenocytes of the Polychæt nephridium. Inside each of these tubes is a delicate flagellum, working down the tube into the lumen of the nephridial canal, while their distal ends are slightly enlarged and give off protoplasmic processes. These heads of the solenocytes, unlike those of the *Actinotrocha* larva, do not have a nucleus, their place seems to be taken by the single large nucleus at the bottom of the funnel which they surround and which Fraipont was the first to describe. There is, therefore, this important difference between the head-kidney solenocytes and those of the excretory organs of *Phyllodoce*, *Nepthys*, and *Glycera*, that whilst in these last there is always one nucleus for each solenocyte, in the head-kidney the solenocytes themselves surround one single nucleus. Woltereck (28) has shown that a similar structure holds for the kidneys of the North Sea larva; but here, instead of one single nucleus in the head of the nephridium, there are many, and in the "Haupt" nephridia, which correspond with the head-kidneys of the Neapolitan larva, they are gathered together in a mass, while in the "Seiten" nephridia they are small and scattered throughout the nephridial canal. These would seem to be of two sorts: one set of nuclei that belong to the cells of the nephridial canal, and another set, deeper staining, belonging to the solenocytes proper. Thus the "Haupt" nephridia, with their round heads and solenocytes and numerous nuclei, bear a strong resemblance to the larval organs of *Phoronis* during certain early stages. In these the nuclei are seen collected in the heads of the nephridial canals before they have taken their position, one in each solenocyte.

In the North Sea larva, WOLTERECK has noticed that the tube of the solenocyte has the peculiar hyaline nature characteristic of these structures in Polychæts. For instance, in fig. 4*b*, he shows the protoplasm of the solenocyte drawn away from the tube itself, which stands out as a stiff hyaline cylinder; exactly the same thing is shown by GOODRICH in his figures of the solenocytes of *Nepthys scolopendroides* (7, Part 2, fig. 27, Plate 9). This hyaline nature of the solenocyte tube I have never been able to observe in *P. neapolitanus*, but doubtless it is the same here also, for, in preserved material, the tubes are the parts of the solenocytes that are best preserved, and this points to their being composed of some firm substance, for the protoplasmic heads can seldom be seen in sections or preserved preparations, being extremely perishable and delicate. Thus, the solenocytes of the *Polygordius* larva, with the exception that each does not possess a separate nucleus, are essentially the same in structure with those of other Annelids. In a recent paper, JANOWSKY (10) has examined the nephridia, and has confirmed a number of the above-mentioned facts concerning their structure.

3. *The Structure of the Head-kidney in the Free-swimming Larva.*

Before considering the early development and gastrulation, it may add clearness to the foregoing remarks to give a brief *résumé* of the anatomical structure of the head-kidney in the free-swimming larva. It can readily be divided into three portions: the canal portion, the head portion, and the web-like funnel portion bearing the solenocytes. Of these, the canal portion is the largest, and is composed of an intracellular tube opening on the exterior on the lower larval hemisphere. This opening is difficult to see on account of its slit-like nature, which allows it to be kept closed, and so almost invisible. In the Neapolitan larva, its position, however, can be determined by the presence of a small amount of pigment in the ectoderm, which marks its situation. One or several strong flagella work down the lumen of the canal from the head portion. The protoplasm of the canal is often granular, and many of these granules are highly refractive. While some nuclei are seen embedded in the wall, no cell boundaries are to be distinguished, although the canal sometimes presents the appearance of being constricted into a number of cells, as shown in FRAIPONT'S (6) Plate 12, fig. 2. At its distal end the canal enlarges into the head portion. Here it forms a sort of ampulla, the end of the canal being closed by a large, darkly-staining nucleus, surrounded by a considerable mass of protoplasm.

Piercing this mass, and surrounding the circumference of the end of the nephridial canal about this nucleus, a number of stiff tubes are seen opening into the ampulla and closed at their free ends, these supporting a delicate web-like membrane between them. Each of these tubes contains a delicate flagellum, working down it into the lumen of the nephridial ampulla; each tube gives off one or several protoplasmic processes at its free end into the blastocœl in which the nephridia lie. With growth, this first simple canal develops a second arm at right angles to the first, so that the tube comes to be **Y**-shaped. At the end of this second arm, one or more funnels, like those of the first arm, may develop. In all cases these funnels, with their accompanying solenocytes, are closed, and do not open or connect with the blastocœl. The action of the flagella in the main canal is intermittent, suddenly stopping, to commence vigorously acting shortly afterwards. The whole organ lies against the longitudinal muscles running to the apical organ, and is held in position by being attached to these strands.

In the light of recent research, these structures can no longer be considered as organs *sui generis*, but are typical examples of the primitive type of true nephridia, as found in many adult Polychæts and *Amphioxus*, and larvæ such as those of *Echiurus** and *Phoronis*.

4. *Early Development to the End of Gastrulation.*†

The recent description of the early stages of segmentation by WOLTERECK (29) renders it unnecessary for me to more than briefly touch on them here. *Polygordius* belongs to that rather limited class of animals with equal holoblastic segmentation of the spiral type, of which the eggs of *Thalassema*, *Lepidonotus*, and *Eupomatus* are well-known examples. In fact, the cleavage of *Eupomatus* resembles so closely that of *Polygordius*, that up to the 32-cell stage one set of drawings would almost do for both. Once cleavage has set in, it progresses rapidly and uniformly till the 64-cell stage. About this time the blastula has already flattened out into its characteristic disc-like condition. Finally, it becomes quite flat (Plate 25, fig. 1). The cells at the margin of the disc, where one surface passes into that of the other, are somewhat larger than the others, and possess conspicuous nuclei. These cells develop the protrochal cilia, which soon become well marked. They also show the presence of large vacuoles, usually one large one, divided into several compartments.

At first the two layers of the blastula lie close together, with more or less obliteration of the original blastocœlic space (fig. 1). Soon, however, the layers separate as in fig. 2, the cells of the trochal margin becoming more vacuolated, and their protoplasm stringy in appearance. By the end of segmentation, the blastula

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* According to SALENSKY (18), the larval nephridia of *Echiurus* are not true protonephridia, but arise from the wall of the cœlom of the first mesodermic segment. The evidence of this, brought forward by SALENSKY, is by no means conclusive, and earlier stages than those examined by him will probably show that the larval nephridia, as in *Polygordius*, have nothing to do with the cœlom.

† For material and methods, see under the heading of Notes, at the end of the paper, p. 224.

is entirely ciliated, and, as development proceeds, these cilia become especially elongated at the margin of the disc, and in the dorsal apical region, where at this early stage they are developed into a tuft of stiff hairs (fig. 4 and fig. 6) as in typical Trochophore larvæ. These apical flagella bear a striking resemblance to the stiff hairs of the apical organ of the *Pilidium* larva, not in appearance only, but in the way in which they are moved simultaneously in one direction, as in this larva. In figs. 3 and 4, the thickening of the ventral plate of the blastula is seen in section, preparatory to its invagination to form the endoderm. Invagination takes place by the pushing in of the ventral plate of the ectoderm (figs. 6 and 7). This depression soon becomes elongated in the future long axis of the embryo, forming an elliptical depression, and finally giving rise to a long narrow blastopore (figs. 11, 12, and 36). Little distinction between the future mouth and anal ends of the blastopore, from which one only of these structures arises, can at first be distinguished. The anal end can be readily determined, however, by being always that portion that is nearest the flattened or indented circumference of the gastrula, as shown in figs. 11, 36. This flattening serves as a means of orientation. The cells of the endoderm plate project into the blastocœl in round masses as invagination takes place, and they are remarkable for their great difference in size (fig. 3). Their nuclei are large and opaque, and are densely crowded with chromatine. As compared with the endoderm cells, they possess relatively less cytoplasm, their nuclei being surrounded by but a thin layer. In their division, it is possible to follow with great clearness all the phases of mitosis, the chromatic filaments appearing darker as division becomes more rapid. In some cases this division takes place so rapidly that some endoderm cells seem as if forced into the blastocœl in the process of gastrulation, where they become separated entirely from the endoderm. This takes place frequently in the case of a large cell situated at the point marked * in fig. 7. A large endoderm cell is sometimes seen free at this point in the blastocœl. Whether this is a normal or abnormal condition due to the rapid growth, I am unable to say, as this cell is as frequently absent as present.

The long, narrow, elliptical blastopore meets first in the middle, so that the blastopore comes to assume the form of a figure of eight. The point at which this meeting takes place is marked * in fig. 12. Of the two portions of the blastopore remaining open, one persists as the future mouth, while the other entirely closes. The first-closed portion of the blastopore becomes the future stomach, which rapidly sinks in from the ectodermic surface while still open at both ends. The opening farthest away from the indented border of the embryo becomes the mouth. These changes can be readily followed by referring to the text-figs. B, C, and D, which represent a series of transverse sections taken at right angles to the long axis of the blastopore. The first section is taken through the oral end of the invaginated endoderm, which is seen to lead into a large and spacious œsophagus,

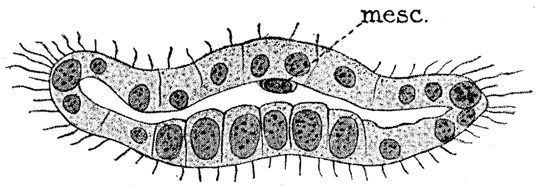

FIG. A.

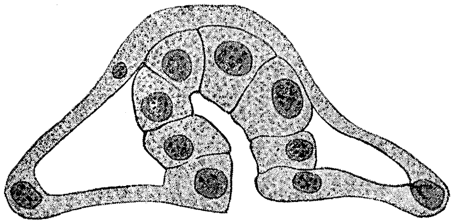

FIG. B.

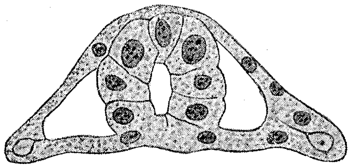

FIG. C.

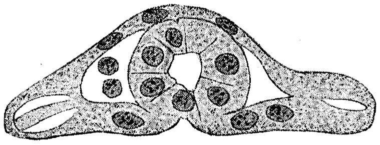

FIG. D.

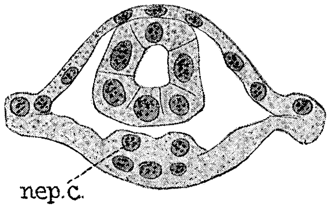

FIG. E.

FIG. A.—Section through a young blastula, showing the presence of a mesenchyme cell in the blastocœl, *mesc.*

FIG. B.—Section through the mouth region of a young larva.

FIG. C.—Section through the stomach region of the same larva.

FIG. D.—Section through the anal region of the same.

FIG. E.—Section through a young larva, showing the nephridial cells on the inner wall of the ventral plate of the ectoderm projecting into the blastocœl, *nep. c.* The gut is shown cut across.

inclined somewhat to one side, which in cross-section is composed of from eight to nine cells. The next section is taken a little further back, between the oral and anal end of the blastopore; here it has closed, and the stomach is seen as a round tube already free from the ectodermic surface which has closed over it. The subsequent section is still further back, through the anal region, and shows this part of the blastopore on the point of closing. Thus the anus is a new formation breaking through after the closure of the blastopore. Until this takes place, the end of the gut remains blind (fig. 8). The closure of the blastopore in the middle is effected by the rapid growth of a conspicuous cell seen in each lip. As this takes place, the embryo becomes much deeper, the apical region projecting from the dorsal region as a marked conical elevation (fig. 7). With the closure of the anal end of the blastopore, the primitive gut is seen as a sleeve-like invagination from the oral aperture or prostoma (figs. 5 and 8). The large vacuolated cells of the ciliated ring are still conspicuous, each cell having one large vacuole divided in the middle by a filament, as shown in text-fig. D. Thus the closure of the blastopore can be described as a form of concrescence, and it is evident that *Polygordius* is but slightly removed from the condition in which the blastopore gives rise to both mouth and anus.

On several occasions I have noticed that the approach of the cells that bring about the meeting of the lips of the blastopore is brought about by the formation of a delicate protoplasmic bridge between the two cells about to touch from opposite sides of the blastopore (figs. 9 and 36). I have seen this thread between these cells repeatedly in sections, as well as in preparations, and there is no doubt that it is thrown out by the cells before they come together. The cells next to meet are the group immediately behind the first ones, thus the blastopore closes progressively from the middle backwards towards the anal end, this being the last to close. By the time all these cells have met they have been invaginated somewhat from the surface, so that they no longer form the exterior lips of the blastopore. Thus the blastopore comes to have a double line of closure—the first or inner one, which forms the gut tract, and the exterior or external one that represents the closing of the ectoderm over this invaginated first part. The closure of the inner blastopore over the region of the future anus is somewhat slower than the closure of the external one at the same point; on this account the gut appears to expand at this end. While these changes are taking place, the mouth part of the gut is moving towards the ciliated border of the larva, so that finally it comes to open in this structure instead of on the ventral surface as at first. This can be readily comprehended by a glance at figs. 37 and 41, which represent the further course of this change at a later date. In fig. 37, the mouth and anus are seen to open directly on the ventral surface; in fig. 41, the anus is seen still holding the same position, while the mouth has moved out to open laterally in the ciliated ring. While the mouth is also at first round, like the anus, it at the same time undergoes flattening, and finally becomes slit-shaped, its corners being drawn out into distinct labia.

The mitotic spindles of the cells closing the blastopore are always placed distinctly at right angles to its surface; that is, they run through its wall, in what is frequently the shortest diameter of the cell. Towards the anal end of the blastopore, four distinct cells are seen arranged about it somewhat asymmetrically, two on the left side, one in the end of the blastopore wall, and one slightly to the right hand side. These cells, I have every reason to believe, give rise subsequently to the cells seen in fig. 13, *neph.* This takes place by their wandering out on the ventral ectodermic plate of the larva as the blastopore closes. Two of these, the large anterior ones, are the cells which subsequently give rise to the nephridia. WOLTERECK (30) (fig. 20) figures four cells at this point, which he designates as $3c$, a right and a left $4d$, and a $3d$ cell, two of which, $3c/2/post/post$ and $3d/2/post/post$, are, according to his account, the rudimentary nephridial cells. As the blastopore closes, these cells can be traced out into the ventral plate, where they take up the position shown in fig. 13, *neph.* The first cell on the left side of the blastopore becomes the cell marked *neph.* 1, the second one on the same side becomes the small round cell on the same side behind it, the one at the end becomes the small cell beside this on the right, while the large one in front is derived from the left wall of the blastopore. The two anterior large cells, as just mentioned, give rise to the nephridia. There can be no doubt on this point, as I have repeatedly traced them into the interior of the blastocœl, where they form the rudiments of the nephridia, as shown in figs. 28 and 37. Concerning the ultimate fate of the smaller cells I am not so certain; they retain for some time the same position, and it is possible they give rise at a later date to the secondary mesoderm, or possibly they are the small cells figured by WOLTERECK (29), immediately behind the nephridia cells, and which he designates as 3*d*/2*post*/*ant*/*ant*/ (fig. 12). Although I have readily followed the history of the two larger anterior cells, whose further development is treated of under the heading of nephridia, the smaller ones are more difficult to follow. These cells are again seen in transverse section in text-fig. E, *nep. c.* According to WOLTERECK (29), the cells mentioned as first closing the blastopore are derived from divisions of 3*c* and 3*d*. As I have mentioned, these cells are very plainly seen in the lips of the blastopore on account of their very large nuclei.

WOLTERECK (29) has pointed out that the early cleavage, like that of Annelids, is at first distinctly radial, the usual five groups of quartette cells being present. The first appearance of bilateral symmetry is brought about by the equal division of 4*d* and 4*b*, which take up positions one on either side of the future median line. The first quartette cells give rise, as in *Podarke*, to the apical rosette, cross and intermediate cells, and trochoblasts; the second quartette, to portions of the gut and rudiments of the germ-bands; the third quartette, to some of the stomatoblasts and some of the mesenchyme—the cells of the nephridia rudiments are also, according to WOLTERECK, to be traced to derivatives of this quartette. While the fourth quartette gives rise to some cells near the nephridia, the fifth takes part in the formation of the mid-gut. In addition to these, WOLTERECK distinguishes a sixth quartette in the eight entomeres. The cells giving rise to the gut, as in fig. 26, are derived in the upper half from the sixth quartette, the lower portion from the fourth, while the ectodermic surface is closed over the invaginated gut by cells derived from the second and third quartettes. Thus the closure of the blastopore is mainly caused by the growth of cells belonging to the second and third quartettes. Of some considerable interest is WOLTERECK'S observation that the definite mesoblast rudiment has its origin in various unpaired and paired cells derived from 2*d*, 3*d*, 3*c*, and 4*d*, in view of EISIG'S (4) observation of a somewhat similar origin in *Capitella* from 3*c* and 3*d*, while in the majority of Annelids it has always been ascribed to 4*d*. As I have made no attempt to determine the cell lineage, I am unable to discuss the results WOLTERECK has obtained, except in so far as these affect obvious features of the larva. Segmentation results in a hollow, ciliated planula, which rapidly flattens out into a flat disc. On this disc-like planula the cilia become elongated on the margin, where they represent the future protrochal cilia. In the apical region they are prolonged in the apical cilia. Gastrulation results in the formation of a long, narrow blastopore, which meets first in the middle and gives rise to the gut by sinking in and becoming free from the ectoderm while still open at both ends. The posterior portion of these openings then closes backwards towards the future anal end, and finally closes entirely. The anus subsequently breaks through at this point. The anterior opening persists as the future mouth and moves from its original ventral position towards the margin of the disc, finally opening in the ciliated ring. The flat, disc-like shape of the larva is lost as the primitive gut forms, and the larva becomes conical. As the anal end of the blastopore is closing, several cells in its lips become a part of the ventral ectodermic plate and wander forwards and outwards from their original position. The first group of these become the parent cells of the nephridia, which subsequently force their way into the blastocœl, there to give rise to the rudiments of these structures. A similar but smaller set of cells behind these take up a somewhat similar position, touching almost the anal end of the gut where it abuts against the ectoderm; these cells probably represent the rudimentary cells of the future mesoblast bands. At this date they are in no way different from the ordinary ectoderm cells amongst which they lie. With the closure of the anal end of the blastopore and the formation of the anus the embryo assumes the essential shape of the larva.

5. *The Larval Mesoderm.*

HATSCHEK (8) first drew attention to certain mesoderm cells seen about the œsophagus in advanced stages. These have the characteristic appearance of mesenchyme, with their irregular forms and numerous processes, being readily distinguished from the ecto- and endodermic tissues against which they lie. HATSCHEK was of opinion that they had their origin in the mesoblast bands, from which they become separated and afterwards distributed throughout the blastocœl. However, had he obtained young larvæ such as those represented in figs. 32, 34, 33, 35, or 30, he could have readily observed their presence long before the appearance of the mesoderm bands, and in larvæ very much younger than these, before even the blastopore had completely closed. These cells correspond to the larval mesoblast of Annelids and Mollusca, and, as in these groups, have probably a similar origin from ectoderm, quite apart from the secondary mesoblast, and for this reason they have been fittingly termed the ectomesoblast.

MEYER (17) has also shown that in the free-swimming stages the mesoderm can be distinctly divided into two classes, the *larval* and the *secondary* mesoblast; the mesoblast of the pole cells of the mesoderm bands, and the larval mesoblast, which subsequently gives rise to the larval muscles, some of which, he claims, persist in the adult as the circular muscles of the body wall and the septum tissues. The secondary mesoblast gives rise to the peritoneal epithelium, gonads, and the longitudinal muscles. In the early pre-larval stages, the larval mesoblast alone is present, as can be seen from an examination of a larva such as that shown in fig. 30; and, as development proceeds, the secondary mesoblast arises from the ectoderm near the anus.

In Annelids, a larval mesoblast has been described in *Capitella, Podarke, Aricia.* In *Eupomatus* and *Pomatoceros* I have seen it present in young stages long before the appearance of the secondary mesoblast. It is seen, just as in the young stages of *Polygordius*, as a number of cells about the œsophagus and sides of the stomach. In the majority of these forms it is highly probable it can be divided, as TORREY has described for *Thalassema*, into a median unpaired, and right and left lateral portions. The median portion arises in the vicinity of the cross cells from the ectoderm, the right and left portions also being ectodermal. In *Pomatoceros* and *Eupomatus*, as I hope to show in a future paper of the present series, it is distinctly divided into these three portions. In them the right and left parts give rise to the right and left head-kidneys of the Trochophore. I believe that these portions are represented in *Polygordius* by the nephridia rudiments, which in origin and growth are directly comparable with the head-kidneys of the Trochophore larva. These organs, in the *Polygordius* and the Trochophore larvæ, are so similar in growth, step for step, that there can hardly be any doubt that they are homologous structures. In fact, the head-kidney strands in *Pomatoceros* so closely agree in arrangement with the lateral portions of the larval mesoblast of *Thalassema*, that TORREY'S (21) fig. 3 might do to illustrate certain stages in the development of *Pomatoceros.* It is not unfair, therefore, to hold that the head-kidney strands of *Polygordius* similarly represent lateral portions of the ectomesoblast. Some confirmation of this view is, perhaps, to be found in the way the head-kidney strands arise, a mode of origin so characteristic of the mesenchyme. According to WOLTERECK, the head-kidneys of *Polygordius* arise from 3*c* and 3*d*; the same cells, TORREY (21) states, in *Thalassema* give rise to parts of the right and left portions of the ectomesoblast.

In *Eupomatus* and *Pomatoceros* the head-kidneys form from ectomesoblast. In *Eupomatus*, the so-called mesoblast bands of HATSCHEK are no more mesoblast bands in the early stages than the strands of the nephridial rudiments of *Polygordius* larvæ (such as those of figs. 28, 30, and 39) are mesoblast bands. The real mesoblast, the cœlomesoblast, arises at a much later date, when the larva is a day or so old; at this stage it is represented by two cells, one on either side of the anus, in the wall of the gut at the point of its junction with the ectoderm. At a comparatively late stage they give rise to the mesoderm bands, which creep up the head-kidney strands towards the œsophagus, establishing an intimate connection with the strands, which, however, is not a primitive condition, as HATSCHEK thought, but one secondarily acquired. MEYER (17) was the first to suggest (p. 471) that HATSCHEK might be mistaken in this, and he was quite right in questioning this statement of the relation of the wandering mesoderm cells of *Eupomatus* to the definite mesoderm. In short, the right and left portions of the ectomesoblast of *Eupomatus* and *Pomatoceros*, in great part represented by the head-kidneys, in origin are quite different from the cœlomesoblast, with which they establish so close a union at a later date. TREADWELL (20) is wrong in arguing that no hard or sharp distinction can be made between the mesoderm of the bands and the scattered cells of the blastocœlic space of the Trochophore. Had he examined the very young Trochophores of *Eupomatus*, *Polygordius*, or *Pomatoceros*, he would readily have seen the presence of these cells of the blastocœl in great numbers before the mesoderm bands had appeared, and from which, therefore, they could not have taken their origin.

More remarkable, however, is the way in which the cœlomesoblast, once it appears, unites with the ectomesoblast, and apparently supplants it. When I say supplants it, this term is, perhaps, somewhat incorrect; for, undoubtedly, almost if not quite all the larval mesoblast survives in the adult; as in the post-trochophoral stages of *Pomatoceros*, when the larva has developed three segments, it is still seen forming muscle strands.

The larval mesoblast was first described as having an origin separate from that of the secondary mesoblast, by LILLIE (14), in *Unio*; here it arises from the second group of micromeres in the A quadrant asymmetrically, but soon takes up a symmetrical position in the cleavage cavity, where it forms the larval adductor muscles. In *Crepidula*, it has been found by CONKLIN arising from the second quartette in the quadrants A, B, and C. In Annelids, it has been found by EISIG (4) in *Capitella*, arising from a portion of 4*d*; in *Aricia* from either the second or the third quartette; while in *Podarke*, by TREADWELL (20), arising from descendants 3*a*, 3*c*, and 3*d*. In *Thalassema* it arises from all the first three quartettes, and from all the quadrants, which indicates its primitive character. In other words, as CONKLIN (2) has remarked, the larval mesoblast is ancestral and might be called the "primary" in distinction to the "secondary" mesoblast. It is not improbable, therefore, as already mentioned, that it corresponds with the general mesoblastic tissue of Platyhelminths, while the secondary mesoblast would correspond with the mesoderm of the genital follicles of these forms. In fact, young larvæ, such as that represented in fig. 30, consist of ectoderm, endoderm, and larval mesenchyme, the definite mesoderm being present only potentially, so to speak, as two cells in the anal region, not different in any way from the other ectoderm cells among which they lie. As the most primitive of mesodermic tissues, we should expect the larval mesoblast of Annelids to possess the "ancestral reminiscence," as WILSON (24) has termed it, that we find it does in reality possess. Its radial origin, in some cases arising from all four quartettes as in *Thalassema*, its early appearance, and its distinct separation in larval forms from the definite mesoderm, all amply testify this. We find, as we might expect, it plays a large part in the formation of blood, blood-vessels, connective and the general skeleto-trophic tissues.

The larval mesenchyme, in *Polygordius*, first makes its appearance as delicate strands thrown into the blastocœl towards the end of gastrulation; they increase in size, and subsequently nuclei wander out into them; after this they soon lose their connection with their parent cells, and become free in the blastocœl. Sometimes, even early in gastrulation, as shown in figs. 4 and 6, they can be distinguished, but usually they arise somewhat later. The exact cells from which they arise I have been unable to determine, as I have not followed the cell-lineage. It undoubtedly arises from the ectoderm while gastrulation is taking place. In proof of this, a small cell is frequently seen close under the apical organ, or what becomes this organ at a slightly later date, apparently arising from the ectoderm as early in gastrulation as the stage shown in text-fig. A, *mesc.* This cell either undergoes division, or is joined in a like manner by other cells, for at a slightly later stage a small collection of cells is usually seen in this region, as shown somewhat diagrammatically in figs. 4 and 6. Their number and size is subject to considerable variation, and they frequently appear at a much later date; they are, however, a constant feature of the larva, being present some time sooner or later. This portion of the ectomesoblast corresponds, as already mentioned, with what TORREY (21) designates, in *Thalassema*, the "median," as distinct from the "right" and "left lateral" portions. Some of it secondarily takes up its position against the endoderm, being so closely applied to it as to simulate the appearance of forming from it. I believe the mesenchyme cells first arise, as I have said, in all cases by the throwing out of protoplasmic processes by the cells composing the inner surface of the blastocœl. In perfectly normal embryos, the blastocœl is seen to be traversed by delicate protoplasmic strands, without nuclei, and I believe these subsequently become independent, as mesenchyme cells, on acquiring ectodermal nuclei. The irregular time of their appearance makes their exact origin difficult to observe. While in some cases they are present early, in others they appear only at a much later date, and they bear little relation to one another, but soon scatter throughout the blastocœl, where they take part in the formation of the larval muscles. In the early stages they are seen closely applied to the mouth or œsophageal region, as in figs. 24, 32, 34, 33, 35, 30. At this time, as already remarked, they are the only mesodermal cells present.

To summarise briefly the larval mesoderm of *Polygordius*: it shows the usual complex origin from the ectoderm, so characteristic of it in the majority of Annelids, and presents no evidence of being derived from any source other than that of the ectoderm. In larval life, it is in large part transformed into the definite musculature of the young worm. Its development, therefore, affords strong evidence in confirmation of the theory advocated by MEYER (17), that in the majority of Polychæts it is to be looked on as phylogenetically the oldest mesodermic tissue and, as such, comparable to the parenchyma of the Turbellaria and the mesoglœa of Cœlenterates.

This is borne out in *Polygordius* by its totally different origin from the cœlomesoblast, and from its arising at a much earlier date. It is present before the cœlomesoblast has appeared, both in *Polygordius* and *Eupomatus*, as well as in such forms as *Echiurus* and *Criodrilus*. There are a number of Annelids in which its genetic history is somewhat obscure, as in *Nereis*, and cases like *Capitella* in which it would seem to arise in a manner different from other forms; these apparent exceptions are not sufficient to outweigh all the evidence, however, in favour of its conception as a structure distinct from the cœlomesoblast. I hope to show, in subsequent papers, that this conception of the mesenchyme, forming as it does an essential part of MEYER's gonocœl theory, explains satisfactorily many anomalous facts concerning the nephridia, almost inexplicable on any other hypothesis. The general mass of embryological evidence of the past few years seems to be directly in favour of this view, and fresh evidence from day to day seems only to strengthen its position. I hope, however, to return to this subject in my final paper, when the facts presented by a number of forms can be properly examined at length and compared.

6. *The Early Development of the Nephridia.*

The rudiments of the nephridia are first distinguishable as two cells on the ventral plate of the ectoderm, lateral and anterior to the anus, as already mentioned. They soon move forwards and outwards, taking up a position midway between mouth and anus (fig. 13). From the ectoderm cells among which they lie they are only distinguishable at this time by their dark nuclei. They are placed more towards the inner than the outer surface of the ectoderm and project somewhat into the blastocœl (figs. 26 and 27). Finally they lie as shown in text-fig. E. Here, when they have assumed this position, each cell divides, the original cell of the nephridial rudiment remaining in the ectoderm while the daughter cell projects into the blastocœl (figs. 15, 16, 17, 23, 25, and 28). A second cell then appears at the distal end of this cell (figs. 32 and 38), so that the nephridial rudiment soon consists of a string of three cells, the cell of one extremity being situated in the ectoderm, while that of the other touches the side of the œsophagus.

The three nuclei of these cells are large and distinct (figs. 38, 29, and 31). The manner in which they arise is somewhat peculiar. The original cell of the nephridial rudiment, situated in the ectoderm, first throws out a protoplasmic strand which attaches itself to the side of the œsophagus (figs. 18 and 25). This strand contains no nucleus at first, but is simply a protoplasmic process of the ectodermic cell. The nucleus of this ectodermic cell then undergoes division, one of the resulting nuclei wandering out into the strand (figs. 27 and 33), while one remains in the mother cell of the ectoderm. Shortly after this a second nucleus appears in the strand (figs. 34–36), but whether this arises from the nucleus situated in the ectoderm, I have been unable to determine. I have never observed the division that gives rise to this second nucleus taking place. It is somewhat incorrect, therefore, to speak of the nephridial rudiments as composed of three cells, when, as a matter of fact, they represent uniform strands of protoplasm in which three nuclei have taken up their positions. While the strands show the subsequent grouping of their cytoplasm about these nuclei, as in figs. 32, 30, and 36, which simulate the appearance of three distinct cells, no actual cell boundaries are to be discerned.

In fig. 24, which represents a transverse section at right angles to the axis of the œsophagus taken obliquely through the margin of the young larva, the section shows the head-kidneys as thin strands. In one of these two nuclei are seen, one close to the œsophagus, and the other in the ectoderm. At a rather later date a third appears. In the other strand the nucleus of the ectoderm alone is present. In fig. 20 the origin, on one side, of one of these nuclei is shown taking place. This section passes through the ventral plate of the larva somewhat obliquely, so only one of these structures is shown, and from the obliquity of the section it appears unusually forward and to one side of the larva. Fig. 14 represents a sagittal section of a larva of the same age as that of fig. 20; here one nucleus is seen, just after division, taking up its position in the strand. Fig. 16 represents a still later stage of the same condition, where one of the nuclei has made its way to the œsophageal end of the strand. Fig. 24 represents a state intermediate between these two conditions. In fig. 10 a still later condition is shown, but a portion only of the nephridium is cut across in this section. While the nephridium seems to be in the one-nucleus stage, examination of the subsequent sections shows that the mouth portion containing the other nucleus is present in the next section. When the nephridium has attained the three-nucleus condition, it presents the appearance as seen in the larva from the ventral surface, as shown in figs. 37 and 41; in side views, looking into the mouth, as shown in fig. 36. The condition of the nephridium under greater magnification is shown in fig. 29. The nucleus remaining in the ectoderm is much darker in colour than those of the strand portion, which gradually assume a fainter and fainter staining reaction as development proceeds. Finally, their cell walls seem almost to have disappeared, while at the same time the appearance of the strand itself has considerably changed. At first the cytoplasm shows a tendency to segregate round the nuclei, producing the appearance of three distinct cells; this becomes less and less marked (figs. 38, 31, 29), and the strand becomes uniformly homogeneous in appearance, the chromatine of the original nuclei apparently collecting in granules at either end of the strand (fig. 40). About this time the lumen of the nephridial canal begins to appear as a faint line in the middle of the strand (fig. 40). The lumen of the canal forms rapidly once it has appeared, and after the 48th hour the cilia in its interior are seen beating down the tube, and the organ becomes functional.

The inner ends of the nephridia are still closely applied to the wall of the œsophagus (fig. 31). About them numerous mesenchyme cells are seen applied, which, however, take no part in the formation of the nephridia or solenocytes, and possess a slightly different staining reaction to the nephridial cells, which enables them to be readily distinguished. The general relationships of the nephridia at this stage can be seen by a glance at fig. 39, no mesoderm bands having yet appeared and the larval muscles being but slightly developed. The stomach is small in proportion to the size of the larva, and the pigment of the ciliated ring is but slightly developed. Careful examination of the inner end of the nephridium at this stage shows no trace of the solenocytes, the head of the nephridium being still closely applied to the sides of the œsophagus. Even in a stage somewhat later than that shown in fig. 40, when the organ is about to become functional, no trace of the solenocytes can be seen.

7. *The Origin of the Solenocytes.*

The observation of the early development and origin of the solenocytes in *Polygordius* is somewhat unsatisfactory, on account of their very minute size and their lack of actively staining nuclei, which might show their presence. I have been able to determine, however, that they are derived as outgrowths of the distal extremity of the nephridial canal, as in *Actinotrocha* larvæ, and that the free wandering mesenchyme cells, seen abundantly in the blastocœl at this time, play no part in their formation. While the mesenchyme cells are often seen clustered about the ends of the canals, where they touch the sides of the œsophagus, they are never seen actually fusing with the canal ends themselves and giving rise to the solenocytes. I have already mentioned how the entire head-kidneys are possibly ectomesoblastic, and so are to be ranked as mesenchymatous structures. The origin of the solenocytes is, I think, the essential point to be determined in their development, as the actual formation of their component parts will be difficult to follow. In this respect, *Polygordius* will probably prove one of the most unsatisfactory of animals for their study. For this, doubtless, the large and numerous solenocytes of the Polychæte nephridium will prove a more suitable subject. It is from the study of the development of the solenocytes in some such form as *Phyllodoce* or *Glycera* that we may hope to learn the details of this process. In *Actinotrocha*, the peculiar shape of the larva, coupled with the great opacity of the cuticle, renders their observation in the living animal difficult, if not almost impossible under normal conditions. In *Polygordius*, their minute size (at least in the Neapolitan species), with their lack of nuclei, are objections almost as formidable.

Shortly after the stage shown in fig. 39 is reached, the growth of the lower hemisphere of the larva draws the œsophageal end of the head-kidney away from the sides of the alimentary tract. When this takes place, the distal end of the kidney still remains for some time attached to the œsophagus by several fine strands of protoplasm, which remain unbroken as the structure is drawn away from the œsophagus; at this time a very rapid growth seems to be taking place in the larva, for much of the ectomesoblast is being transformed into muscle. At this time I have repeatedly examined the free end of the nephridium, and I have not found any trace of the solenocytes. Only some time later have I been able to determine their presence, when they are seen distinctly on the ends of the nephridial canal, to all appearance derived directly as outgrowths of this structure; at this time no mesenchyme cells are present about the nephridia, and there is not the slightest indication that the free mesenchyme of the blastocœl takes any part in the formation of the solenocytes or the nephridial canals, as already mentioned.

We have now reached a stage which corresponds roughly with the early larvæ figured by Hatschek (8), Meyer (17), and Fraipont (6), a stage after which I have not sought to follow the fate of the larval organs.

A point of almost equal importance with the origin of these solenocytes is that of their ultimate fate and the means by which the larval nephridia of the succeeding segments acquire openings into the cœlom. It is well known that in the adult *Polygordius* the permanent nephridia are composed of delicate canals running between the junction of the longitudinal with the oblique muscles, and turning out to open to the exterior on the epidermis. In front it opens into the cœlom of the next segment, Goodrich (7), by means of a small nephrostome, one lip of which only is ciliated. This opening is remarkable for its size, and it belongs, in all probability, to the category of a true nephridial opening, a nephrostome, as the genital products of *Polygordius*, as in other Archiannelids, escape by rupture of the body wall, but the exact nature of this opening is at present doubtful.

Fraipont (6), in his monograph, states (p. 83): "Les organes segmentaires du tronc d'après ce que j'ai pu voir ont une double origine. L'extrémité périphérique de chacun d'eux provient d'une invagination de l'épiblaste. La partie du canal située dans la couche somatique du péritoine et l'entonnoir terminal ont une origine mésoblastique," and, again, "le mésoblaste est représenté de plus au niveau des muscles obliques par une masse de cellules assez confuse. C'est dans ce groupe de cellules situées au-dessus des muscles obliques contre les champs musculaires longitudinaux que se différencieront les entonnoirs des organes segmentaires et plus tard encore les organes sexuels. C'est un simple épaississement du péritoine" (p. 72). Should this last prove true, the nephrostome would be a structure of different origin from the canal portion of the nephridium, as Fraipont claims. This point, at least, needs further corroboration, and it is to be hoped that Woltereck, in his forthcoming work, will deal with this point.* These changes take place long after metamorphosis, when the young worm has already taken up its habitat in the sand, and belong, therefore, to a stage in the development beyond the scope of the present work.

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* Since the present paper has been read, an interesting paper has been published dealing with this point. See Hempelmann, "Zur Morphologie von *Polygordius lacteus*," 'Zeit. f. Wissen. Zool.,' vol. 84, p. 527, 1906.

8. *The Mesoderm Bands: the Cœlomesoblast.*

The mesoderm bands of the *Polygordius* arise so late, relatively, in development, in comparison with the head-kidneys, that their consideration hardly comes within the scope of the present research. From the time the head-kidneys become functional until the appearance of the mesoderm bands, there is a short period in the larval life-history during which the larva undergoes considerable change in shape; this is brought about by the formation of the anal papilla, which gives it a more conical outline. Its examination, shortly before this change takes place, indicates that the mesoderm bands have not yet appeared. Careful examination of the anal region shows the presence of two large nuclei, one on either side of the anus, which probably represent the rudiments of these structures. These nuclei or cells are very unlike the teloblasts HATSCHEK (8) has described as giving rise to these bands, and at no later period of development can I ever find them as well marked as indicated in his figures. His figures, 57, 58, and 59, in fact, are very misleading in this respect.

The real pole cells of the mesoderm bands, if such we can call them, lie close under the ectoderm, against its inner surface, almost touching the gut. They are most satisfactorily seen in ventral views of the larva seen *en face*, and are conspicuous for their large dark oval nuclei and lack of surrounding cytoplasm. These nuclei are the same as those seen in fig. 13 behind the rudiments of the nephridial cells. In side views they are seen against the gut, as shown in fig. 30. As the larva changes from the condition shown in fig. 13 to that of fig. 30, these cells, I believe, move backwards till they take up their position finally against the gut. Close to these cells, but not to be confounded with them, are two large cells seen side by side on the surface of the ectoderm. They are clear and vacuolated, and possess small granular nuclei quite unlike the real mesoderm cells. They undoubtedly correspond to the anal vesicle cells of the Trochophore larva, as WILSON (26) has pointed out, and have nothing to do with the mesoderm bands. It is highly probable that both FRAIPONT (6) and HATSCHEK (8) have mistaken them for the pole cells of the bands; this may account for the unusual prominence of the pole cells in the drawings of these investigators. The real pole cells, situated in a deeper plane, do not lie directly in the ectoderm.

The growth of the mesoderm bands takes place in essentially the same manner as in the Trochophore larva. In *Pomatoceros* and *Eupomatus*, contrary to HATSCHEK'S statements, it is equally as difficult to distinguish the pole cells as in *Polygordius*. Teloblasts similar to the teloblasts of the germ bands of *Clepsine* do not exist in any of these forms. In them the bands arise from two cells, one on either side of the anus, which in the early stage differ so little from their surrounding cells as to be quite indistinguishable from them. By division, they give rise to the bands of their respective sides, but their manner of division is not that of a true teloblastic one. Once the bands have begun to appear their growth is rapid, and they soon reach their full development, creeping up the head-kidney, as shown in fig. 42. In the living state, the cell outlines of their individual cells are indistinct, and one can see nothing but the small round regular nuclei which denote their presence. MEYER (17) has shown how soon after their appearance the bands become divided and broken up into the mesodermic segments, and the close relation that is established between the ectomesoblast and these segments. The cœlom sacs appear in these segments remarkably early and soon develop a peritoneal lining. According to MEYER, the ectomesoblast also forces its way in between the septa, taking considerable part in the formation of the septa wall.

He finds the larval mesoblast to have a slightly different staining reaction from that of the definite mesoderm, which enables the anastomosis of these two structures to be followed in considerable detail. It results in a general mesodermic tissue which, in late stages, is a veritable synthesis of ecto- and cœlomesoblast.

I have shown how at an early stage the pole cells of *Polygordius* lie on the ventral ectodermic plate, while at a later date they have moved back to come into relation with the gut. Their final secondary position, as in fig. 30, might thus be taken for their primary position if it were not for the information furnished by earlier stages. In the Trochophore larva, I believe, much the same series of changes takes place, only in their secondary position these cells come to lie actually in the wall of the gut at the point of its junction with the ectoderm. They are derived from the lips of the closing blastopore at a point where, as a matter of fact, it is hard to decide whether they are ecto- or endodermic. MEYER has found, in Annelids, that the mesoderm bands in some forms lie close in relation with the endoderm or gut, while in others they lie close against the ectodermic wall. Whether this difference of position of the pole cells in *Polygordius* and *Eupomatus* accounts for this arrangement, I cannot say. Certainly, no great morphological importance can be attached to this position of the pole cell in the gut wall, seeing that it is not a primary condition. HATSCHEK, in 1877, first suggested, in the embryology of *Pedicellina*, that the pole cells of the mesoderm bands were phylogenetically to be regarded in the light of germ cells. As such we might expect they might show some primitive relationship with the endoderm. In many cases the older school of embryologists considered they had ample evidence of this in the development of many Annelids. Subsequent investigation, however, has shown this to be by no means a common process. MEYER (17) has recently found, in *Lopadorhynchus*, they form from the ectoderm. It is doubtful, therefore, if the endodermic origin of the mesoderm has any significance in relation to the primitive origin of the germ cells from this layer in Cœlenterates.

9. *The Comparison of the Development of the Larval Nephridia of* Actinotrocha *and* Polygordius.

Before proceeding to summarise the results of the present paper, it may be of slight interest to compare the development of the larval organs of the *Actinotrocha* larva with those of *Polygordius*. I have tried to illustrate this in the following diagrammatic figures (p. 222). The right-hand diagram of fig. F shows the diverticulum of the nephridial pit of the *Actinotrocha* larva arising as a shallow fold in the pit-wall. This fold is the rudiment of the future nephridium. The left-hand diagram of the same figure shows the corresponding stage in the *Polygordius* larva, the nephridial rudiment being represented by a single cell in the ectoderm. In the subsequent diagrams, different stages in the growth of the nephridia in the two forms are shown arranged opposite one another. In both animals the process is the same, and they bear a close resemblance to one another in the development of their larval nephridia. In each case these structures arise from a cell or cells situated in the ectoderm, which, by growth into the blastocœl, give rise to the nephridial canals, these in turn give rise to the solenocytes. Whether we choose to call these structures mesodermal, from their situation in the blastocœl, or ectodermal, from their origin, seems to me to be quite immaterial; the essential process of their evolution in the life-history of both forms is the same. It may be held that the nephridial cells do not really represent the rudiments of the future nephridia until they have taken up their position in the blastocœl, and thrown out filose strands towards the œsophagus; prior to this they are simply cells of the ectoderm, differing in no particular from other ectoderm cells. Once they have taken up their position in the blastocœl, however, they come under the heading of ectomesoblast. I have pointed out* in the *Actinotrocha* larva the larval nephridia arise entirely from the ectoderm; that the wandering mesoderm cells of the blastocœl take no part in their formation. I have emphasised, perhaps unduly, this purely ectodermal origin. It may likewise be held here that once the rudiments of the organs project into the blastocœl from the ends of the folds of the nephridial pit they no longer represent ectodermic but mesodermic structures. To settle questions like these definitely, in terms of the germ-layer theory, seems perfectly impossible.

In *Phoronis*, the mesoderm mostly takes its origin from the endoderm. It would therefore correspond with the cœlomesoblast of Annelids. The question arises, is it possible to distinguish an ecto- or larval mesoblast in *Phoronis*? At present it would seem doubtful; it is true, we might compare the nephridial diverticulæ of *Phoronis* to the right and left portions of the ectomesoblast of the Trochophore larva, while the median portion is possibly represented by the subnural gland of the Actinotrochal hood of *Phoronis*. In the first place, the origin of the nephridial diverticula of *Phoronis* as distinct folds of the ectodermic surface is quite unlike the origin of the

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* Part I, 'Mittheil. a. d. Zool. Stat. zu Neapel,' vol. 17, 1906, p. 487.

individual cells of the ectomesoblast of Annelids, and it is doubtful if the subneural gland of *Actinotrocha* plays any part in the elaborations of the mesenchyme of the hood. This, however, by no means precludes the possibility of a portion of the mesenchyme of *Phoronis* arising from the ectoderm. Whether such a process really takes place can only be determined by the very close examination of living and carefully fixed material. The mesenchyme arising, as it does, in such small irregular cells, may readily simulate an erroneous appearance of arising totally from the endoderm, as it does to a certain extent in *Polygordius*. In *Phoronis*, the endoderm gives rise to the mesoderm, and therefore it is comparable, not to the ectomesoblast of Annelids, but to the cœlomesoblast alone. At present, the balance of evidence seems to be in favour of regarding it as purely endodermic in origin, as in Echinoderms.

The remarkable coincidence in the cell-lineage of the head-kidney cells of *Polygordius*, given by WOLTERECK (28), and portions of the lateral ectomesoblast of *Thalassema*, according to TORREY (21), is indeed striking. It is rendered all the more so by the conditions presented by *Pomatoceros*—in many ways, from the developmental standpoint, a connecting link between the two forms. The force of this resemblance cannot be appreciated fully until drawings of the early Trochophores of *Pomatoceros* and *Thalassema* are compared. It then seems impossible to escape the conclusion that the string of cells seen running from the œsophagus in *Pomatoceros* towards the anal end of the gut represents portions of almost similar strings of cells in *Thalassema*. WOLTERECK (29, p. 388, foot-note) has gone so far as to suggest that

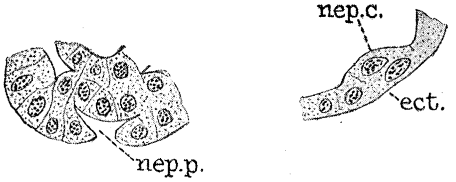

FIG. F.

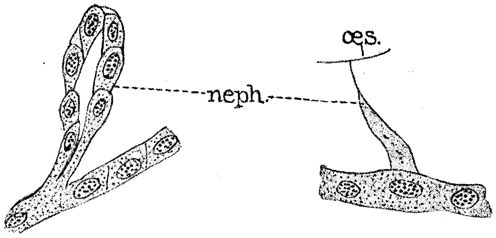

FIG. G.

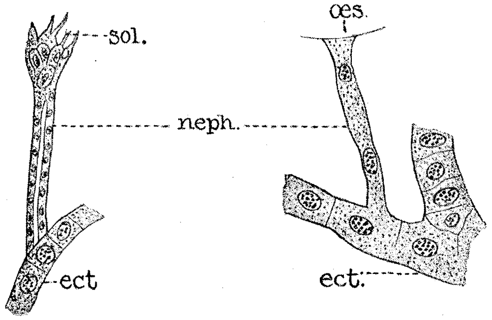

FIG. H.

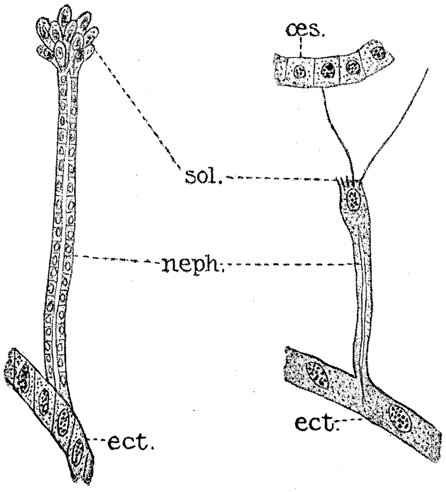

FIG. I.

Diagrammatic Series of Figures representing the relationships of the developing nephridia in the *Actinotrocha* larva and in the *Polygordius* larva: *œs.*, œsophagus; *ect.*, ectoderm; *neph.*, nephridium; *sol.*, solenocytes; *nep. p.*, nephridial pit; *nep. c.*, nephridial cell.

these cells in *Thalassema*, as in *Pomatoceros*, represent primitive nephridial rudiments. TORREY (21), however, seems to have conclusively proved that no larval excretory organs are present in the Trochophore of *Thalassema*, and this recalls the total absence of larval organs in the Trochophore of the Annelid *Podarke*. I have examined the Trochophore of *Podarke* in both living and fixed material as well as in sections, and I can only confirm TREADWELL'S (20) statement of their total absence.

In *Thalassema*, TORREY (21) suggests (p. 216) that "the excretory function seems to be relegated to a number of globular mesenchymatous cells, which are either loosely attached to the body-wall and muscles or float freely in the primary body-cavity. These are especially prominent in the three- or four-day Trochophore. They soon become stained a yellowish-brown colour and in the oldest Trochophores contain refractive granules." It is strange that in *Dinophilus*, as I hope to show shortly in a forthcoming paper, the primary body-cavity contains numerous yellowish-brown excretory cells, and in young *Sipunculus* larvæ the body-cavity is similarly crowded with cells; yet each of these forms possess larval nephridia, and in the case of *Dinophilus* they are of the primitive solenocyte-bearing type. It is doubtful if the presence of the brown concretions, therefore, explains the absence of the nephridia in *Thalassema*. I can suggest no other satisfactory explanation of their absence in *Thalassema* or *Podarke*, especially as in *Echiurus* larval organs are present and probably are referable to the primitive solenocyte type.*

10. *Summary.*

Segmentation results in a flat ciliated blastula. The invagination of the ventral plate of this blastula gives rise to a somewhat similarly shaped gastrula, which becomes conical in shape as development proceeds. The blastopore is from the first long and narrow, closing first in the middle; one of the resulting openings remaining as the mouth of the future larva, while the other closes entirely; the anus forms

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* Of considerable interest in relation to the mesoderm and ectomesoblast of Annelids is the recent publication of WIERZEJSKI'S (23) admirable paper on the embryology of *Physa*. He shows that in this Mollusc there is a remarkable similarity in the position and growth of the larval mesenchyme and the secondary mesoderm to the growth of these structures in Annelids. When we consider the absence of definite mesoderm bands in the Mollusc, this similarity is all the greater. While in most Molluscs the kidney rudiments have been attributed in origin to the ectoderm, in *Physa* they arise from the secondary mesoderm (which WIERZEJSKI calls the primary). From his very clear figures and descriptions it seems impossible that he should have mistaken the cells from which they arise. Yet the close proximity of the secondary mesoderm to the larval mesenchyme at the point where they arise makes one feel that such a mistake could be readily made. We know, however, so little of the true nature of the so-called "kidneys" of Mollusc larvæ that it is not necessary to suppose that their origin from the cœlomesoblast is a contradiction to the condition possessed by *Polygordius*. These structures, after all, probably belong to the category of cœlomoducts and, as such, are portions of the cœlom and not nephridia; in which case their origin from the cœlomesoblast is quite what might be expected. WIERZEJSKI'S figure of the kidney, however, seems to show it closed and very similar in many respects to the primitive flame-cell nephridium of Annelids.

subsequently at the point where the last portion of the blastopore has closed. The nephridial rudiments are first recognisable as two cells on the ventral plate of the ectoderm on either side, close to the region of the line along which the blastopore has closed, and anterior to the anus. These cells project into the blastocœl, giving rise to two strands, which run up to the œsophagus on either side. As growth proceeds, they are differentiated into the canals of the head-kidneys. The solenocytes form late, as outgrowths of the ends of the canals, and after the organs have become functional. At this time the mesoderm bands are only appearing. During the early history of the head-kidneys, no trace of the mesoderm bands can be observed. With the appearance of the bands, the larva reaches a stage subsequent to which the growth and fate of the head-kidneys have already been investigated.

The growth and development of the head-kidneys clearly show they are not cœlomic or in any way connected with the cœlom. This, with their primitive solenocyte-bearing character, plainly indicates their nephridial nature, in distinction to the modified portions of the cœlom; the so-called nephridia of *Peripatus* and some Molluscs. While no cœlom is present in the segment to which they belong, a reason, it may be held, why they appear independent of it, a condition secondary, not primary, their development, nevertheless, proves this condition to be primary. Their origin before the cœlom has appeared, and their growth from the ectoderm at such an early stage, clearly testifies to this. If cœlomic in origin, they should hardly be found present in the absence of the cœlom. Their development affords no evidence that they were connected with the cœlom at a former date in the animal's history.

The real homologues of the nephridia of *Polygordius* are to be sought, not in the cœlomic ducts of the Cœlomata, but in the flame-cells and protonephridia of lower forms. As in the Turbellaria, these flame-cells, in their ultimate analysis, are referable to the mesenchyme or parenchyma; it is of some interest to find considerable evidence, in *Polygordius*, for regarding the head-kidneys similarly as special portions of the mesenchyme or ectomesoblast.

11. *Notes on Material and Methods.*

The material used was obtained at the Naples Zoological Station in the spring of 1902; the adult worms containing the sexual products being broken up in small jars of fresh sea-water, when the ripe eggs and spermatozoa readily separate out. The sexual products remain suspended in the water, while the broken fragments of worm and *débris* fall to the bottom of the jar, when they can be readily drawn off. The jars are set aside till fertilisation has taken place. Usually some three or four hours after the sexual products have been brought together the first signs of cleavage appear. The eggs are then stirred up, and washed in several changes of sea-water to remove unnecessary spermatozoa, then set aside. Development proceeds rapidly and steadily till the third day, after which the larvæ cease to grow unless some means is adopted of supplying them with more food than they can obtain from ordinary sea-water. If not fed, after the third day they gradually diminish in size and finally break up at the end of a few weeks, having made no further progress in development.

As the adult worms are by no means very abundant at Naples, and the number of eggs obtained from a single worm is never very great, I experienced considerable difficulty in obtaining sufficient material. On this account I adopted the most economical method of studying the material I obtained. I found the combined celloidin-paraffin method of sectioning very useful, as by the use of this method very little material is lost in the process of embedding, and it is possible to section the minutest larvæ one at a time if necessary. It also has this advantage, that very small sections can be traced in a rapid manner consecutively on the slide, by means of the celloidin. On this account I did not adopt the usual plan of dissolving it out, but allowed it to remain on the slide as a means of readily finding the sections. Material was fixed either in Flemming's strong solution, or Hermann's. For staining, hæmacalcium and other hæmatoxylin mixtures are most useful. For larvæ to be studied whole, dilute paracarmine, followed by slightly acid alcohol, gives very satisfactory results.

While the larva of *Polygordius* is found in the "tow" abundantly during only the months of February, March, and April, it is possible to rear the larva from the egg throughout all the spring and summer months. After the middle of September I had some difficulty in obtaining the adults, so I have been unable to determine whether the sexual products are developed all the year round. The normal discharge of these products seems to be restricted to the spring and, possibly, the summer months. The free-swimming larvæ are found only during the spring and summer months along the eastern coast of North America and in the North Sea, while in the Mediterranean they are found in February, March, and April, as already mentioned. During the month of May, 1900, I obtained a number of *Polygordius* larvæ from the "tow" at Woods Holl, Massachusetts, which I had the opportunity of comparing with the larvæ found at Naples; from these they can hardly be distinguished, and in all essential features, as I have already mentioned, they are the same.

I have considered it best in the foregoing work not to mention the various stages by age, as development is affected by temperature to such an extent that there is some uncertainty in designating stages by this means. The progress made in development in warm weather is twice as great as in the cold months of the early spring. It is possible that under natural conditions development may be still more rapid; for this reason time is a somewhat uncertain manner of designating them. COWLES (3) states that he found the larva of *P. appendiculatus* go through metamorphosis in the course of a night under favourable conditions. He says, "It surprised me to find that fully-developed swimming larvæ, taken at night, and measuring slightly less than 1 millim. in length, had metamorphosed by the next morning. They were at this time 2 millims. in length, showing an increase of 1 millim. in length" (p. 125).

The time, however, required under laboratory conditions for the larva to reach the following stages after fertilisation is, roughly, as follows: Completion of segmentation, 12 hours; completion of gastrulation, 20 hours; formation of anus, with wandering of mouth to the ciliated ring, 24 hours; nephridia distinguishable at 22 hours, but the flagella are not developed till after the 48th hour. The ages of many of the larvæ shown in the plates are given under the descriptions, but these in many cases are only relatively correct, as they belong to various batches of material that have developed under different conditions.

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13. EXPLANATION OF PLATES.*

LETTERING.

a. = anal opening.
œs. = œsophagus.
al. = alimentary canal.
blas. = blastocœl.
bl. = blastopore.
ect. = ectoderm.
end. = endoderm.
h.k. = head-kidney.
h.k.c. = head-kidney canal.
neph. = nephridial cells, the rudiments of the head-kidneys.
m. = mouth.
mesc. = mesenchyme.
mesd. = mesoderm bands.
seg. c. = segmentation cavity.
sol. = solenocytes.
Stm. = stomach.

PLATE 25.

Fig. 1.—Section of a *Polygordius* larva 18 hours after fertilisation, showing the flat condition of the morula stage. The cilia on the external surface are not represented.

Fig. 2.—Section of a stage slightly later than that shown in fig. 1, showing the segmentation cavity.

Fig. 3.—Section showing the thickening of the ventral surface of the larva preparatory to invagination.

Fig. 4.—Commencement of invagination and formation of the gastrula.

Fig. 5.—A later stage of fig. 4, showing the gastrula in horizontal section.

Fig. 6.—Drawing of a whole preparation, showing invagination of the endoderm, with the presence of mesenchyme cells in the segmentation cavity.

Fig. 7.—Section of a gastrula.

Fig. 8.—Section of a larva 36 hours old, showing the primitive gut. Sagittal section; the anal end of the blastopore is closed.

Fig. 9.—Horizontal section through a larva 36 hours old. The closure of the blastopore is shown by the two meeting cells in the middle. Several mesenchyme cells are also shown applied close to the ectoderm.

Fig. 10.—Section through larva 48 hours old.

Fig. 11.—A larva seen from the upper surface, 20 hours old.

Fig. 12.—Larva seen from the upper surface, a section of which is shown in fig. 7.

Fig. 13.—Section of a larva cut horizontally, showing the rudiments of the head-kidney in relation with the ventral plate of the ectoderm, the

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* All the figures have been made under a 2-millim. Zeiss oil-immersion objective with No. 4 eye-piece. The figures are only roughly proportional in size to one another.

section just cutting the ventral ectodermal surface in the segmentation cavity. The anterior large cells are the nephridial cells, while the two small ones may represent the rudimentary mesoderm band cells. These cells are in the ectoderm. By examining the series of sections, this section is seen to be below the gut and does not touch it.

Fig. 14.—Section of a larva 24 hours old.

Plate 26.

Fig. 15.—Oblique section through a larva, showing head-kidney on one side.

Fig. 16.—Section passing through a larva 25 hours old in the axis of the alimentary canal.

Fig. 17.—Horizontal section through a larva.

Fig. 18.—Coronal section through the œsophagus, showing the head-kidneys on either side.

Fig. 19.—View of a larva of the same age as fig. 30, seen from the oral end. The head-kidneys are seen on each side of the oral opening.

Fig. 20.—A horizontal section through the ventral surface of a larva 24 hours old, the section being somewhat oblique, showing the head-kidney in connection with the ectoderm on one side; the section is below the level of the alimentary canal and mouth.

Fig. 21.—The next section to that shown in fig. 10, Plate 25.

Fig. 22.—Section obliquely through the mouth region, showing the relationships of the head-kidneys on each side.

Fig. 23.—Section somewhat similar to that of fig. 16, showing the connection of the head-kidney with the ectoderm, the part in connection with the œsophagus being cut off in this section.

Fig. 24.—Section somewhat similar to the former one, but in a larva 48 hours old.

Plate 27.

Fig. 25.—*Polygordius* larva, the earliest stage at which the head-kidney is recognisable as a small strand connecting a large cell in the ventral ectoderm with the œsophagus.

Fig. 26.—Very young larva of about the same stage as the last, in which a large cell in the ectoderm, midway between mouth and anus, is the rudiment of the nephridium.

Fig. 27.—Early stage, in which the head-kidney is clearly developed as a strand stretching from the region on either side of the anus to the œsophagus.

Fig. 28.—A stage later than the last, in which the head-kidney is more clearly developed.

Fig. 29.—Head-kidney strand, early stage.

Fig. 30.—A later stage than fig. 28, in which there has been considerable increase in size. Numerous mesenchyme cells are seen applied to the œsophagus. The head-kidney is seen as a strand connecting with the œsophagus at one end, and with a conspicuous cell in the ectoderm at the other. The nucleus shown near the anal end of the intestine where it joins the ectoderm is one of the polar mesoderm cells.

Fig. 31.—Head-kidney strand, early stage.

Fig. 32.—A stage in which the larva has changed its shape considerably, having lost the former flat form and become more cubical. The head-kidney is shown as two cells.

Fig. 33.—A stage of about the same age as the last, in which the head-kidney appears as a thin strand.

Fig. 34.—Slightly later stage than the last.

Fig. 35.—A larva considerably older than the preceding stage. The adult larval form is beginning to be assumed. The head-kidney is composed of two distinct cells in the blastocœl. Certain mesenchyme cells are seen applied to the intestinal tract in this and the former figures.

PLATE 28.

Fig. 36.—View of a larva 23 hours old, seen from the upper surface. The partial closure of the blastopore is seen in the middle.

Fig. 37.—Larva 48 hours old, seen from the ventral surface; the head-kidneys are seen on each side of the œsophagus running up to the surface of the ectoderm.

Fig. 38.—Head-kidney strand, early stage.

Fig. 39.—A late stage. The nuclei of the head-kidney have disappeared, it has become somewhat granular at each end, and a faint lumen has appeared in the middle. Shortly after this the cilia in its interior are developed.

Fig. 40.—A drawing of the head-kidney, the same age as the larva figured in fig. 39.

Fig. 41.—A larva about 48 hours old, seen from the ventral surface; head-kidneys are seen on each side of the œsophagus as fine strands composed each of three cells.

Fig. 42.—Fully developed free-swimming larva, seen from the ventral surface. The head-kidneys, with the mesoderm bands, are seen on either side of the anal opening. To the head-kidneys run the muscle strands from the apical region.

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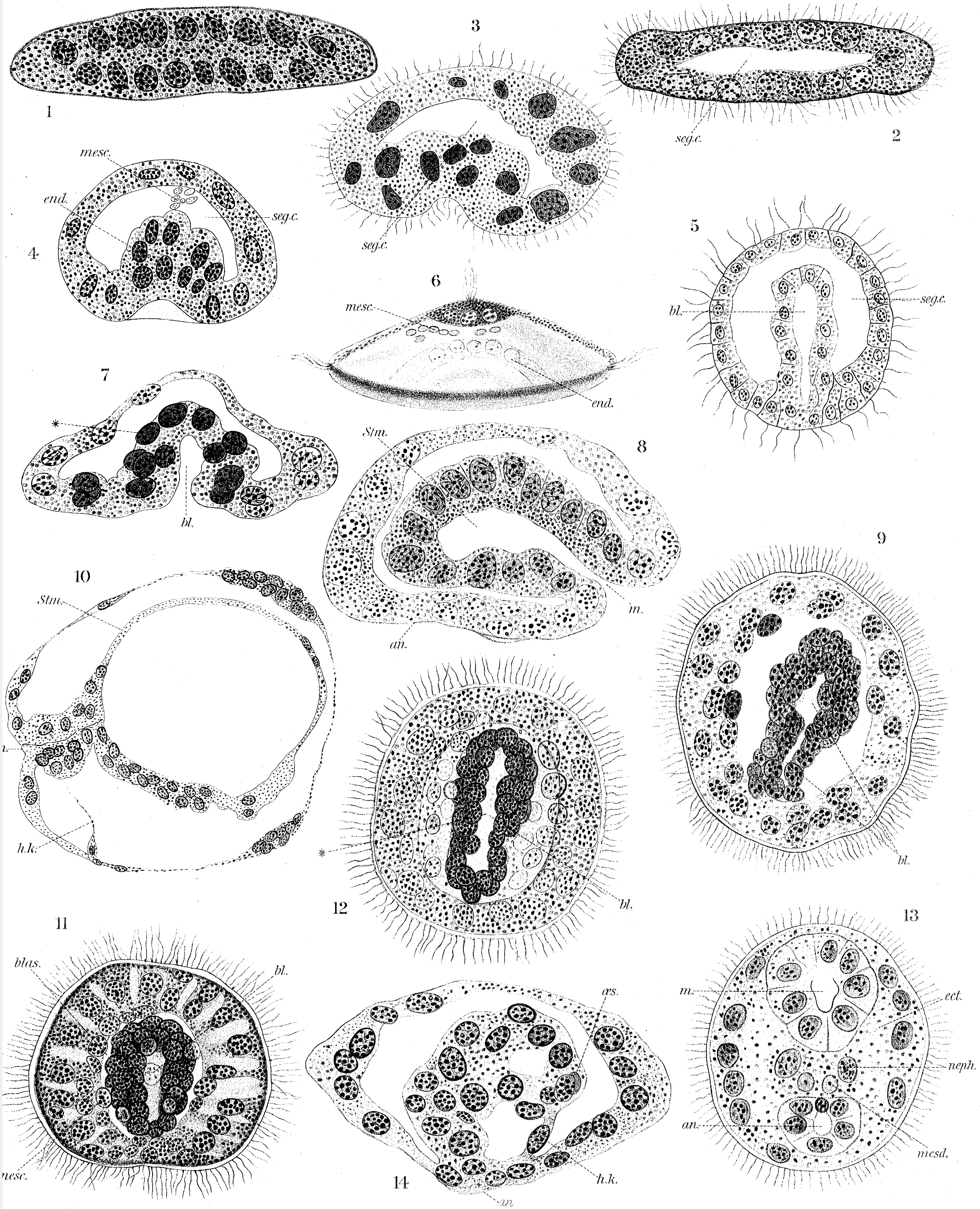

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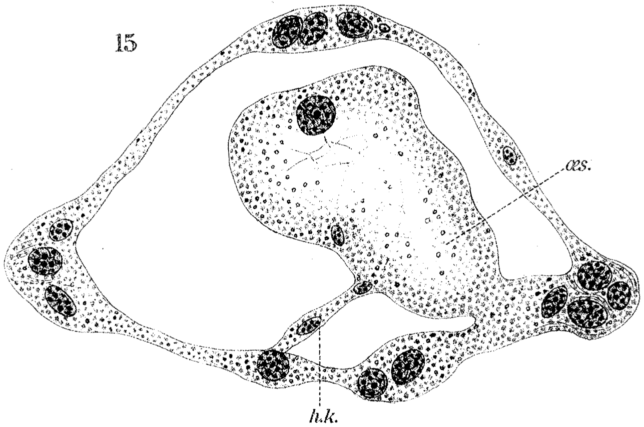

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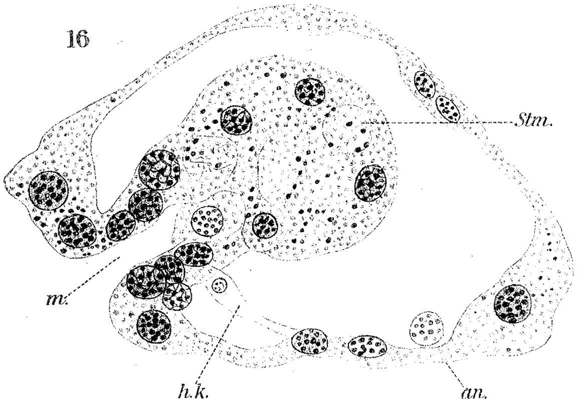

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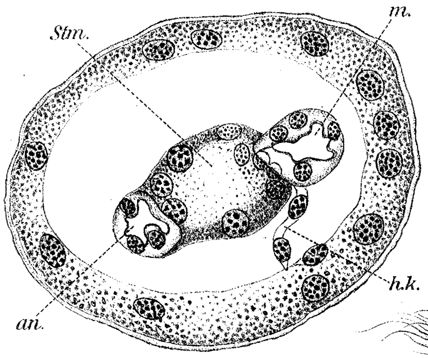

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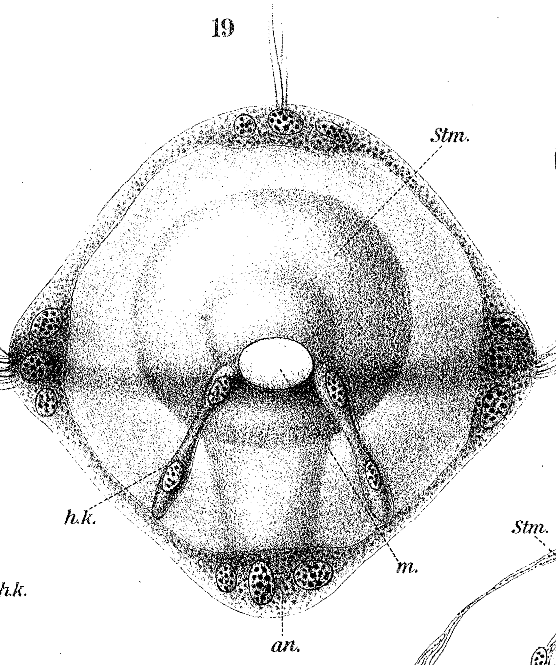

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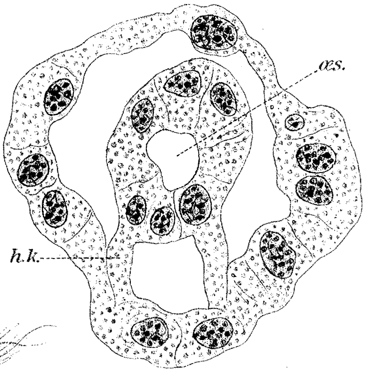

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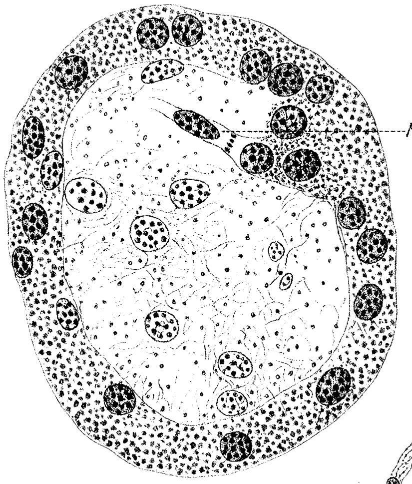

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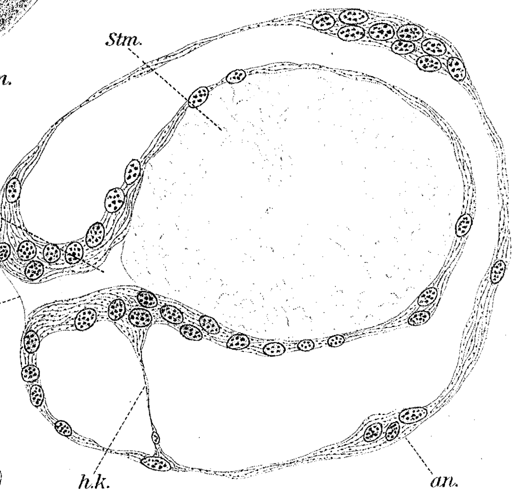

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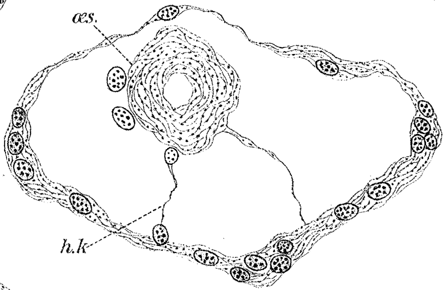

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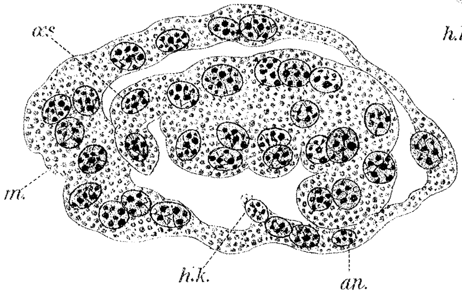

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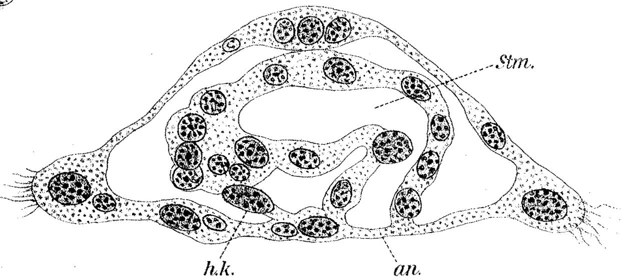

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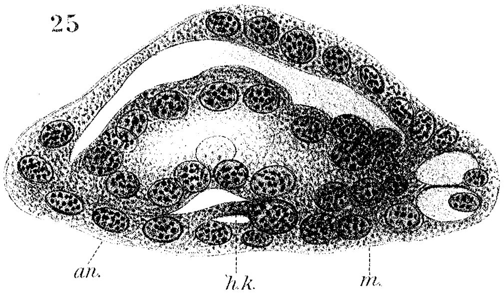

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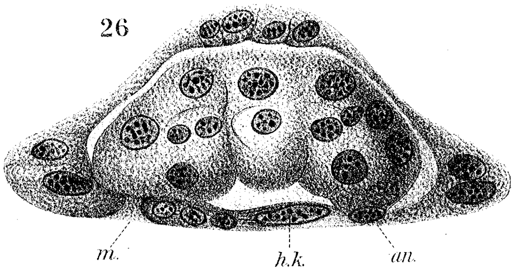

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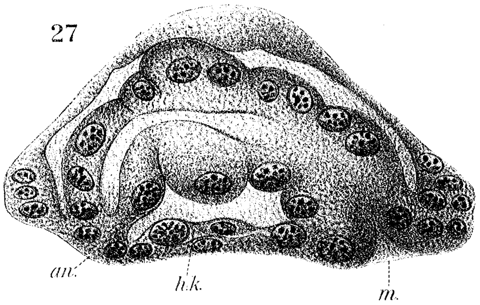

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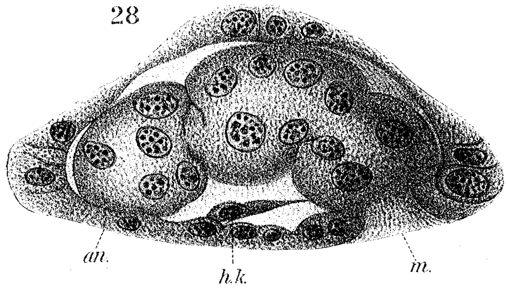

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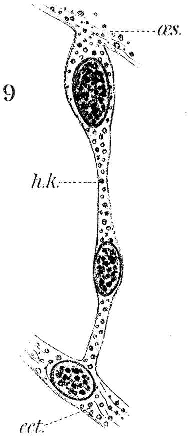

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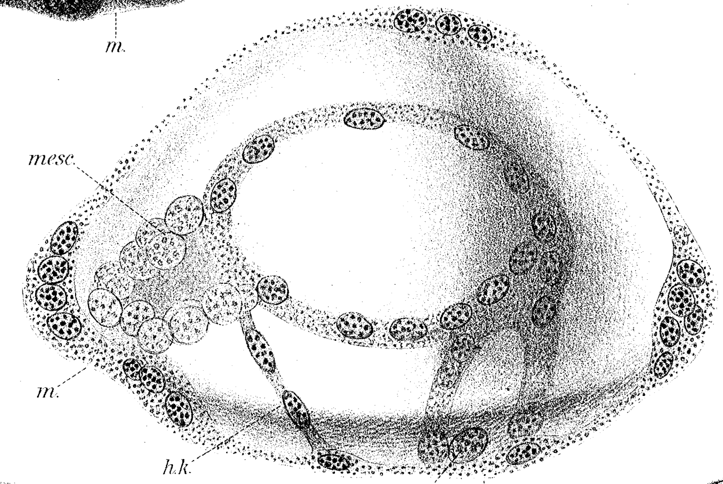

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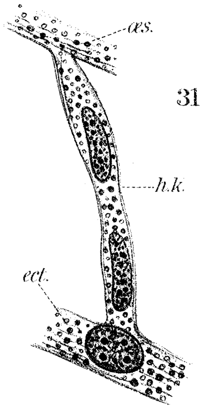

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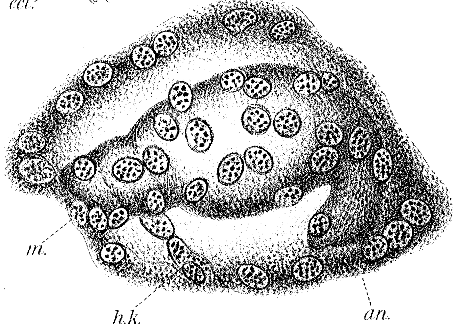

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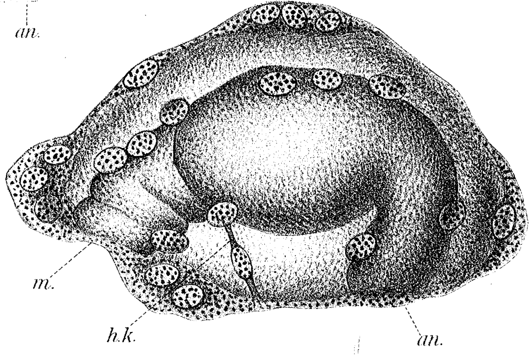

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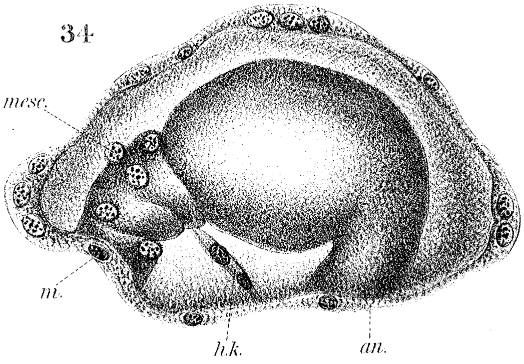

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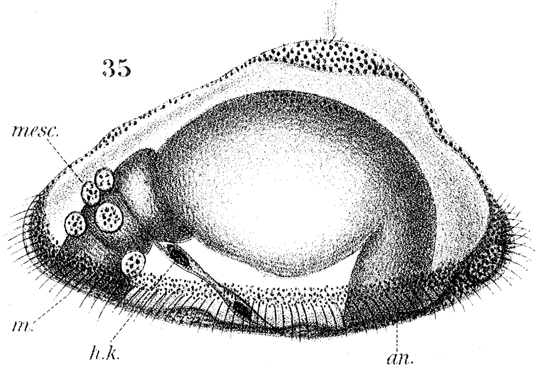

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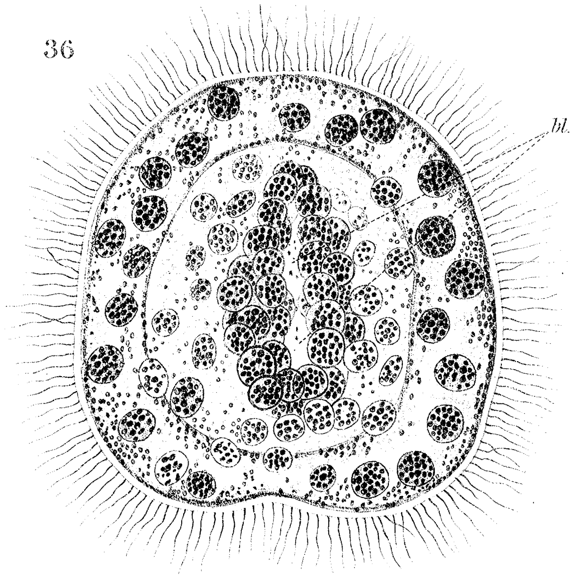

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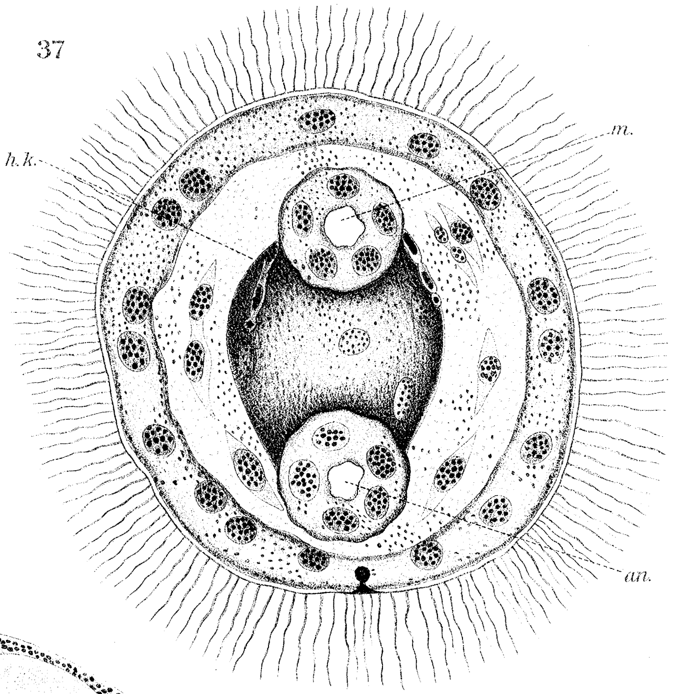

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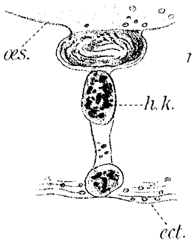

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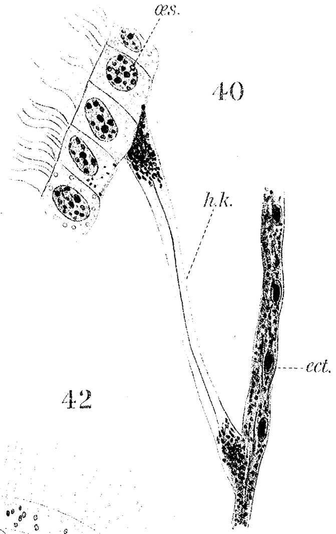

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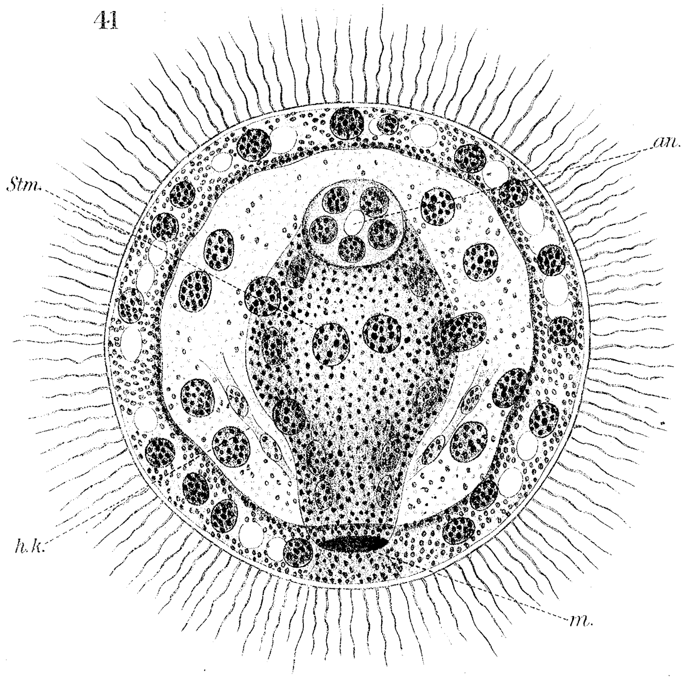

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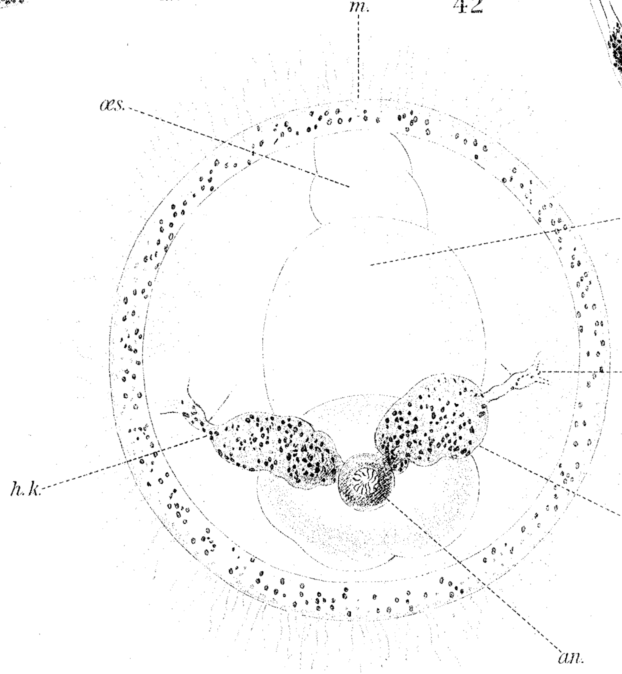

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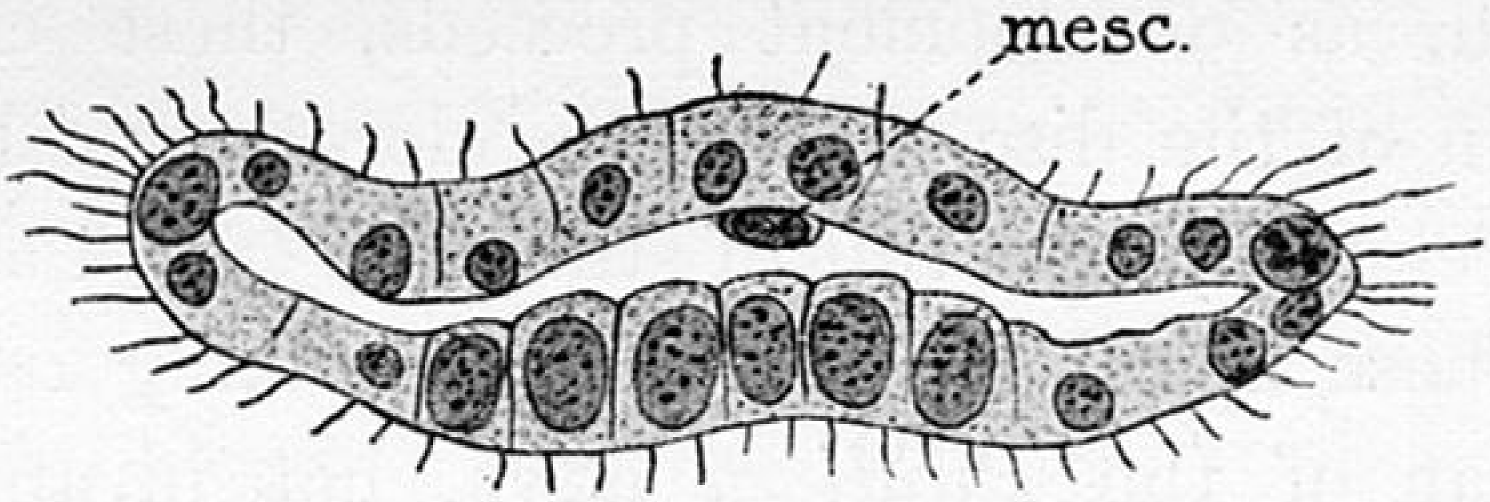

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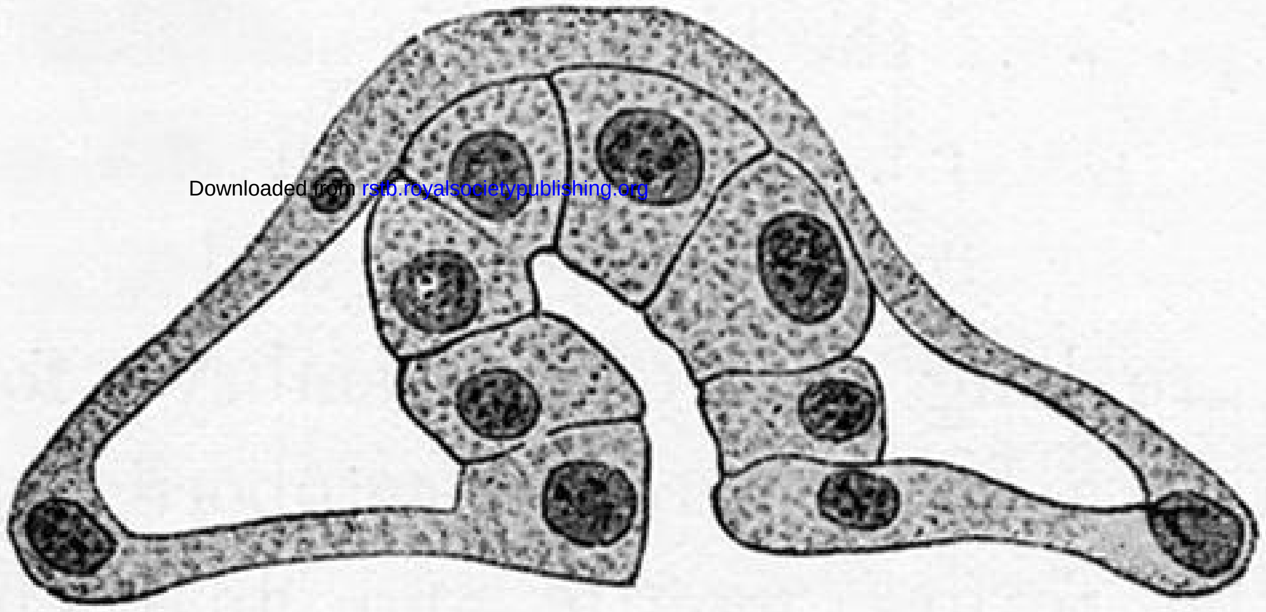

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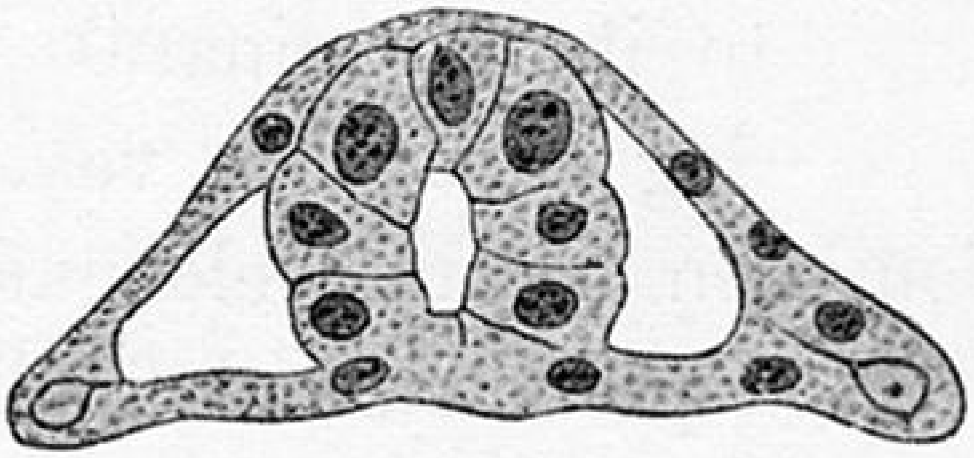

Fig. C.

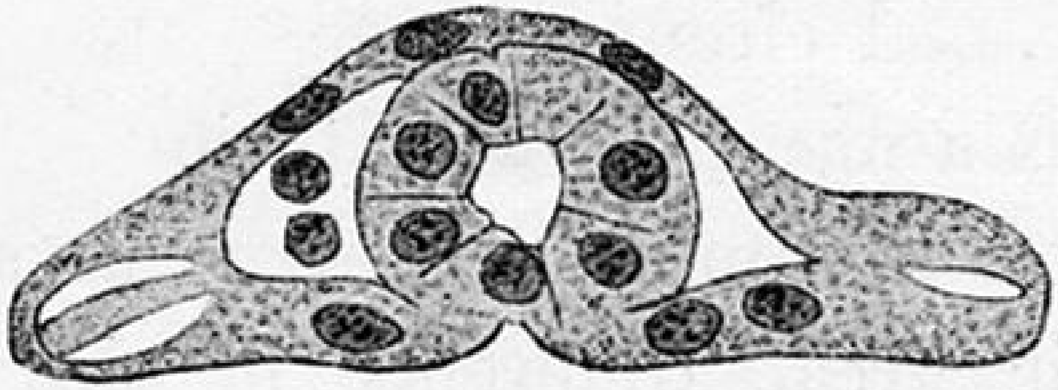

Fig. D.

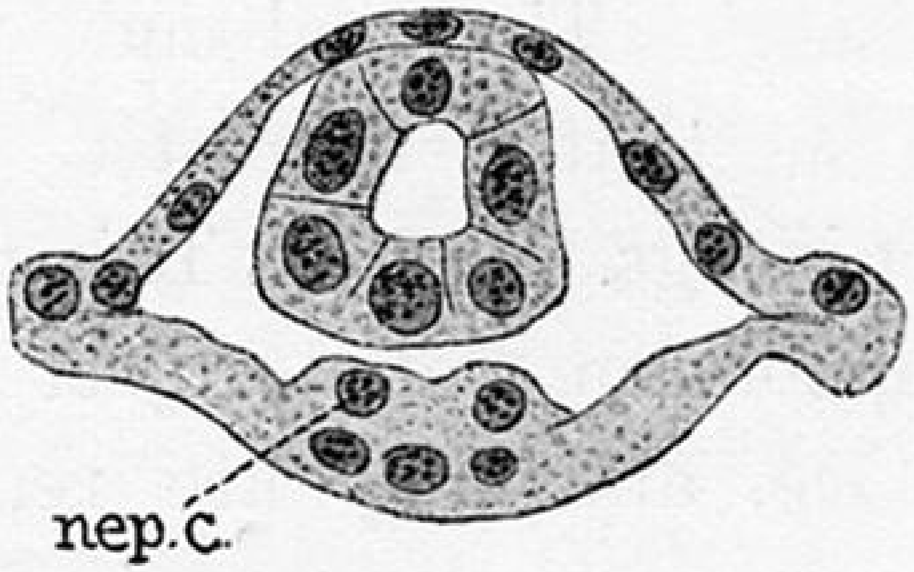

Fig. E.

Fig. A.—Section through a young blastula, showing the presence of a mesenchyme cell in the blastocœl, *mesc.*

Fig. B.—Section through the mouth region of a young larva.

Fig. C.—Section through the stomach region of the same larva.

Fig. D.—Section through the anal region of the same.

Fig. E.—Section through a young larva, showing the nephridial cells on the inner wall of the ventral plate of the ectoderm projecting into the blastocœl, *nep. c.* The gut is shown cut across.

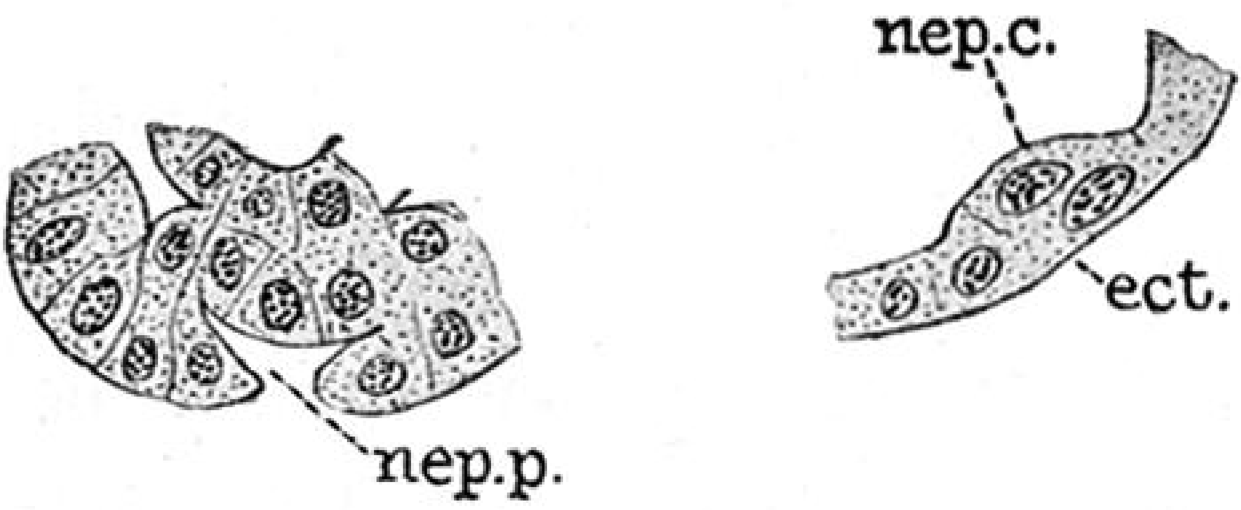

FIG. F.

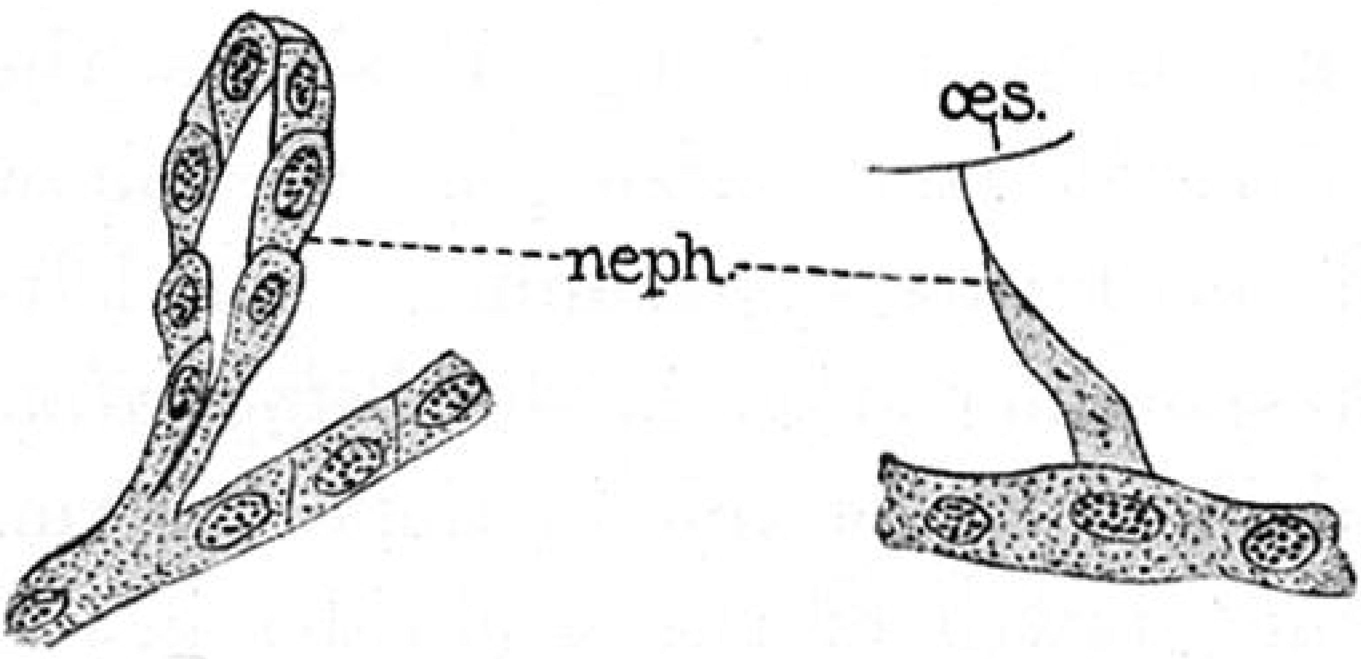

FIG. G.

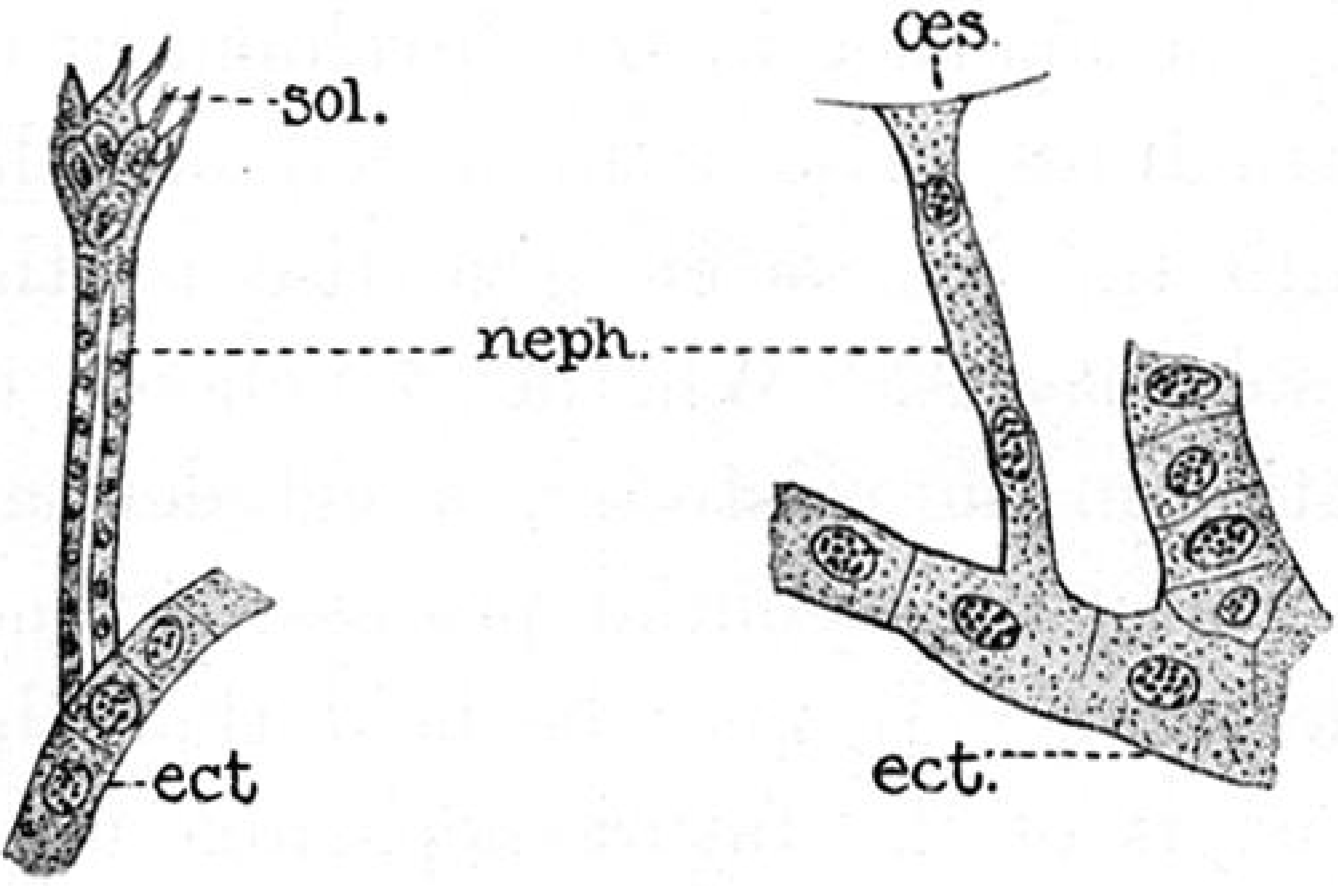

FIG. H.

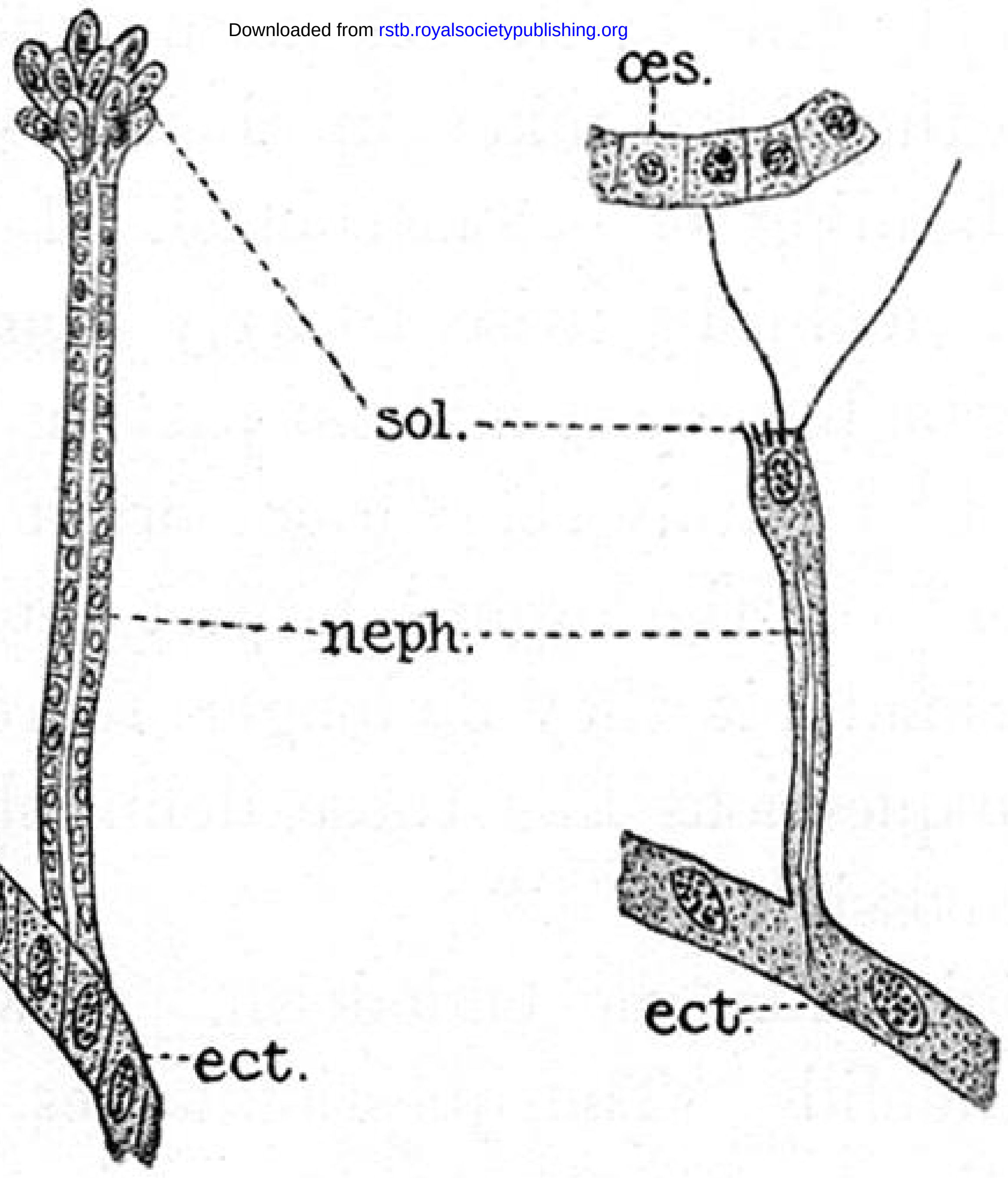

FIG. I.

Diagrammatic Series of Figures representing the relationships of the developing nephridia in the *Actinotrocha* larva and in the *Polygordius* larva: *œs.*, œsophagus; *ect.*, ectoderm; *neph.*, nephridium; *sol.*, solenocytes; *nep. p.*, nephridial pit; *nep. c.*, nephridial cell.

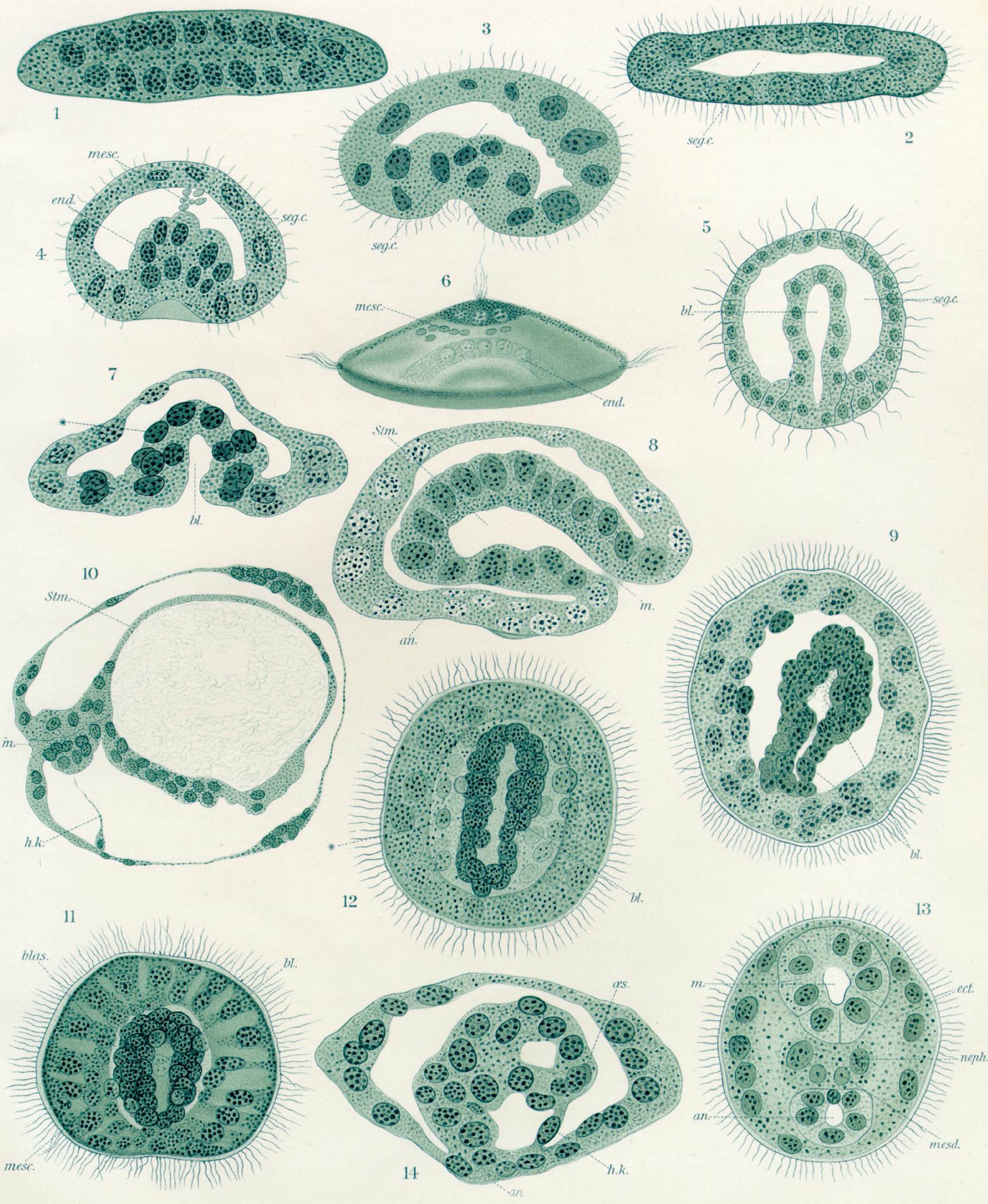

PLATE 25.

Fig. 1.—Section of a *Polygordius* larva 18 hours after fertilisation, showing the flat condition of the morula stage. The cilia on the external surface are not represented.

Fig. 2.—Section of a stage slightly later than that shown in fig. 1, showing the segmentation cavity.

Fig. 3.—Section showing the thickening of the ventral surface of the larva preparatory to invagination.

Fig. 4.—Commencement of invagination and formation of the gastrula.

Fig. 5.—A later stage of fig. 4, showing the gastrula in horizontal section.

Fig. 6.—Drawing of a whole preparation, showing invagination of the endoderm, with the presence of mesenchyme cells in the segmentation cavity.

Fig. 7.—Section of a gastrula.

Fig. 8.—Section of a larva 36 hours old, showing the primitive gut. Sagittal section; the anal end of the blastopore is closed.

Fig. 9.—Horizontal section through a larva 36 hours old. The closure of the blastopore is shown by the two meeting cells in the middle. Several mesenchyme cells are also shown applied close to the ectoderm.

Fig. 10.—Section through larva 48 hours old.

Fig. 11.—A larva seen from the upper surface, 20 hours old.

Fig. 12.—Larva seen from the upper surface, a section of which is shown in fig. 7.

Fig. 13.—Section of a larva cut horizontally, showing the rudiments of the head-kidney in relation with the ventral plate of the ectoderm, the section just cutting the ventral ectodermal surface in the segmentation cavity. The anterior large cells are the nephridial cells, while the two small ones may represent the rudimentary mesoderm band cells. These cells are in the ectoderm. By examining the series of sections, this section is seen to be below the gut and does not touch it.

Fig. 14.—Section of a larva 24 hours old.

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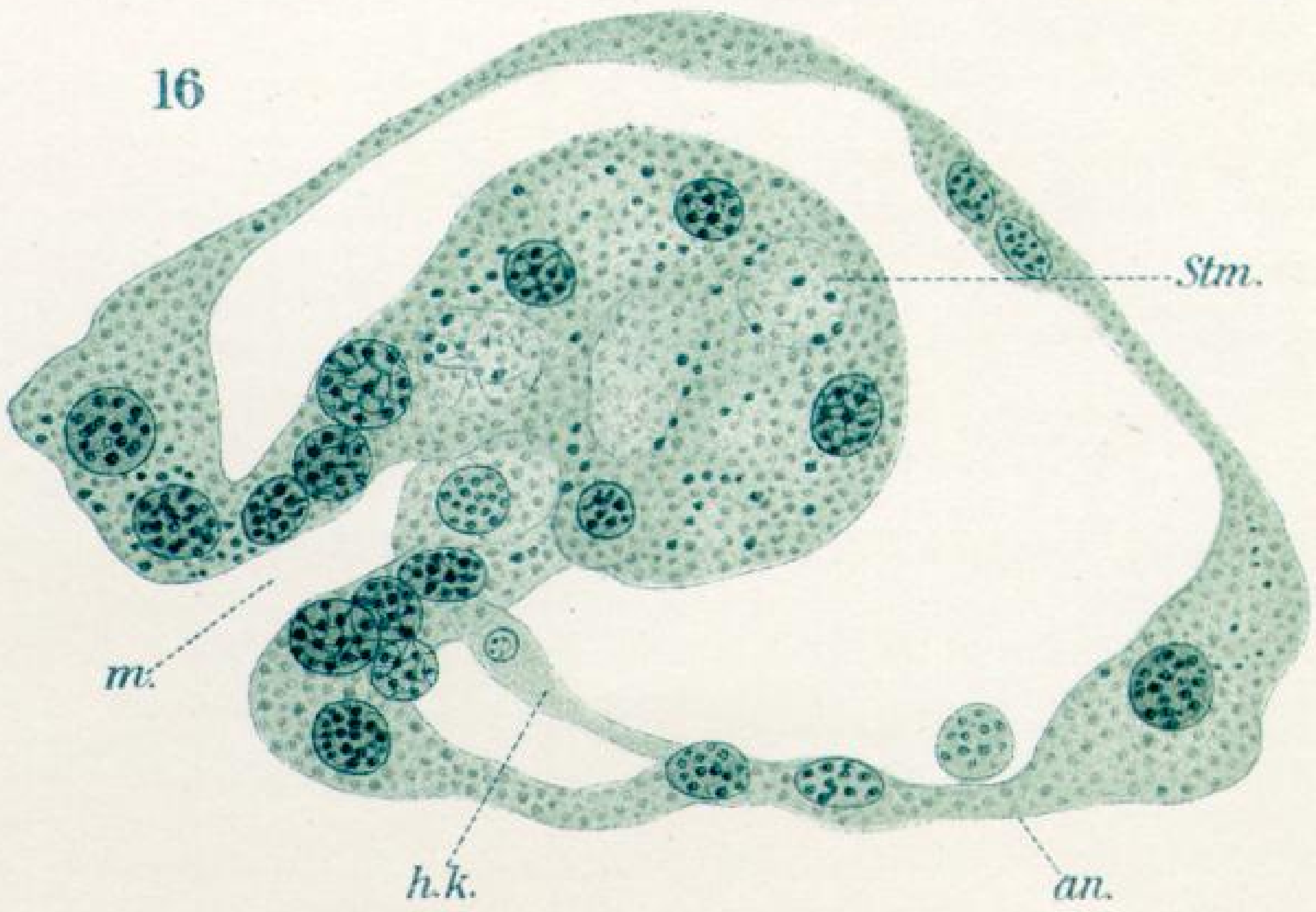

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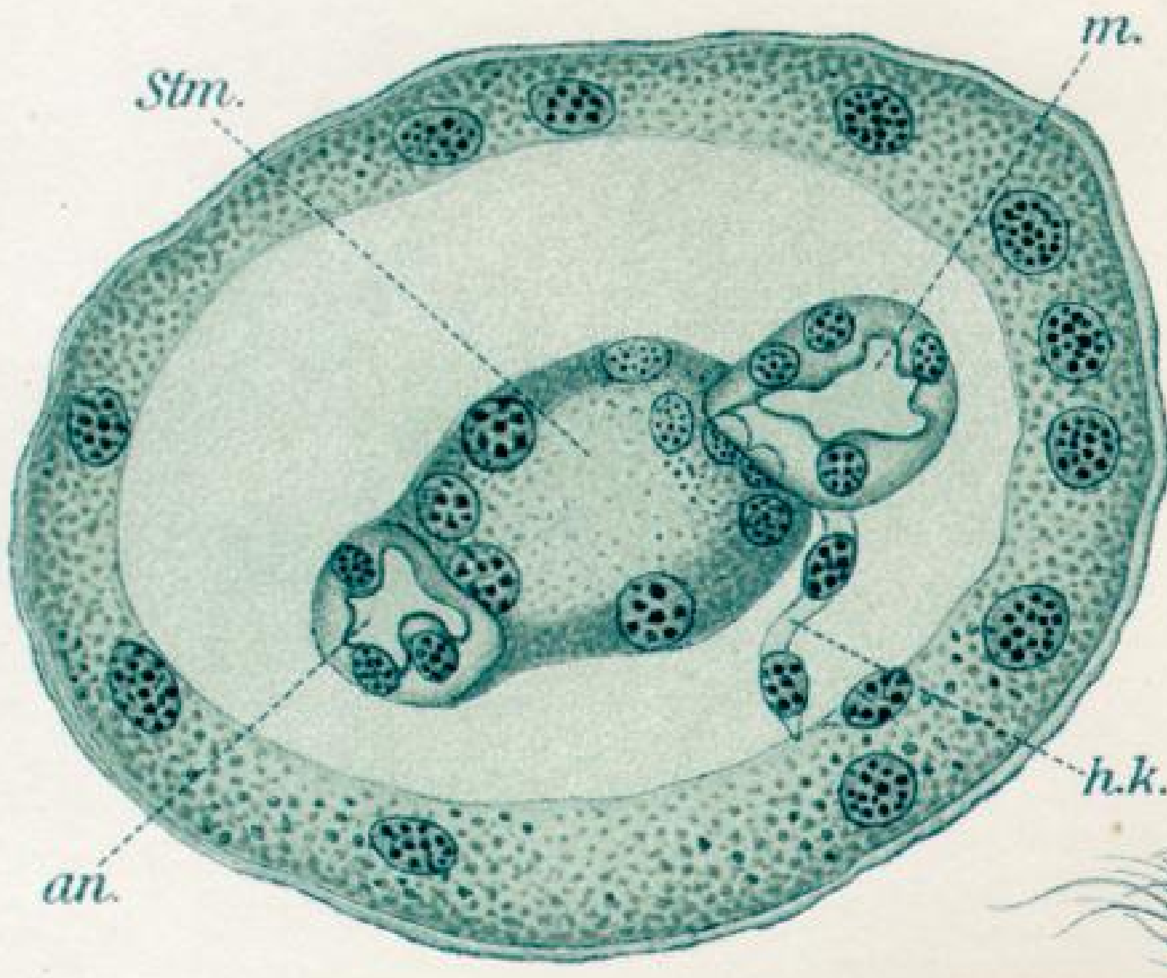

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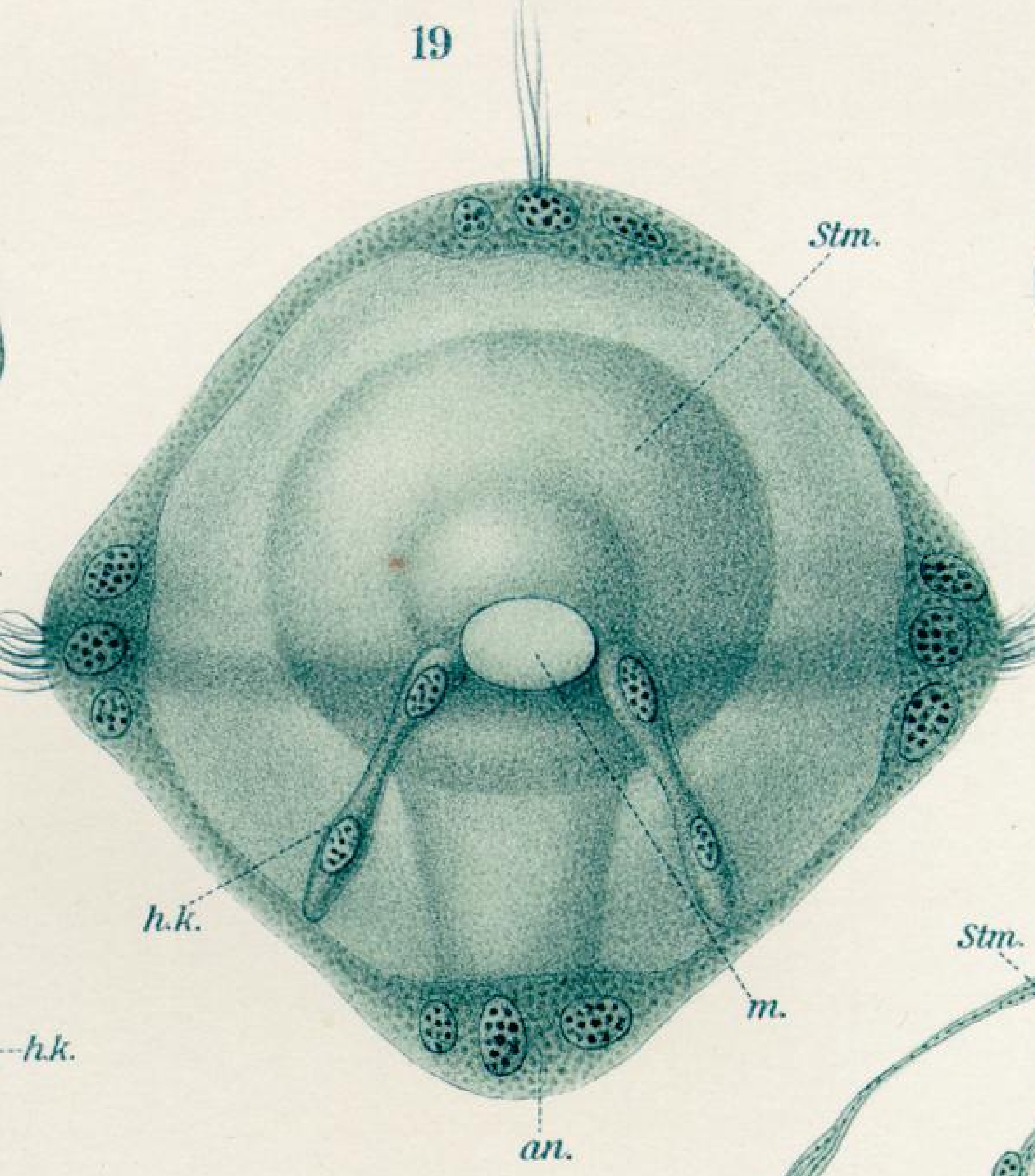

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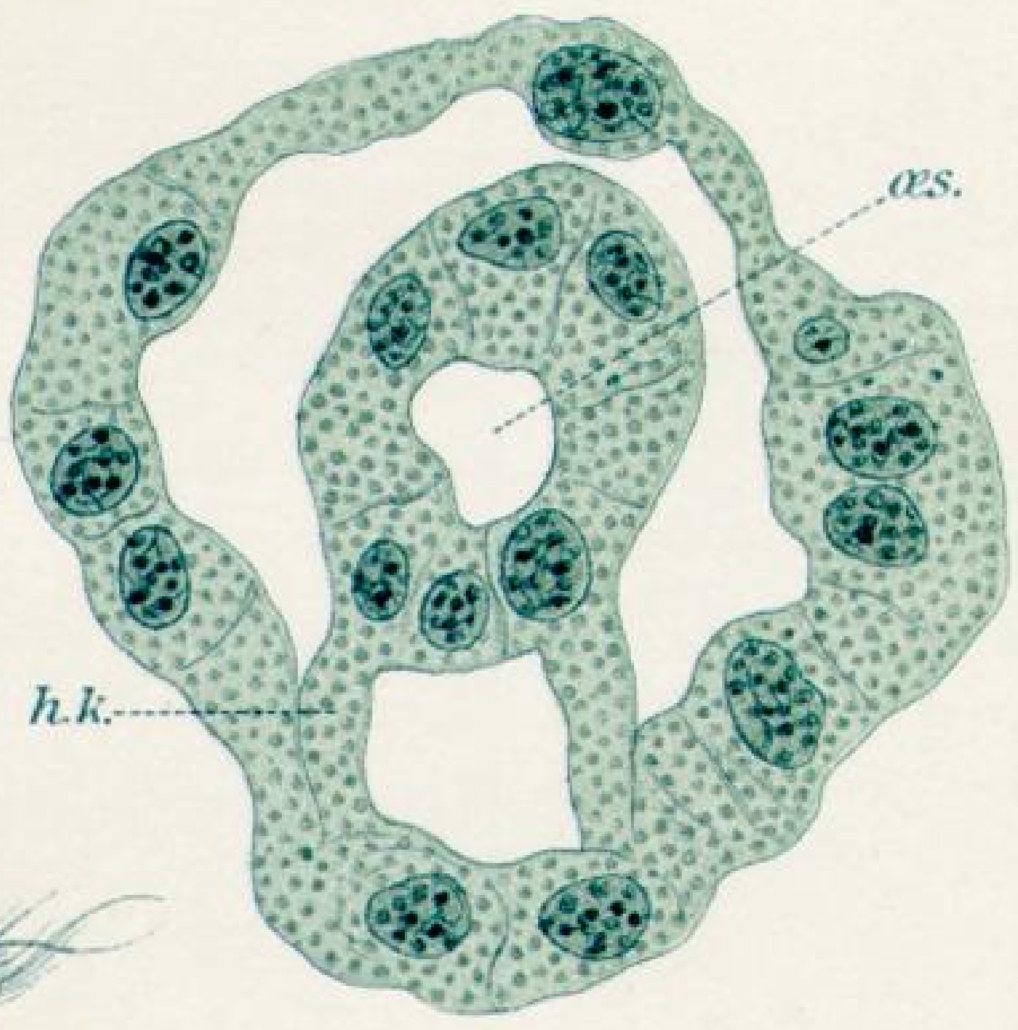

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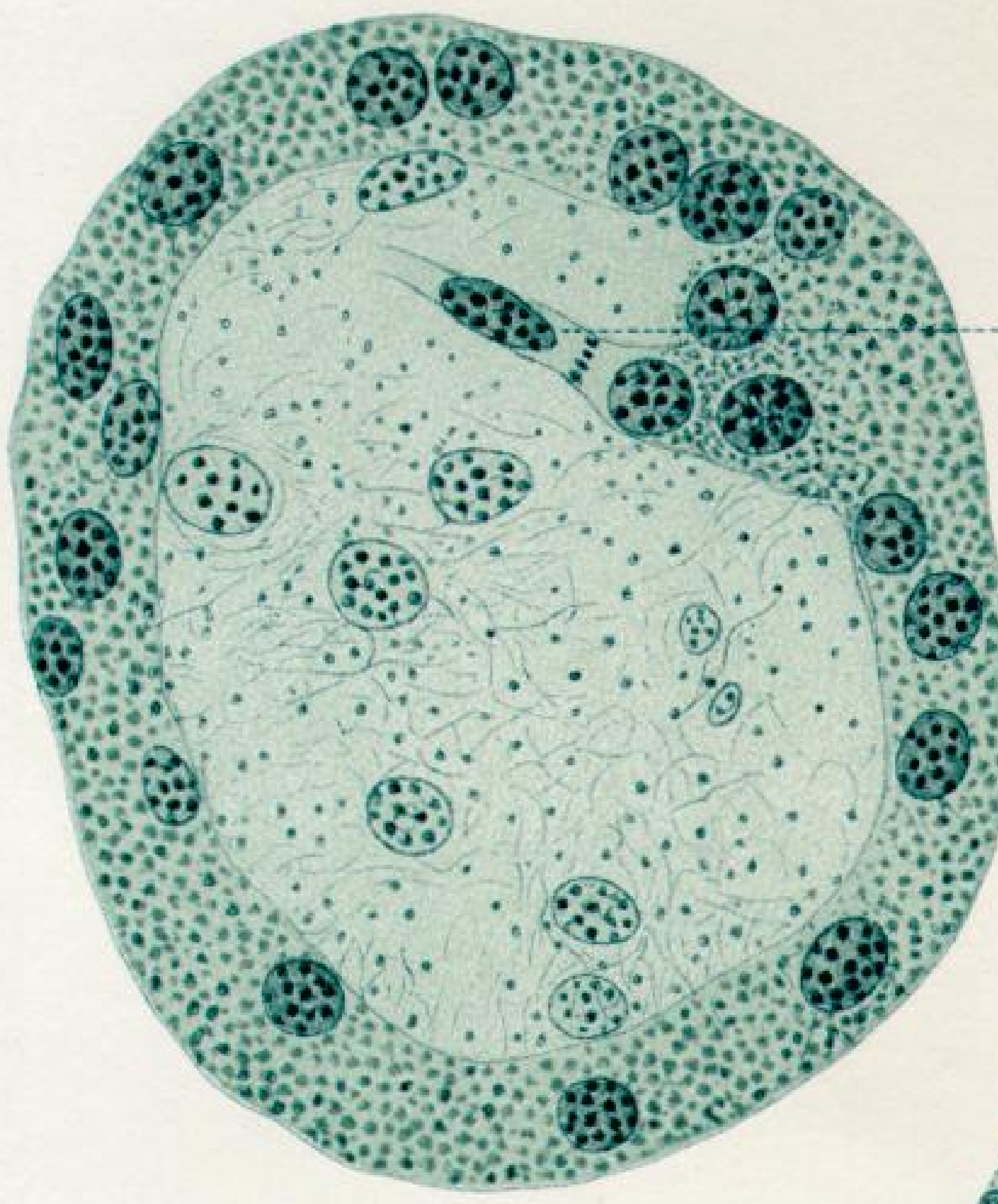

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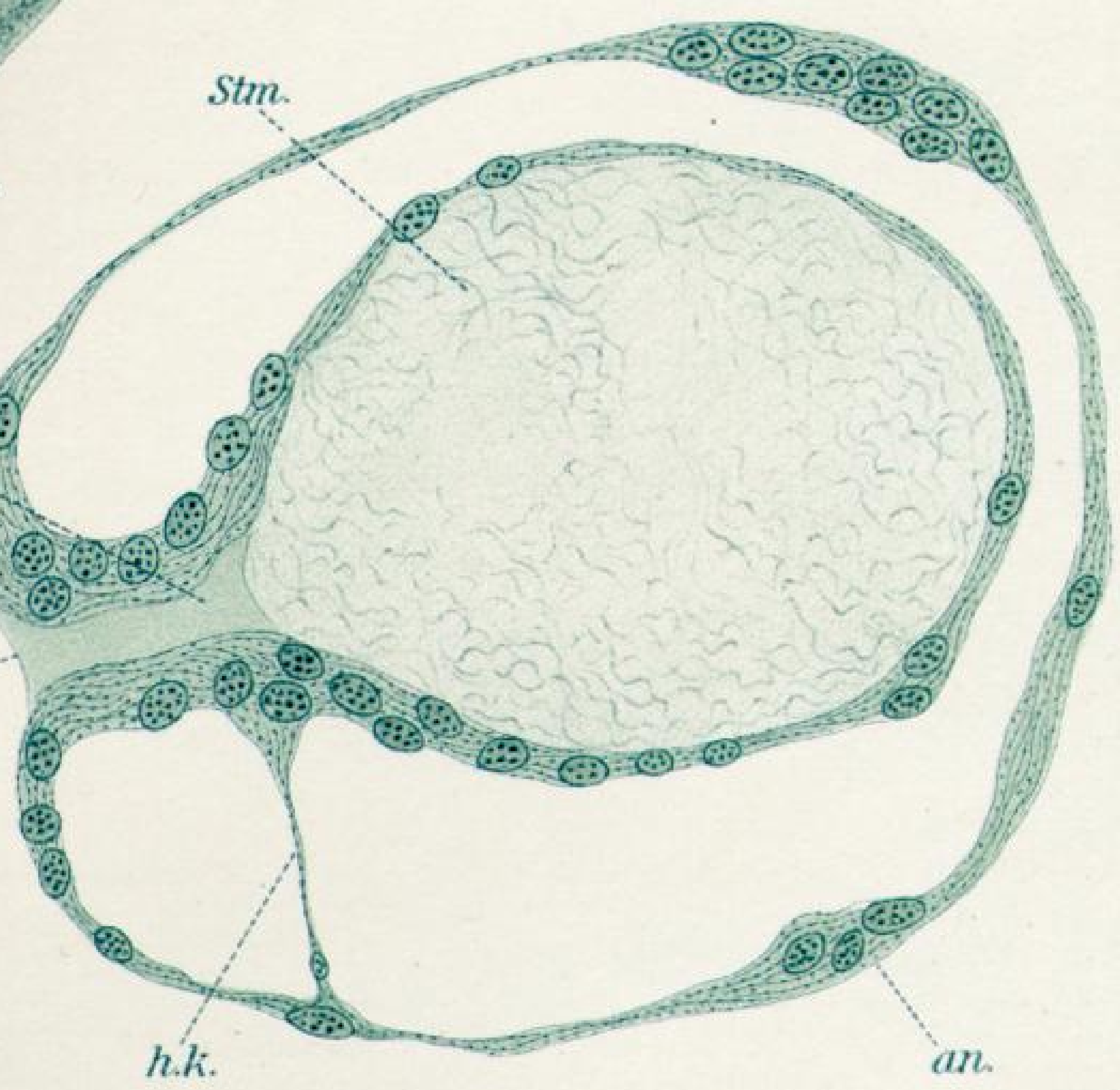

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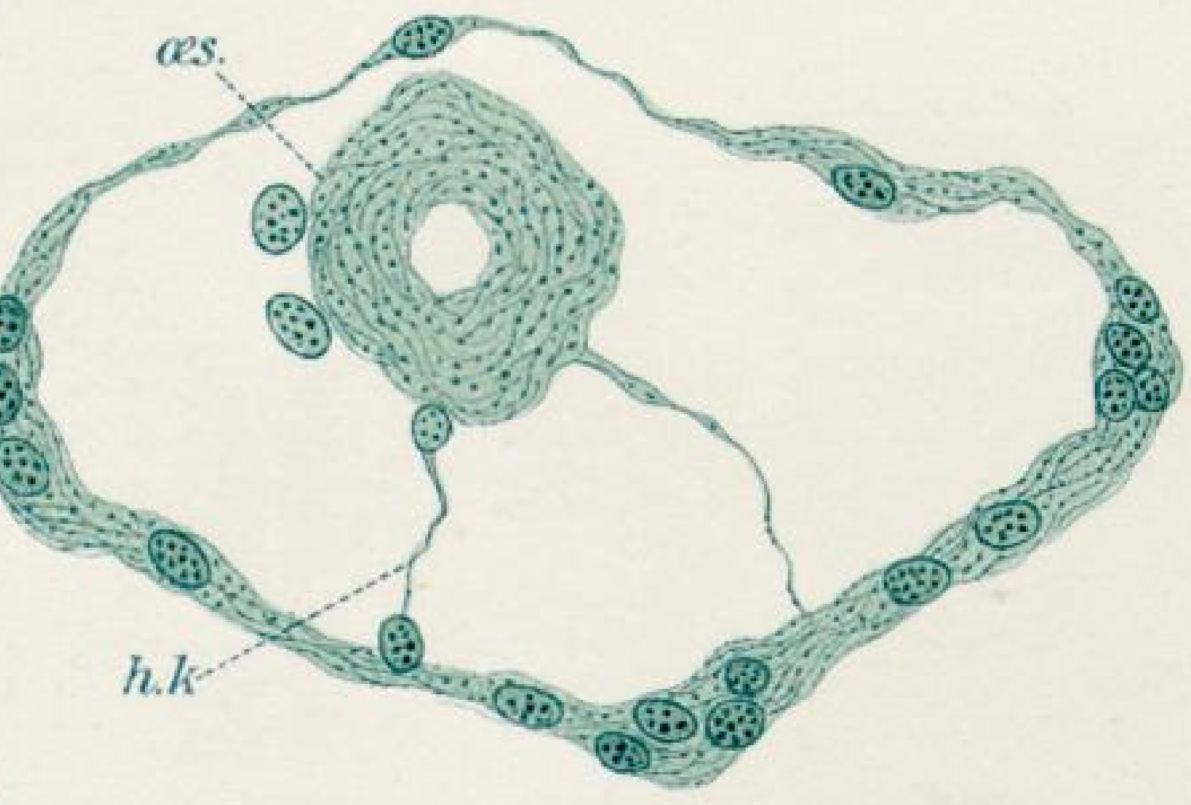

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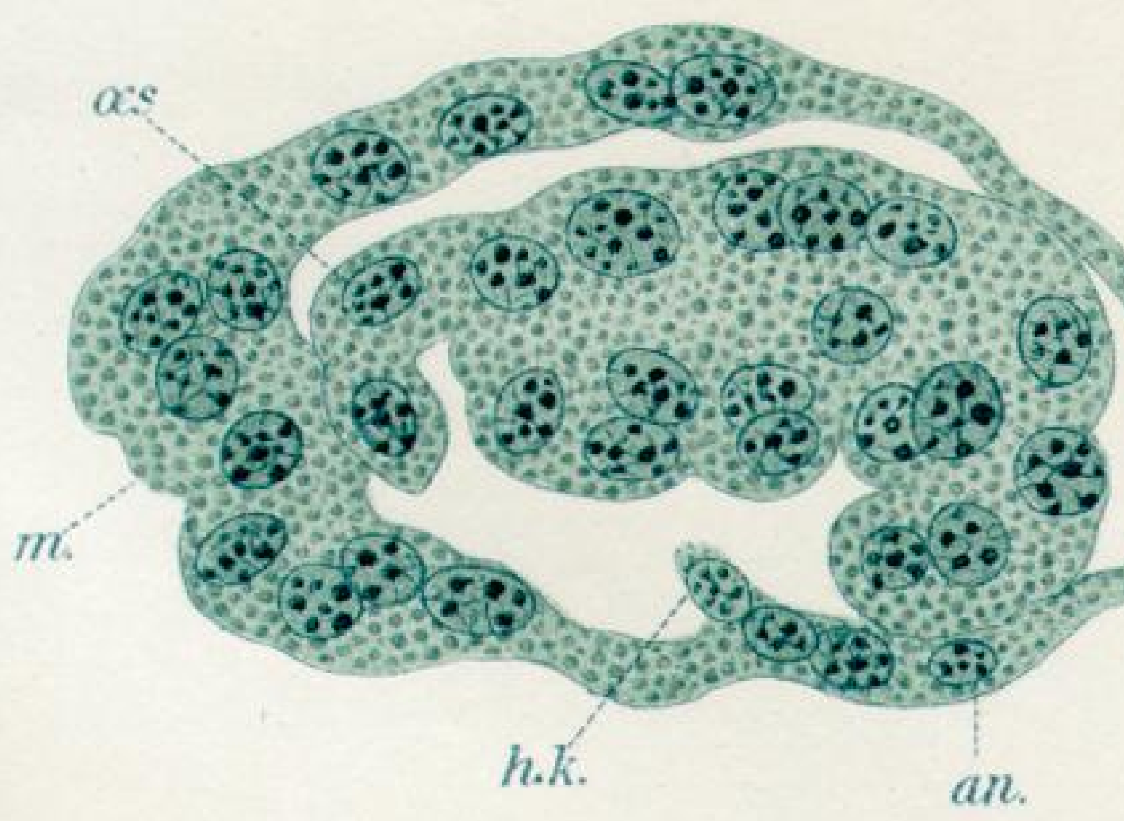

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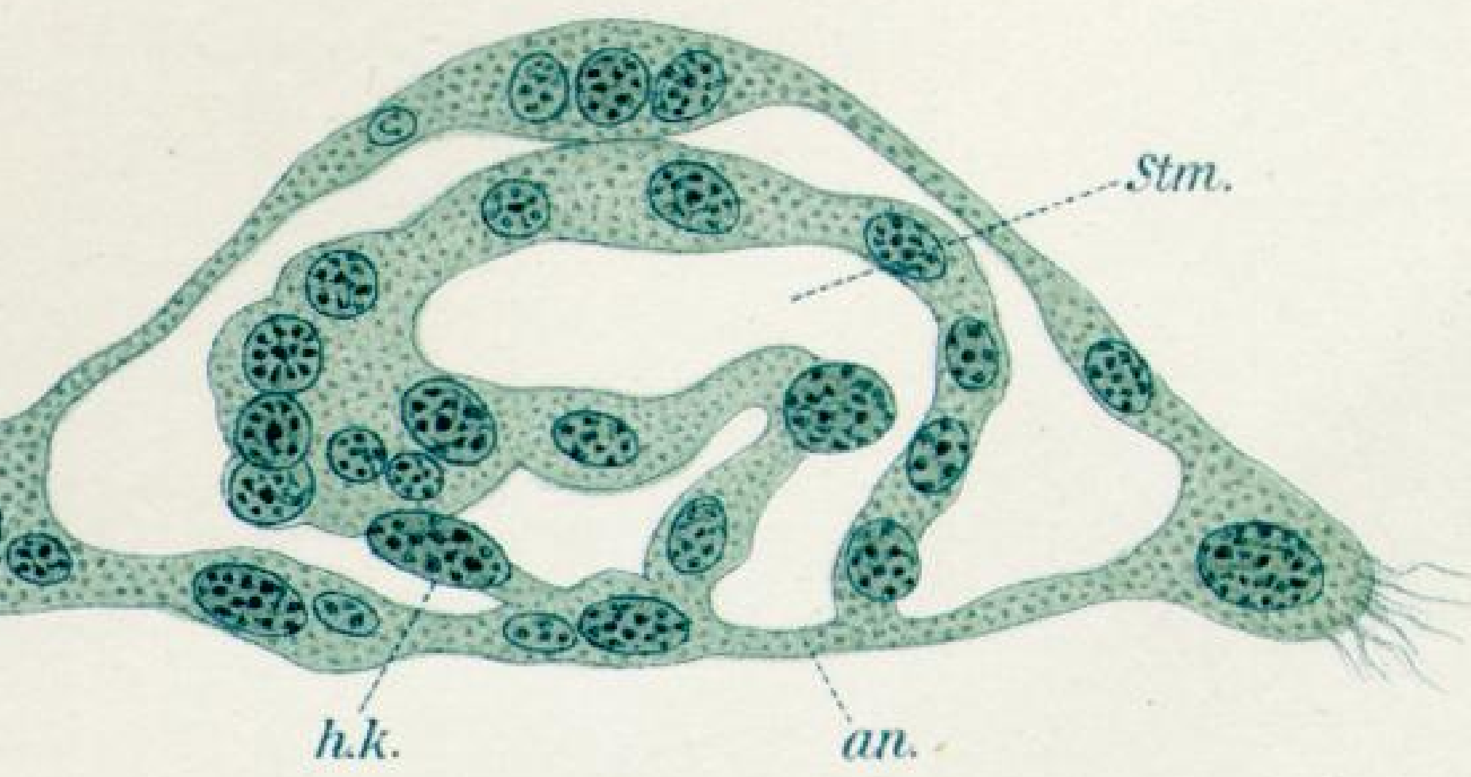

Plate 26.

Fig. 15.—Oblique section through a larva, showing head-kidney on one side.

Fig. 16.—Section passing through a larva 25 hours old in the axis of the alimentary canal.

Fig. 17.—Horizontal section through a larva.

Fig. 18.—Coronal section through the œsophagus, showing the head-kidneys on either side.

Fig. 19.—View of a larva of the same age as fig. 30, seen from the oral end. The head-kidneys are seen on each side of the oral opening.

Fig. 20.—A horizontal section through the ventral surface of a larva 24 hours old, the section being somewhat oblique, showing the head-kidney in connection with the ectoderm on one side; the section is below the level of the alimentary canal and mouth.

Fig. 21.—The next section to that shown in fig. 10, Plate 25.

Fig. 22.—Section obliquely through the mouth region, showing the relationships of the head-kidneys on each side.

Fig. 23.—Section somewhat similar to that of fig. 16, showing the connection of the head-kidney with the ectoderm, the part in connection with the œsophagus being cut off in this section.

Fig. 24.—Section somewhat similar to the former one, but in a larva 48 hours old.

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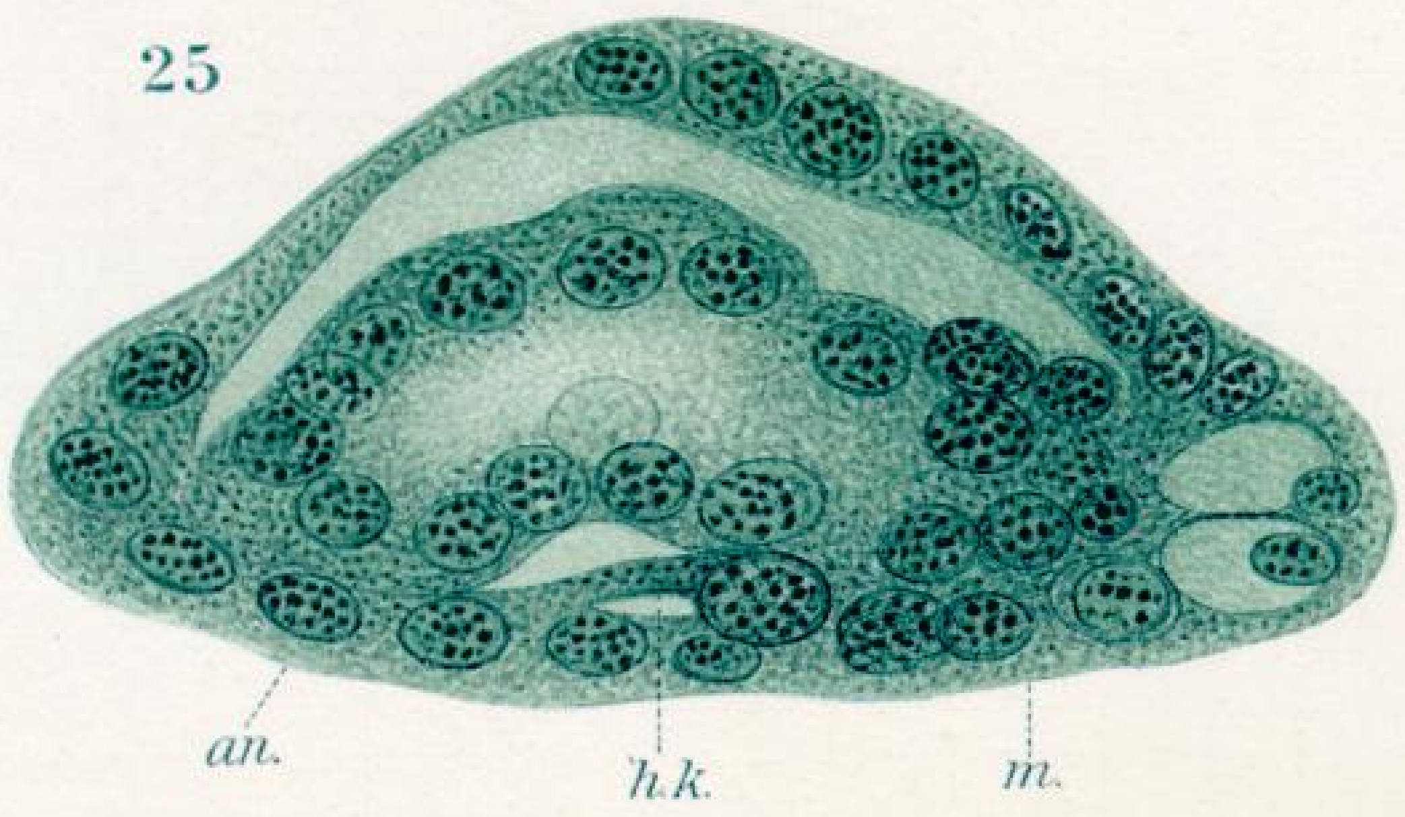

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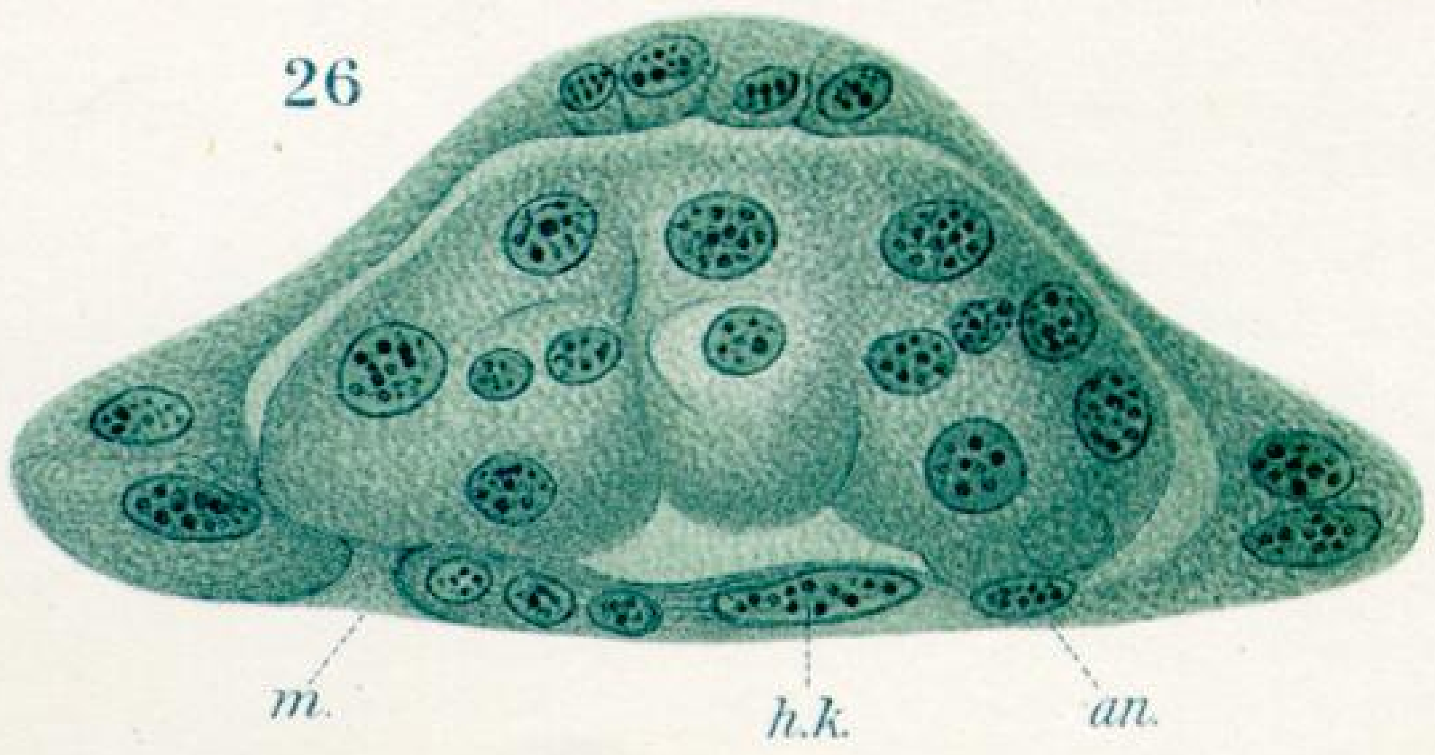

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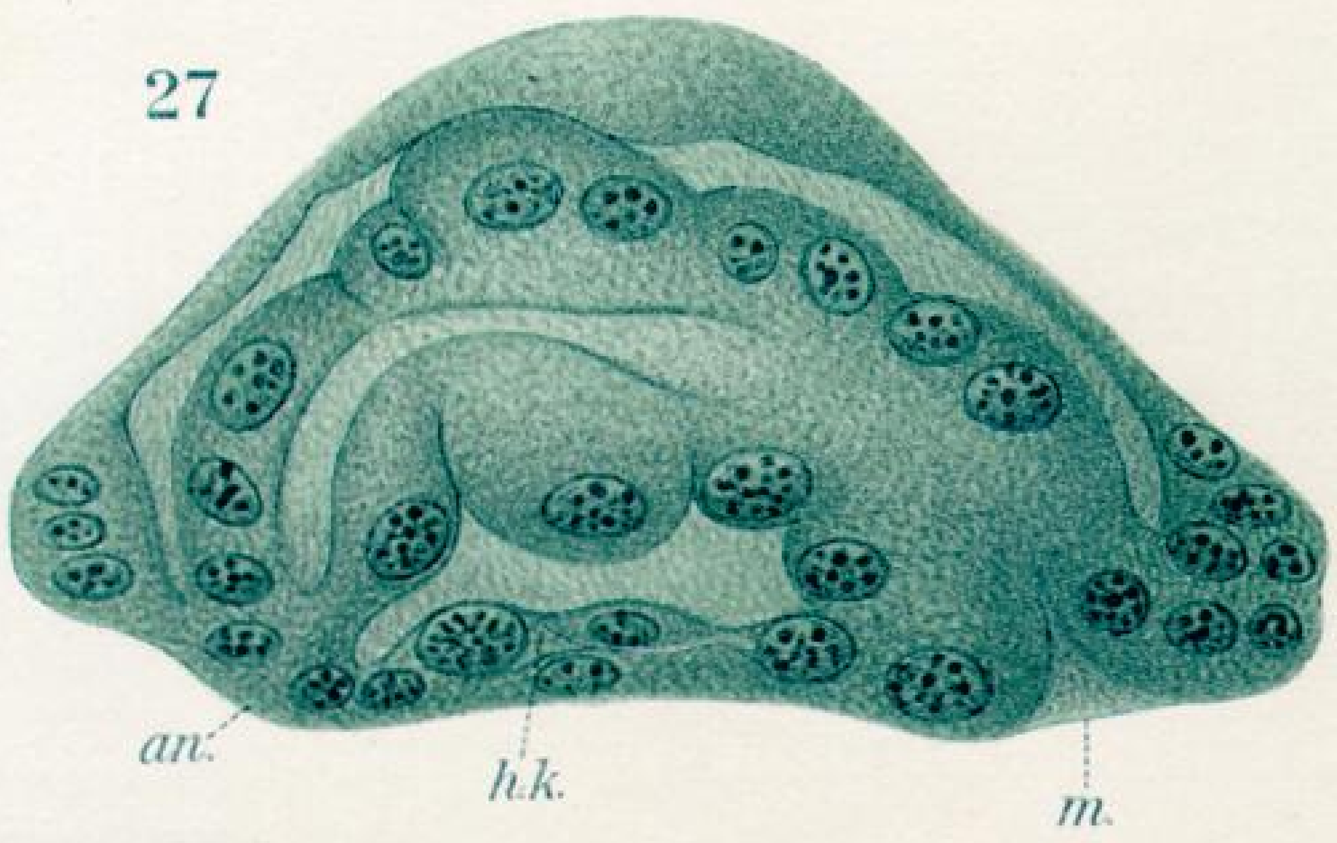

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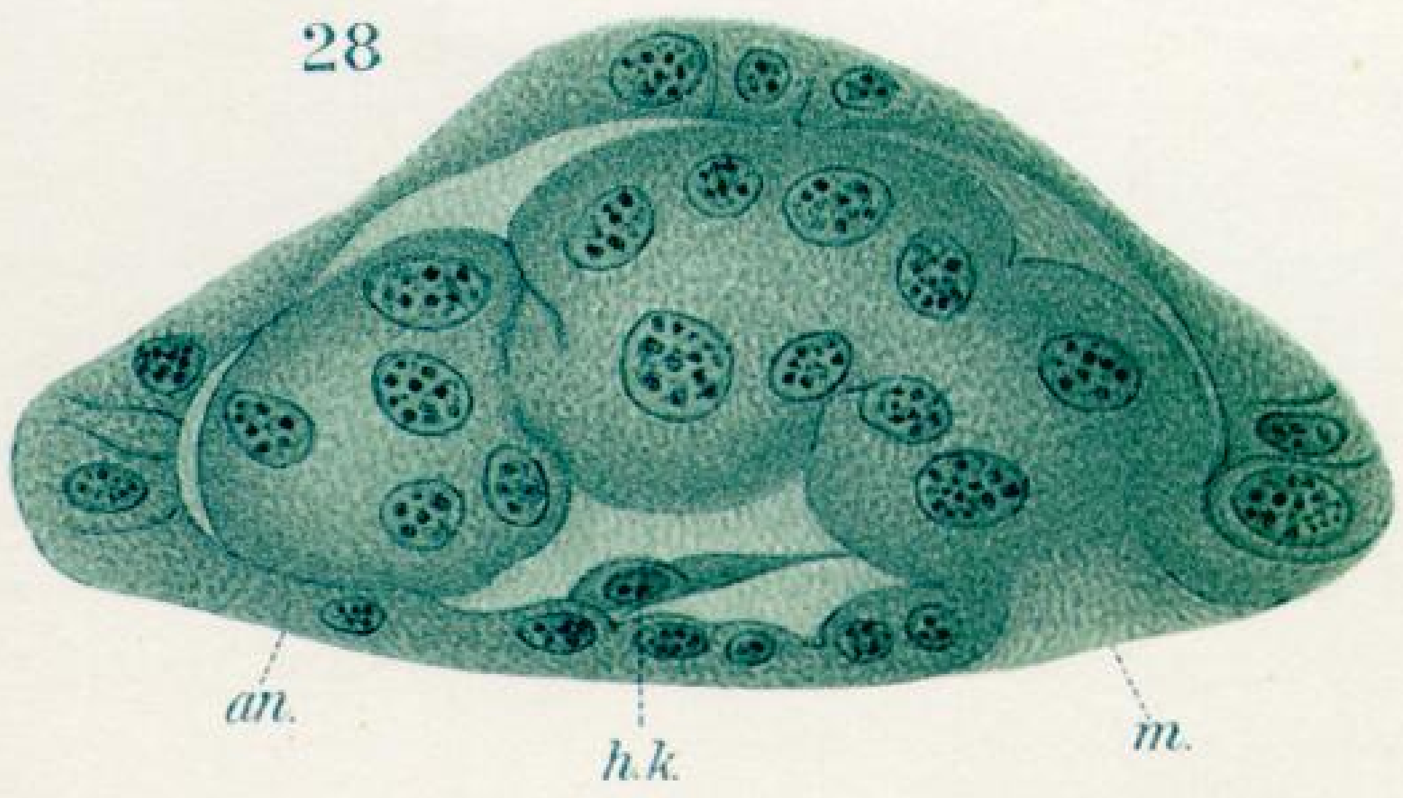

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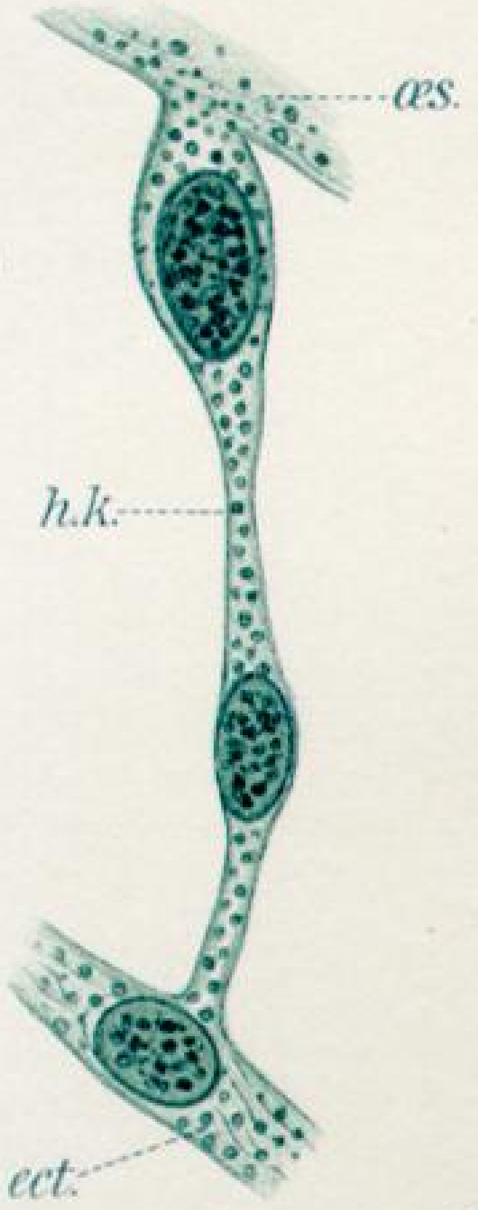

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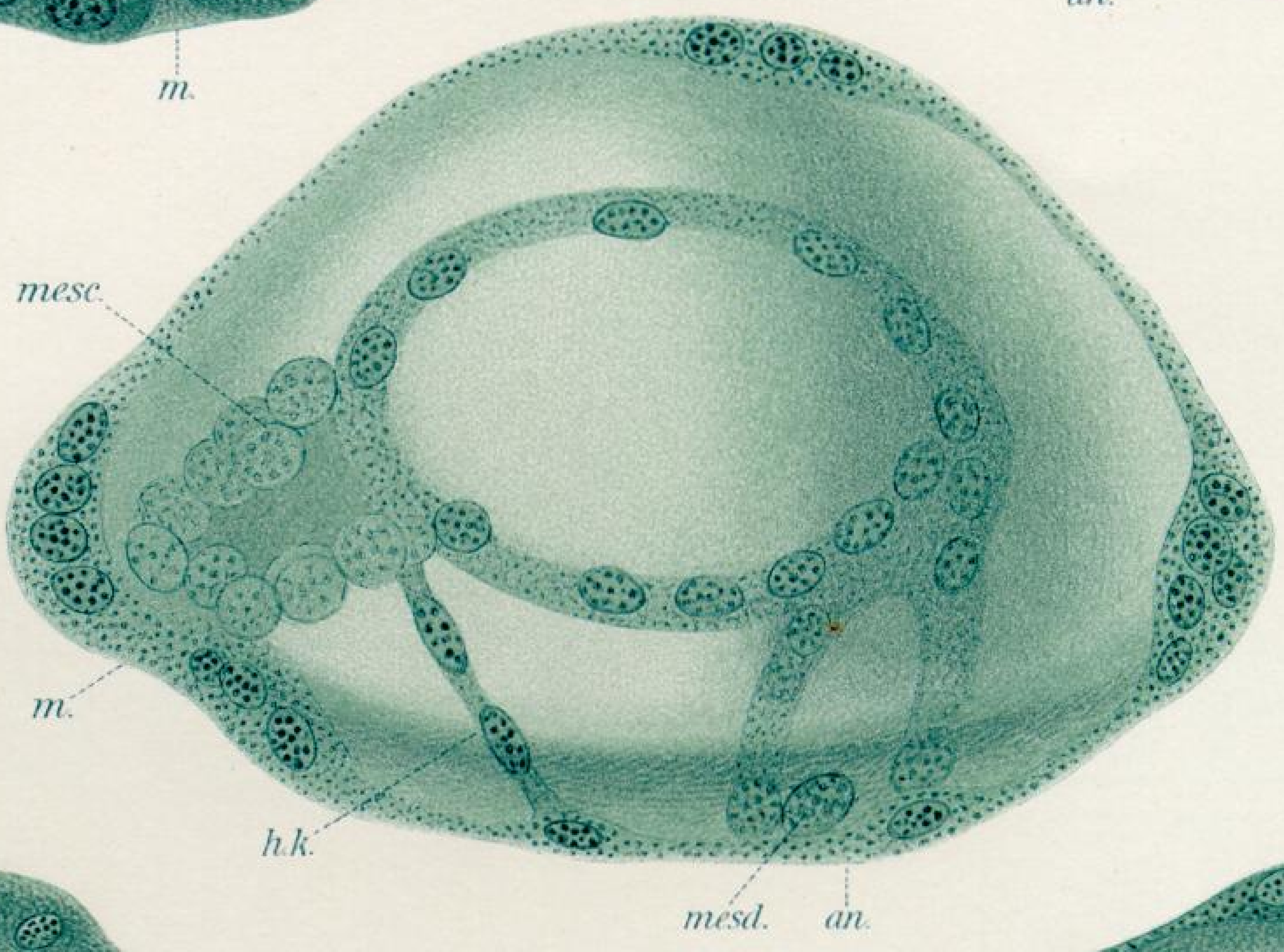

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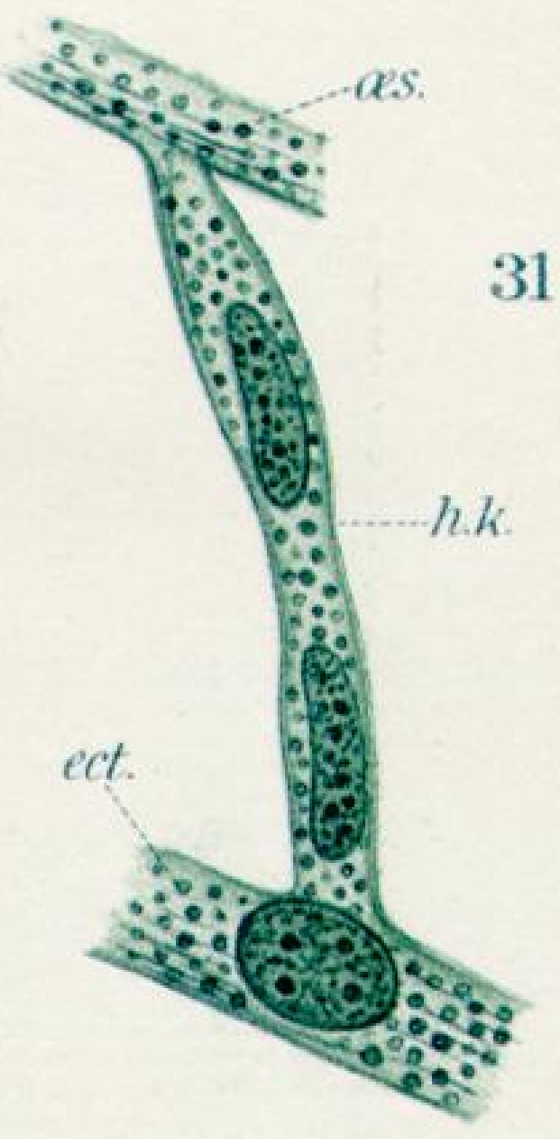

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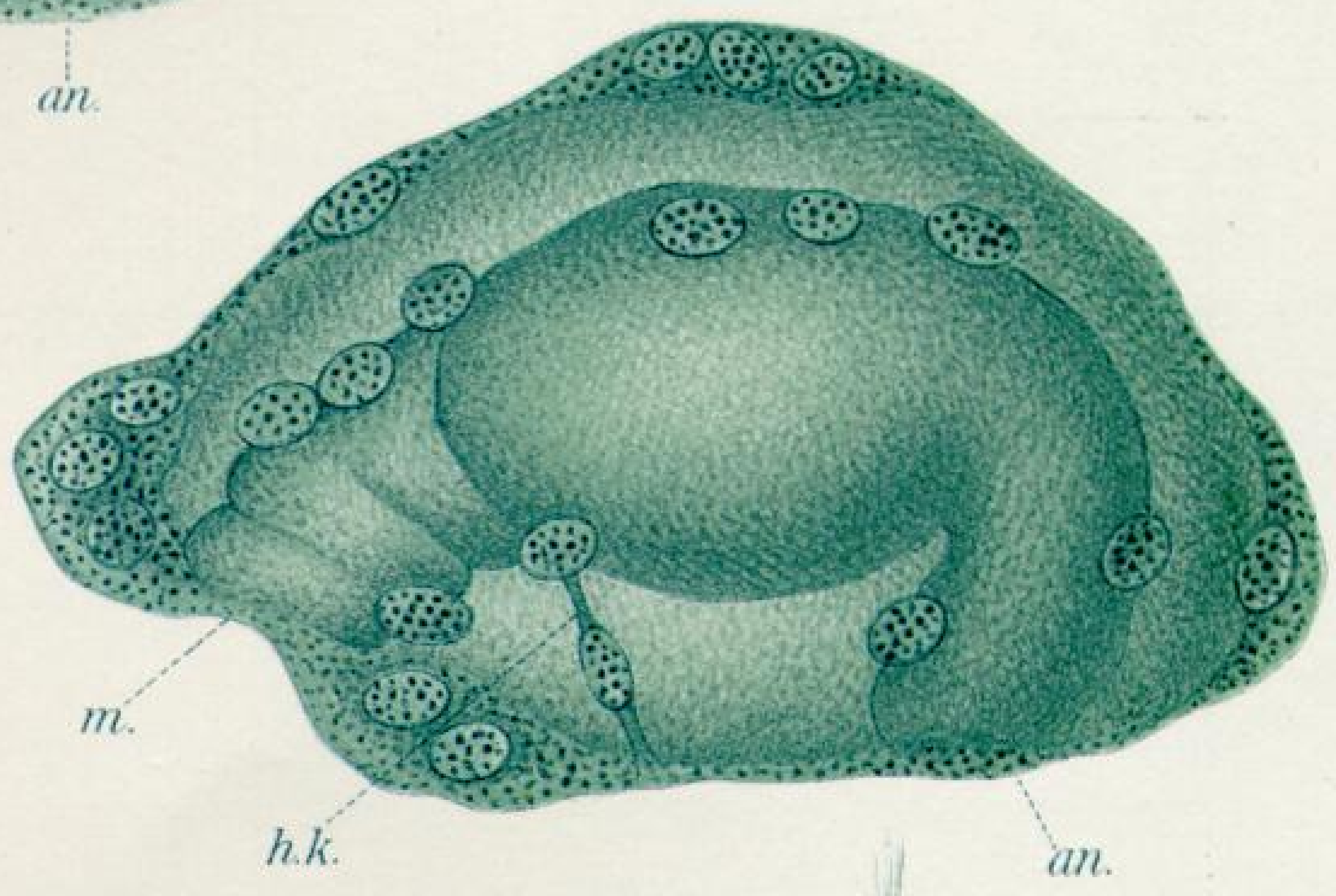

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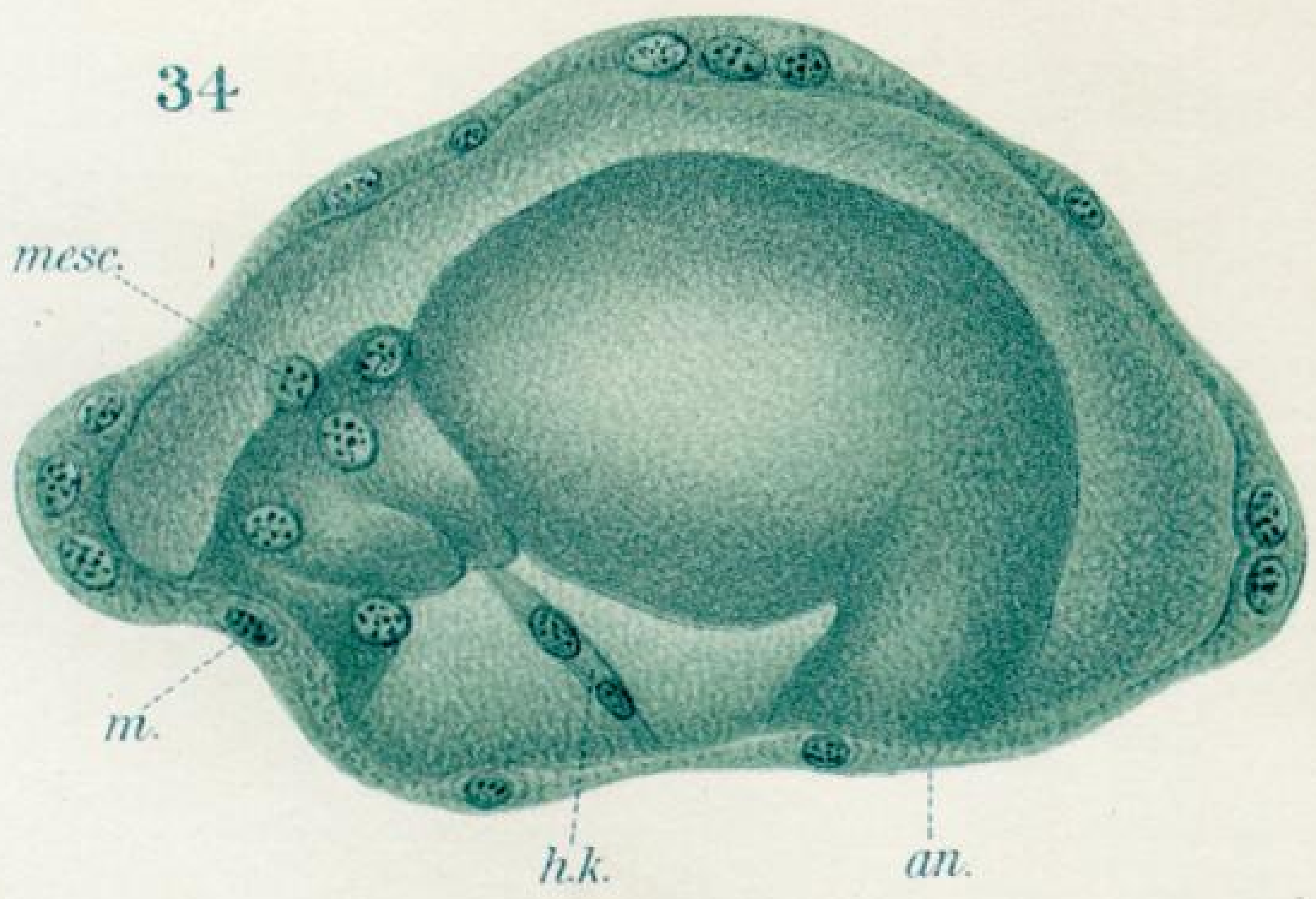

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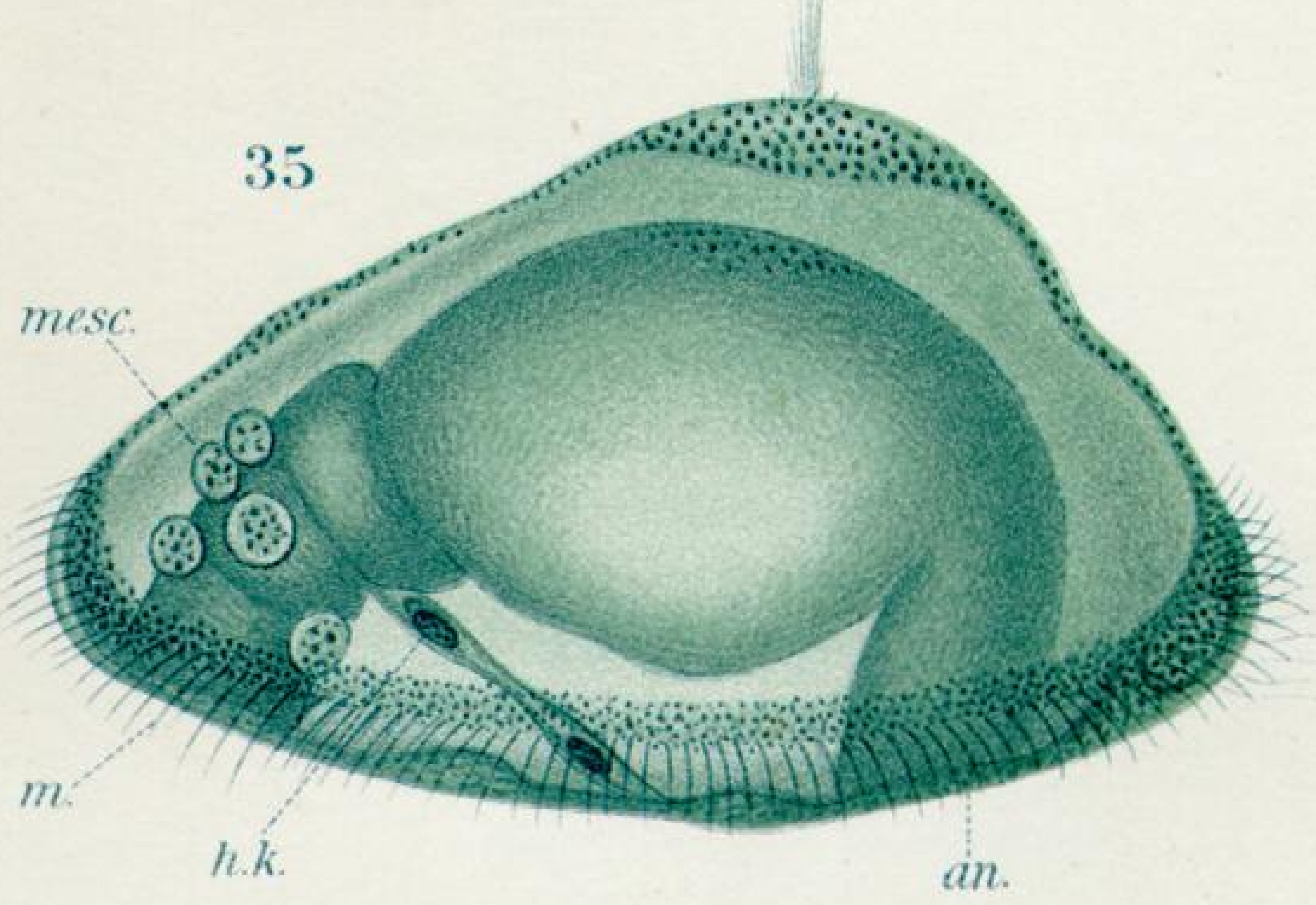

PLATE 27.

Fig. 25.—*Polygordius* larva, the earliest stage at which the head-kidney is recognisable as a small strand connecting a large cell in the ventral ectoderm with the œsophagus.

Fig. 26.—Very young larva of about the same stage as the last, in which a large cell in the ectoderm, midway between mouth and anus, is the rudiment of the nephridium.

Fig. 27.—Early stage, in which the head-kidney is clearly developed as a strand stretching from the region on either side of the anus to the œsophagus.

Fig. 28.—A stage later than the last, in which the head-kidney is more clearly developed.

Fig. 29.—Head-kidney strand, early stage.

Fig. 30.—A later stage than fig. 28, in which there has been considerable increase in size. Numerous mesenchyme cells are seen applied to the œsophagus. The head-kidney is seen as a strand connecting with the œsophagus at one end, and with a conspicuous cell in the ectoderm at the other. The nucleus shown near the anal end of the intestine where it joins the ectoderm is one of the polar mesoderm cells.

Fig. 31.—Head-kidney strand, early stage.

Fig. 32.—A stage in which the larva has changed its shape considerably, having lost the former flat form and become more cubical. The head-kidney is shown as two cells.

Fig. 33.—A stage of about the same age as the last, in which the head-kidney appears as a thin strand.

Fig. 34.—Slightly later stage than the last.

Fig. 35.—A larva considerably older than the preceding stage. The adult larval form is beginning to be assumed. The head-kidney is composed of two distinct cells in the blastocœl. Certain mesenchyme cells are seen applied to the intestinal tract in this and the former figures.

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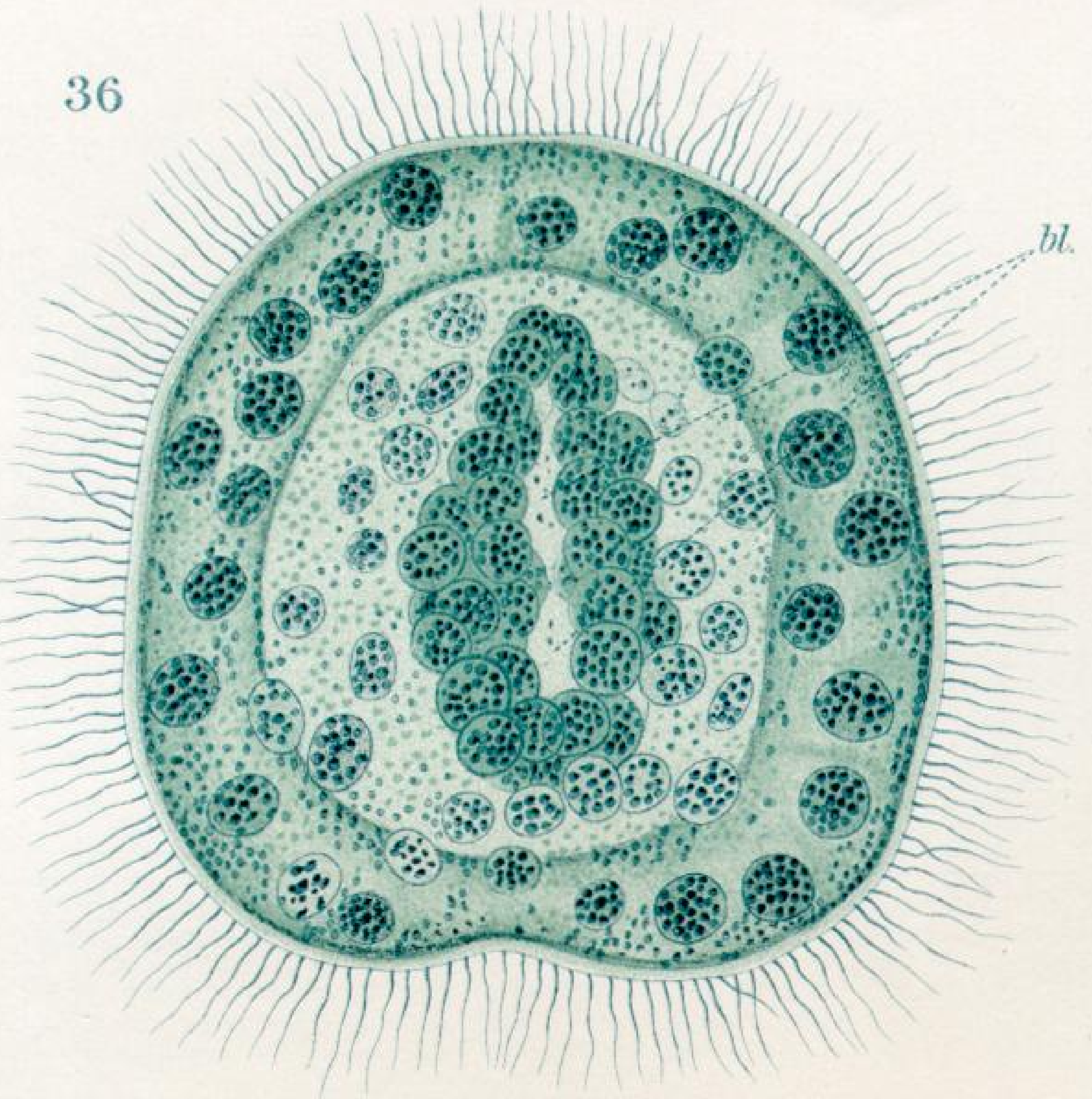

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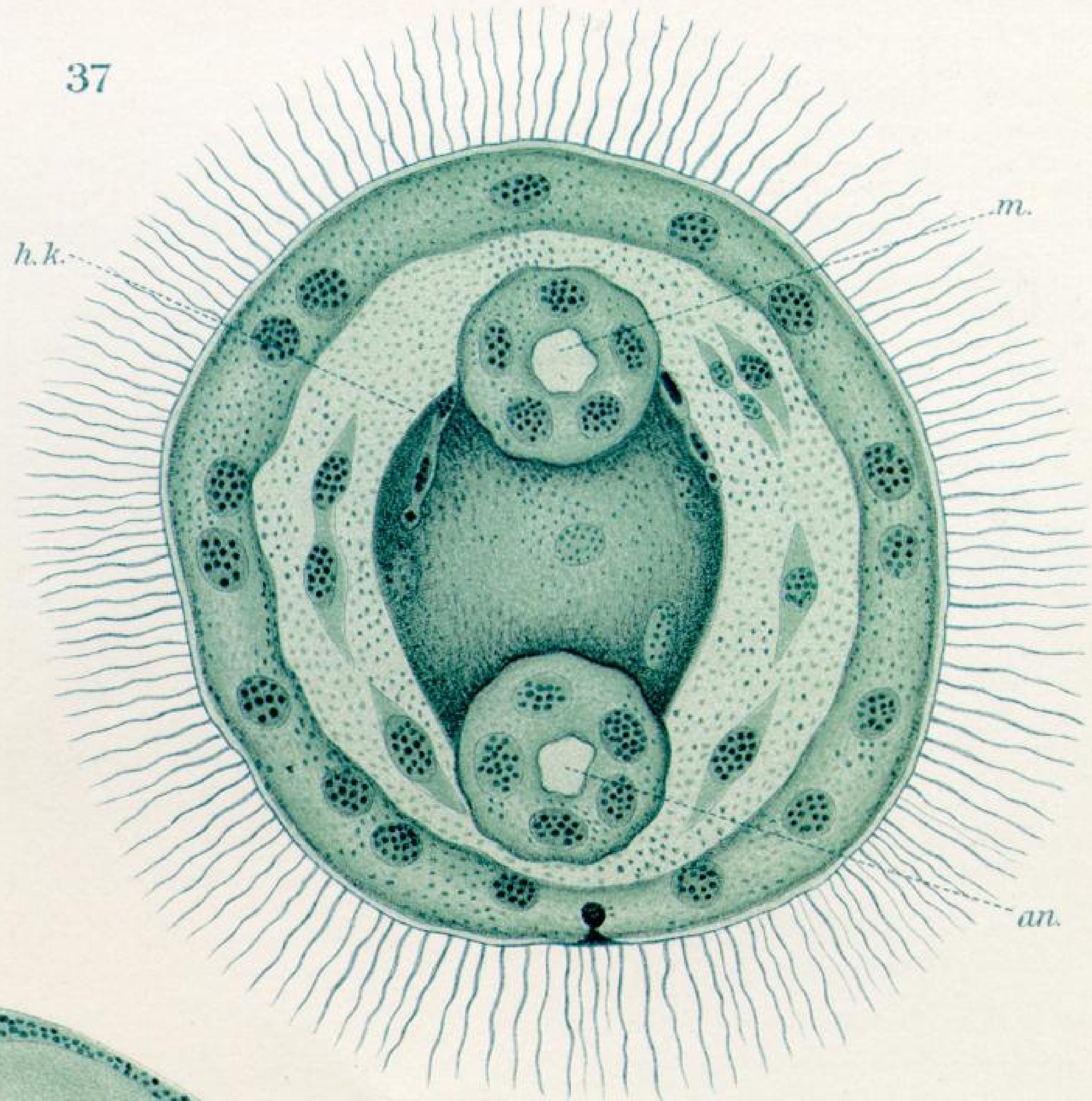

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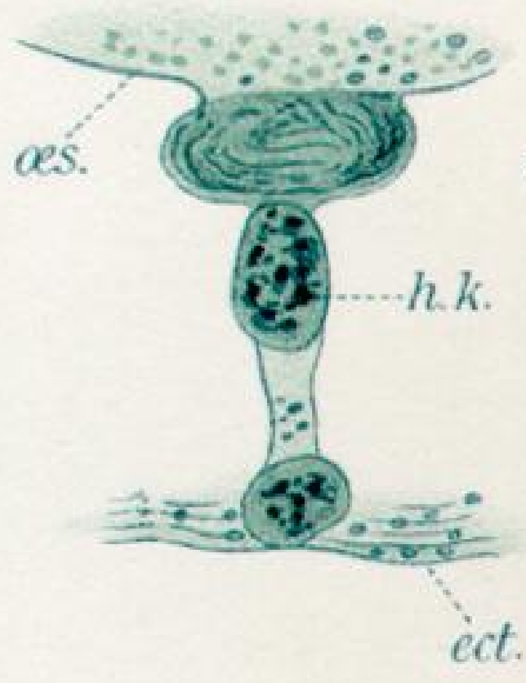

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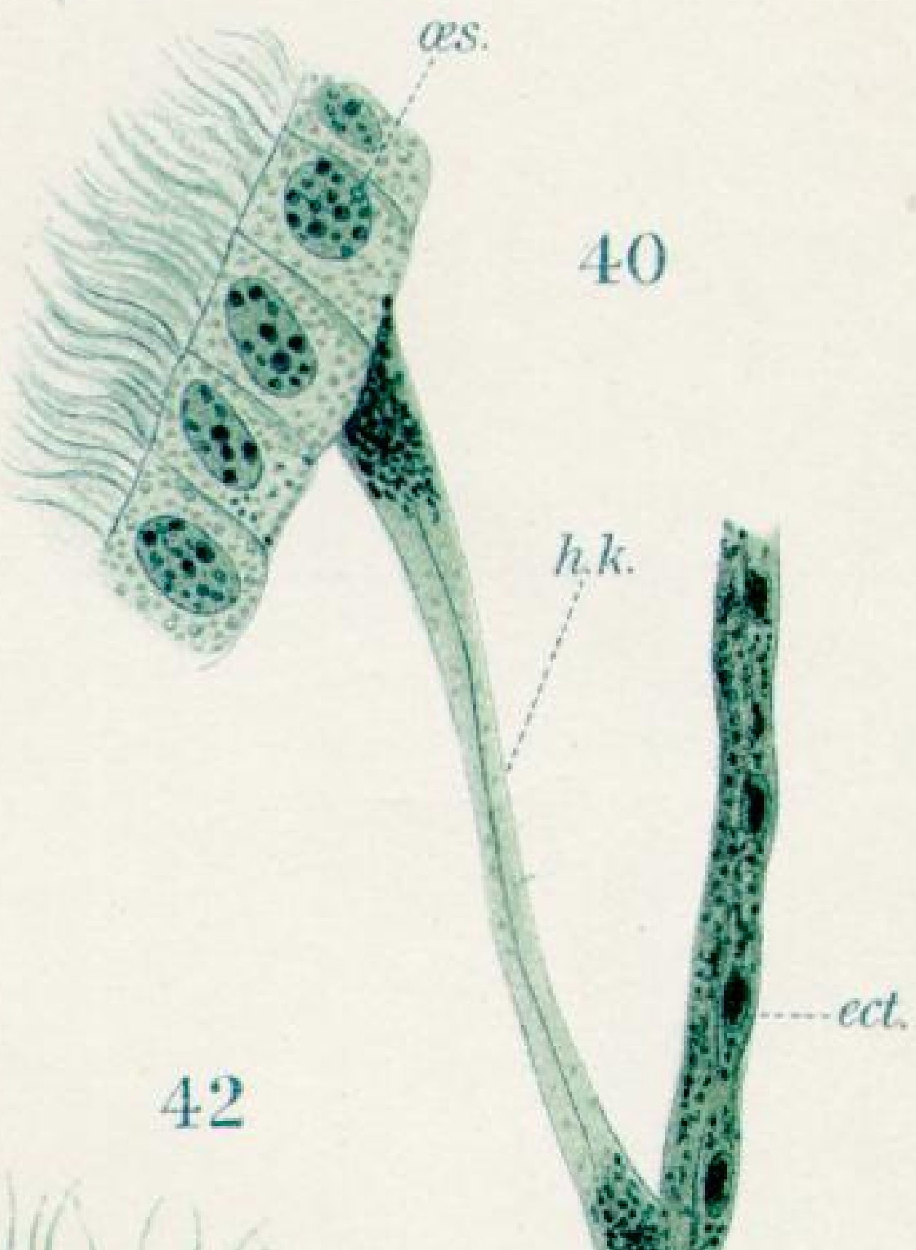

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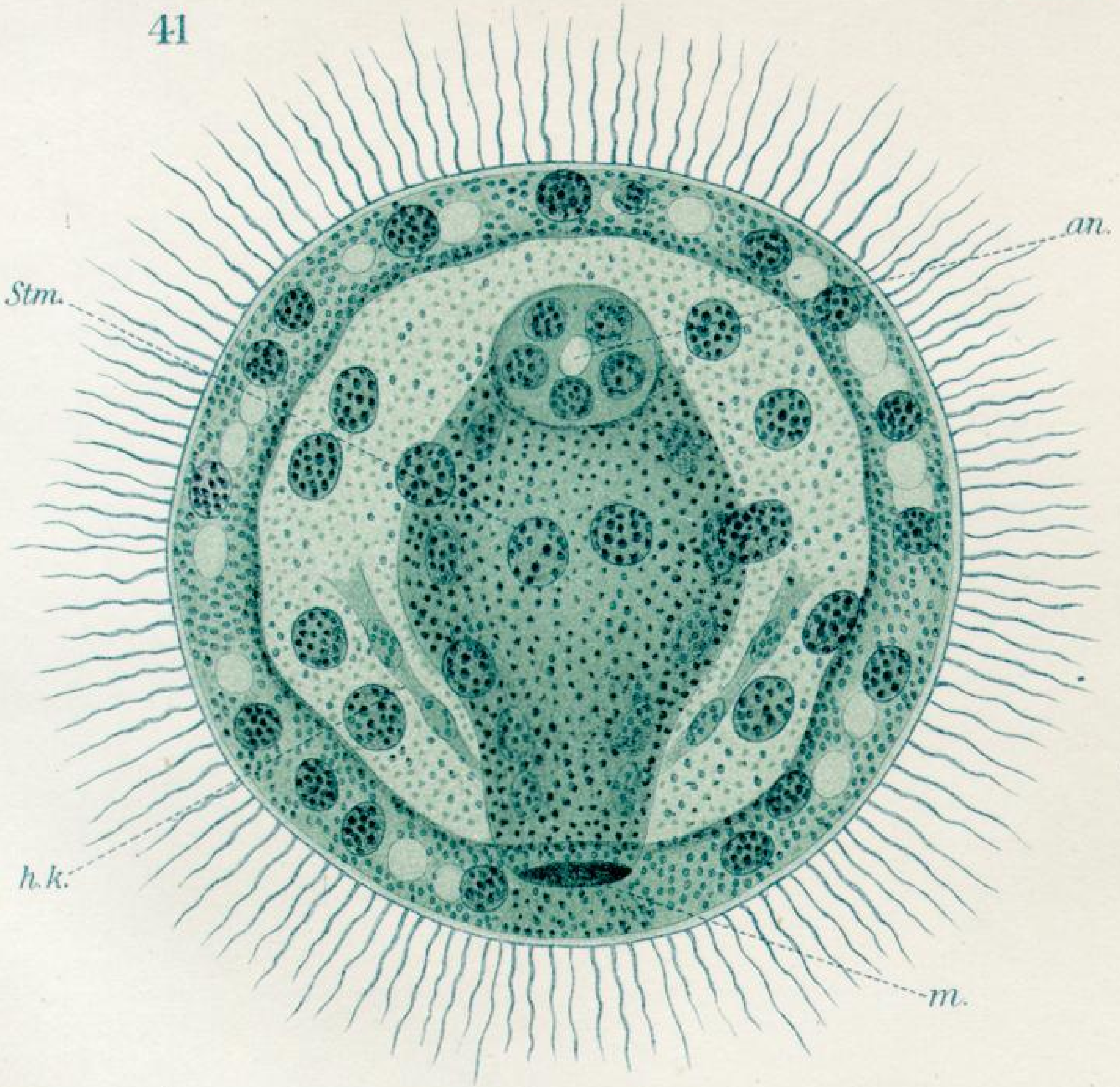

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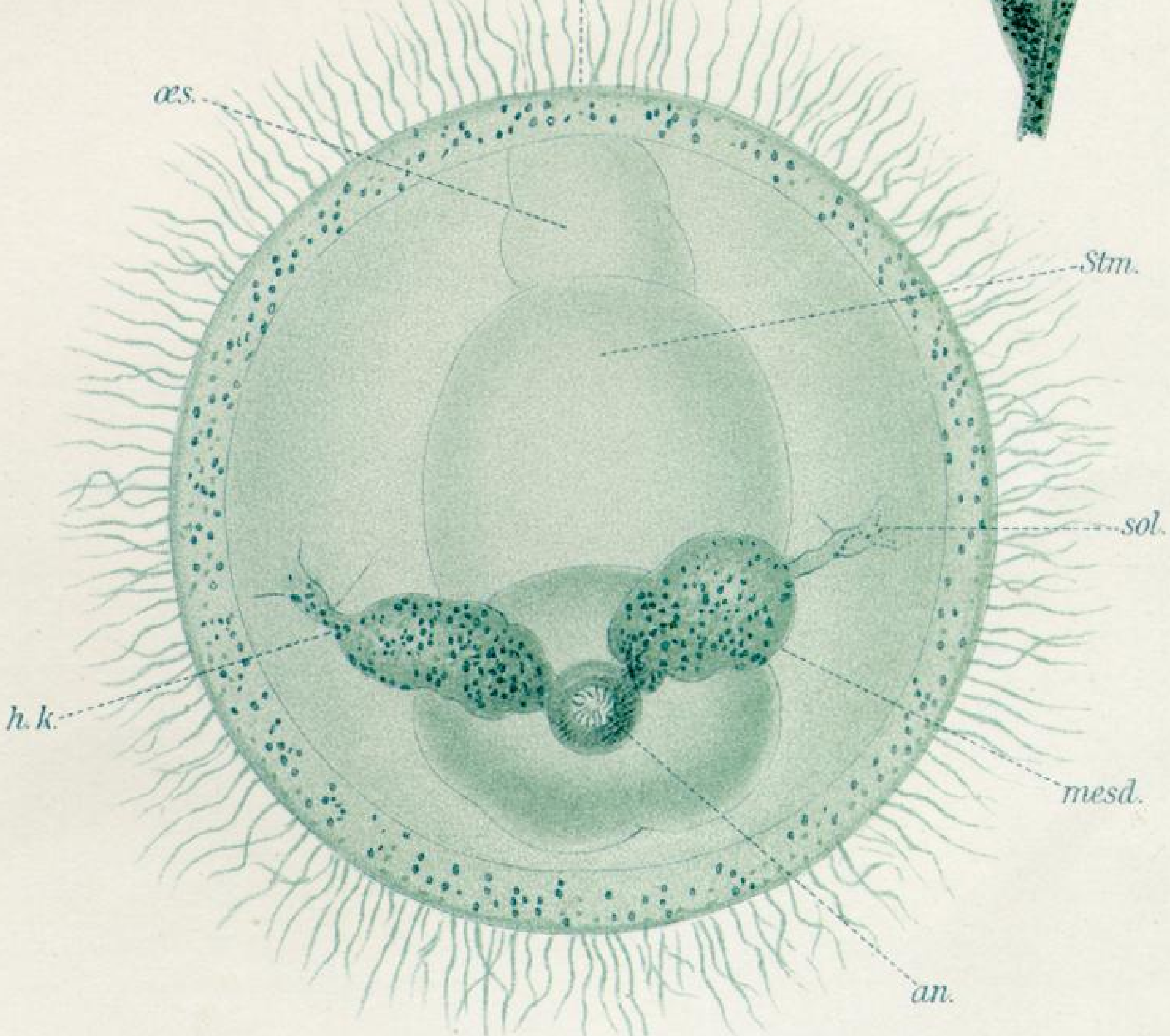

Plate 28.

Fig. 36.—View of a larva 23 hours old, seen from the upper surface. The partial closure of the blastopore is seen in the middle.

Fig. 37.—Larva 48 hours old, seen from the ventral surface; the head-kidneys are seen on each side of the œsophagus running up to the surface of the ectoderm.

Fig. 38.—Head-kidney strand, early stage.

Fig. 39.—A late stage. The nuclei of the head-kidney have disappeared, it has become somewhat granular at each end, and a faint lumen has appeared in the middle. Shortly after this the cilia in its interior are developed.

Fig. 40.—A drawing of the head-kidney, the same age as the larva figured in fig. 39.

Fig. 41.—A larva about 48 hours old, seen from the ventral surface; head-kidneys are seen on each side of the œsophagus as fine strands composed each of three cells.

Fig. 42.—Fully developed free-swimming larva, seen from the ventral surface. The head-kidneys, with the mesoderm bands, are seen on either side of the anal opening. To the head-kidneys run the muscle strands from the apical region.