Observations on the Development, Structure and Metamorphosis of Actinotrocha.

By

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With Plates XXV-XXX.

Introductory.

Since the discovery of *Actinotrocha* by JOHANNES MÜLLER in 1846, this peculiar larval form and its mother animal, *Phoronis,* have been made the subject of investigations by many distinguished authors such as WAGENER ('47), GEGENBAUR ('54), KROHN ('54), SCHNEIDER ('62), METSCHNIKOFF ('72, '82), E. B. WILSON ('81), and FOETTINGER ('82). Among more recent writers CALDWELL ('85), MC'INTOSH ('88), BENHAM ('88), ROULE ('90, '96), CORI ('91), and E. SCHULTZE ('97) may be mentioned as having published important contributions; while MASTERMAN ('97) has made quite an elaborate study of the animal with the view of establishing its relationship to *Balanoglossus* and the Chordata in general. As, however, in spite of all these works there still existed many gaps and unsatisfactory points in our knowledge of this interesting animal, the investigation, of which an account is given in the following pages, was undertaken, and though the results are far from exhaustive, I hope they will help to advance our knowledge of the subject.

My study was begun in the summer of 1898 during a stay at the Misaki Marine Biological Station and later was continued at that Station as well as in the Zoological Institute of the Science College.

At Aburatsubo, a small inlet close to the Station, is found a species of *Phoronis*, which has been named by Dr. OKA ('97) *P. ijimai*.* Its colonies adhere to the overhanging ledges of rocks near the shore. As the water at the place is always calm and at low tides recedes so as to almost expose the ledges, the animals can be easily collected. During the greater part of the year, eggs and young embryos, clustered together, in what may conveniently be called *embryonal masses*, are found adhering to the lophophoral crown of the adult, one on each side of the median line. These furnished materials for the study of fertilization, segmentation and the early larval stages. The larvæ in the Actinotrocha stage are found swimming in the inlet and are caught with the surface net. As will later be fully described, there occur four kinds of the larvæ, which no doubt represent as many species, including the common *Phoronis ijimai*.

The specimens, both adult and larval, were killed with the saturated solution of corrosive sublimate in 1% acetic acid or with Flemming's fluid. Of the various colouring methods tried on the sections, double-staining with eosin or safranin and Delafield's hæmatoxylin gave the most satisfactory results.

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* For a discussion of the status of this species, see *Supplementary Notes.*

took place in the body-cavities, and accordingly, as Cori remarks, he must have believed that self-fertilization prevails in *Phoronis*. Cori considers this as highly improbable, but does not bring forward any positive facts in contradiction of it, his inference being drawn solely from facts observed in other marine Metazoa.

Since Kowalewsky's valuable researches ('67), it has generally been accepted that the nephridia serve also as oviducts. Thus Benham says that he saw an ovum attached to one side of the nephridial funnel and further mentions that Kowalewsky observed eggs moving through the nephridial canal towards the exterior. Unfortunately both observers failed to elucidate what stage of development these eggs are in.

In *Phoronis ijimai* mature sexual elements are constantly discovered throughout about one half of the year (from November to May or June). By carefully examining a living colony of that species during this period, it will soon be perceived that some individuals differ slightly from the rest in the aspect of the *foot* or *body*. We see in them a moniliform series of small white specks shining through the skin in the uppermost part of the body. These are the ova ready to escape to the exterior through the nephridia. It must have been such individuals that were observed by Kowalewsky and Benham. The body-cavity, in which the ova lie, corresponds to the rectal chamber near the anterior end of the body. I have endeavoured to ascertain whether these ova are fertilized or not, and have at last succeeded in ascertaining that they are in a stage prior to the extrusion of polar globules, —the primary oocytes, in Boveri's terminology. In the fresh state, they are spherical or somewhat elliptical in shape and perfectly opaque by virtue of the abundant yolk-granules contained in the vitellus. It is characteristic of these ova that the nucleus, which

Before proceeding further, I beg to tender my sincere thanks to Professors MITSUKURI and IJIMA for their kind supervision of my work and for their painstaking revision of my manuscripts.

Contents.

I. The Early Development of the Phoronis Larva.

a. NOTES ON FERTILIZATION.

Phoronis is, as is well known, a hermaphrodite, in which *both the male and female sexual elements mature at nearly the same time.* But few authors seem to have studied the animal during its breeding season, so that our knowledge of its sexual organs and, consequently, of its fertilization has remained very imperfect, as was pointed out by CORI ('91). The only existing statement as to how and where fertilization is accomplished in *Phoronis* is that of KOWALEWSKY ('67). This author thought that fertilization is situated, not in the centre, but near the periphery, is always in the meta- or ana-phase of Karyokinesis (fig. 17). In such an ovum the chromosomes are constantly found to be *six in number*, each being dumb-bell shaped with the two ends directed towards the poles. Fig. 18 represents a portion of the section passing through the equatorial plane of the nuclear figure. It is evident that these eggs are in preparation for the extrusion of the first polar globule. As shown in the above figure, the finely granular protoplasm of the vitellus contains thickly and uniformly distributed yolk granules, which have a strong affinity for eosin.

That the eggs in question are mature is further demonstrated by the fact that I succeeded in artificially fertilizing them and in rearing out of them normal embryos which grew to certain advanced stages of development.

If we now examine the embryonal masses, which, as has been mentioned, are found attached one on each side of the tentacular crown of the adult *Phoronis*, we find that the embryos which are farthest away from the nephridial pores are the most advanced in development and that they are found in successively younger and younger stages as we approach the pores, until we reach such eggs as have just been fertilized or perhaps even such as have not yet been fertilized at all. But even the youngest eggs found in the mass present an appearance very different from those found in the body-cavities, the former being invariably at a stage after the expulsion of one or two polar globules. In the egg taken from the mass and shown in fig. 19, two polar globules have already been formed; these are situated close together just inside the vitelline membrane.

On the other hand, if we examine by means of serial sections through the posterior region of an adult, where the stomach and the sexual organs lie grouped together, a number of large eggs are frequently found, floating freely in the coelomic fluid of the body-cavities. These eggs do not differ in any respect from those in the nephridial region as regards the size, the appearance of the karyokinetic figure, or the number of chromosomes.

The facts above stated plainly point to the following conclusion :—*The oogonia fall into the body-cavities by a dehiscence of the ovarian walls and here develop until they reach the stage of primary oocytes. These travel gradually upwards to the nephridial region, retaining meanwhile the nuclear figure formed for the production of the first polar globule. Reaching that region, the primary oocytes are destined sooner or later to be carried by way of the nephridia to the exterior, where they become fertilized by spermatozoa from other individuals.*

Reserving an account of the spermatogenesis and ovogenesis for a future occasion, I may here refer to a few facts observed by me relative to the process of fertilization. When the two sexual elements are artificially brought together, numberless spermatozoa soon attach themselves to the surface of the ovum. About 10 minutes afterwards, the first polar globule makes its appearance, followed soon afterwards by the second. Meanwhile a small clear spot, probably marking the place where the male element has entered, appears on the surface of the egg; it is however observable for only a very short time. Both figs. 19 and 20 are sections of ova taken from the embryonal mass. The ovum given in fig. 19 is fully mature and ready to be fertilized; close to the polar globules rests the large female pronucleus. The ovum represented in fig. 20 belongs to a stage of fertilization in which the two pronuclei stand closely side by side. The larger female pronucleus has a nuclear membrane irregular in contour. The intensely stained chromatin pieces are in both nuclei dispersed without any apparent order throughout the finely granular nuclear substance. At one spot outside the male pronucleus, there is visible a small and clear archoplasmic (?) space surrounded by a set of exceedingly fine radial rays. The two polar globules of this egg were distinctly visible in other sections which have not been figured.

b. NOTES ON SEGMENTATION.

Our knowledge of the mode of segmentation in *Phoronis* is far from being satisfactory. METSCHNIKOFF ('82) gives no account of the process. FOETTINGER ('82), if one may judge from his figures, seems to have seen the egg undergoing to taland unequal segmentation. According to CALDWELL ('82), the segmentation "*proceeds with considerable regularity*" (*l.c.*, p. 374); ROULE ('90) says "*l'ovule fécondé subit une segmentation totale fort régulière......*" (*l.c.*, p. 1147). E. SCHULTZE ('97) simply says "*Ich sah das Ei sich total und unäqual furchen*" (*l.c.*, p. 6).

My observations of the process were made on eggs found in the embryonal mass as well as on those artificially fertilized. As the former showed comparatively rarely the earlier stages of the segmentation, it was necessary to have recourse to the latter for filling up the gaps of observation.

Soon after the formation of the second polar globule and the disappearance of the micropyle-like spot the first cleavage line makes its appearance, passing on one side of the polar globules (figs. 1 and 21). At this stage I can not perceive any difference in size and structure between the two blastomeres. The second cleavage plane passes at right angles to the first (fig. 2 *b*). It is a remarkable peculiarity of *Phoronis* eggs that two sister blastomeres derived by the division of a mother blastomere, never undergo the next division simultaneously, so that between any two consecutive stages having an even number of blastomeres there intervenes an intermediate stage with an odd number of the same. This phenomenon occurs even at the second cleavage ; thus just before the egg attains the four-cell stage, there exists a stage of three cells, such as is seen in fig. 2 *a*. Among the later stages, those of 5, 7, 9.........cells are of constant occurrence. Consequently it is scarcely admissible to say that the segmentation proceeds with considerable regularity.

Caldwell ('82) has asserted that the first differentation of the future blastoderm into the ectoblast and entoblast is observable as early as in the four-cell stage. He says : "*At the stage of 4 segmentation-spheres a division into two smaller clear and two larger opaque cells indicates the future ectoderm and endoderm*" (*l.c.*, p. 374). At the corresponding stage of *Phoronis ijimai* I have not been able to discover any appreciable difference in the size of its cells (see fig. 2 *b*). Following the 4-cell stage, the division of the blastomeres in the equatorial plane puts the egg on the way to the 8-cell stage. According to my own observations, the above mentioned difference in size of the blastomeres becomes first perceptible at this stage. Fig. 3 shows a side view of an egg with 8 blastomeres ; it will be seen that the upper four blastomeres are very slightly smaller than the lower four. I could not however, at that period, recognize any difference in the cell-contents of the two classes.

The irregularity of division, which, as before mentioned, becomes more and more pronounced as segmentation advances, tends to gradually obscure the orderly arrangement of the cells. At the 16-cell stage the regular arrangement is still, though less distinctly, maintained, while at the 32-cell stage it is quite disturbed (fig. 4). From this period on, the polar globules can no longer be detected.

In the earlier stages of segmentation, the blastomeres are found in close contact with one another, leaving no noticeable space or segmentation-cavity between them. After they have increased to about 32 in number, the blastocœle and its opening to the exterior (fig. 4, *bl.c.*) become recognizable. The embryo at the morula stage is somewhat oblong in shape and has a quite spacious blastocœle, and the blastocœlic pore (*bl.c.*) is distinct on the ventral side (fig. 5). However, this pore disappears at an advanced morula stage, and apparently the vitelline membrane also, nearly simultaneously with it. At any rate both have altogether passed out of sight at the next stage, that of the blastula. In fig. 23, which represents a median section of a young morula (the outline of which has undergone mutual compression by the crowding together of embryos), the pore (*bl.c.*) is cut through and appears as a slit-like passage between two of the bounding blastomeres.

In the blastula (fig. 25) the wall consists of cylindrical cells and encloses a tolerably wide blastocœlic cavity, which is now at its greatest development. In this stage, the bilateral symmetry of the future larva is already established. It has an oblong plano-convex form, the flattened face of which corresponds to the future ventral face; and its ends, one somewhat broader than the other, indicate respectively the future anterior, and posterior, ends. The cells of the wall are all cylindrical in form, as shown in fig. 25, those of the ventral side being slightly larger than those on the convex dorsal side. The nuclei in all the blastodermal cells are always situated in a peripheral position.

Plasmic corpuscles.—A noteworthy fact with regard to the blastula is that in its older stage a certain number of small and non-nucleated plasmic spheres is almost constantly met with in the blastocœlic cavity (fig. 25, *pl.co.*) These have been first described by FOETTINGER under the name "corpuscules mésodermiques." According to this author, these corpuscles are free nuclei imbedded in a common protoplasmic mass which is supposed to fill up the blastocœle, each corpuscle bocoming a mesoblast cell, after appropriating to itself a certain portion of the surrounding protoplasm. This view of FOETTINGER, which certainly can not be accepted at the present day, was, I believe, partially due to the then defective technique. His method consisted in pouring dilute acetic acid over the living embryo, and this, as the author himself was well aware, is highly detrimental, in that it frequently breaks up the blastomeres into fragments. The corpuscles described by him from so early a stage as that with only 8 blastomeres must have been simply produced by fragmentation, the result of his drastic treatment. The common protoplasmic mass supposed to be present in the blastocœle, was probably nothing but a coagulum.

Again the *mesodermzellen* which METSCHNIKOFF ('82) found in the blastocœlic cavity of the blastula are certainly not true mesoblast cells but rather certain spheres similar to FOETTINGER's "corpuscules mésodermiques," as was rightly pointed out by CALDWELL. Recently E. SCHULTZE ('97) published a short paper entitled "*Ueber die Mesodermbildung bei Phoronis,*" in which he writes as follows:—"*Schon auf dem Stadium der rundlichen Blastula sehen wir einige Mesodermzellen im Blastocœl aufsitzten*" (*l.c.*, p. 6). It seems to me that SCHULZE has fallen into the same mistake as METSCHNIKOFF.

Lastly, CALDWELL ('82) has entertained a view quite different from those of other writers. According to him, the bodies in question are not present as such in the blastocœle, but are in reality only the cut ends of blastoderm cells projecting into the cavity and as such of course have nothing to do with the true mesoblast.

In the *Phoronis* studied by me, the plasmic corpuscles are present only in the highly advanced blastula (fig. 25, *pl.co.*). They are usually round in shape and very much smaller in size than any of the blastoderm cells, but as to structure, they do not show any deviation from the latter, except in the important respect that they have no nucleus. Although I tried with them all the available nuclear stains, the presence of any chromatic substance in them could in no instance be detected. In an earlier stage such a free floating sphere has never been met with. Instead of it, some unusually elongate blastoderm cells (*pl. co.*, fig. 24), such as were found by CALDWELL, were discovered protruding their inner end into the blastocœle. The nucleus of these cells commonly lies in the periphery as in all other cells of the more ordinary shape. In my opinion, the proximal ends of the elongate cells break off from the main cell-body and fall into the blastocœle, where they undergo degeneration, breaking into ever smaller and smaller spheres. By examining serial sections of an advanced stage like that of fig. 25, it is easy to convince one's self that there exists no connection whatever between the spheres and the blastoderm cells. The spheres, or the plasmic corpuscles, are clearly distinct bodies and not mere ends of blastoderm cells cut off in the process of microtomizing as was supposed by

CALDWELL. Their small size and the total absence of nuclear substance make it easy to distinguish them from the true mesoblast cells.

The corpuscles are still frequently discovered in the blastocœlic cavity at the beginning of gastrulation, together with a few mesoblast cells. But in an advanced gastrula they have wholly disappeared, possibly having been absorbed by the blastoderm cells. I think the temporary co-existence of the plasmic corpuscles and of the true mesoblast cells in the blastocœle of the gastrula, has led some previous authors to confound the two elements. As to the significance of the corpuscles, I can at present offer no opinion "*unless they be merely an excess of supply of nourishment analogous to food yolk*" as has been suggested by CALDWELL ('82, *l.c.*, p. 18).

c. GASTRULATION AND MESOBLAST-FORMATION.

In this section, I shall first describe what I conceive to be the true history of gastrulation and mesoblast-formation, and then pass on to a discussion of the views of other writers. The two developmental processes are so intimately related to each other, that it seems best to treat them together.

First as to external changes. The bilateral symmetry of the plano-convex blastula becomes more clearly marked than before when the gastral invagination begins on the ventral or the flattened side. The initial depression occurs over the whole ventral wall, so that a saucer-shaped embryo is produced. At first it is so shallow as to be perceived with difficulty in the surface view. Soon it deepens, becoming deepest at a point somewhat nearer to the broader end than to the narrow end of the embryo. The deepest portion may conveniently be called the *central depression*. Fig. 6 represents the ventral view of an embryo in which the invagination has become visible from the outside, the central depression being most deeply shaded in the figure. In a slightly more advanced stage, as the original wide depression grows deeper, the external opening is gradually drawn together and at a certain stage (fig. 7) becomes transformed into an almost triangular blastopore situated at a position slightly anterior to the centre, as was the case with the central depression. The anterior side of the triangular blastopore is somewhat rounded by curving uniformly outwards, while posteriorly the two other sides gradually approach each other so as to meet at a point which may be called the apex of the triangle. Leading backwards from this apex, there runs in the median line the so-called primitive groove. This latter and also the triangular shape of the blastpore are occasioned, in my opinion, simply by the blastopore, originally broadly open, becoming narrowed by the special activity in the lateral posterior parts of its posterior section. In other words, the cell-multiplication of the ectoblastic layer is carried on especially in the last mentioned parts, so that there the pressure, which is exercised by the ectoblast towards the invaginated layer, is more marked than in the anterior and lateral borders of the blastopore. As the result of the above phenomenon, the definitive blastpore is pushed further anteriorly; and consequently, the archenteric cavity deepens in the posterior direction, as shown in fig. 29. The above consideration is supported by the results of actual measurements of the size of the embryos concerned. In spite of the fact that the embryo has developed considerably the body-length does not show any significant increase, remaining all the while at about 0.12 *mm.* on an average. This shows that the growth is lost in the curvature of the body.

When the growing larva reaches the stage represented in fig. 8, the blastpore assumes a narrow transversely directed, slit-like form. That portion of the larval body lying in front of the blastpore—which is the persistent larval mouth—protrudes more or less prominently forwards and ventrally, so as to acquire the form characteristic of the preoral lobe of Actinotrocha. In such an advanced gastrula, the primary gut-cavity is well established and can be plainly traced through the wall in the surface view. If the larvæ of such an early stage of development be taken out of the embryonal mass and set free in water, they will swim about by means of the well developed cilia, which cover the whole external surface.

Fig. 9 represents a side view of a larva, in which the preoral lobe has grown to a very considerable size. The nephridial pit, which is an ectoblastic invagination just in front of the posterior end of the gut, is now distinctly visible from the outside. In short, the larva may be said to possess the inceptive characters of an Actinotrocha.

I will now proceed to describe the internal changes accompanying gastrulation. The earliest symptom of this process can be seen in sections before it can be detected from the surface. It consists at first in a peculiar disposition of those blastodermic cells which constitute the ventral portion of the blastula wall. This portion not only shows a shallow concavity, but also the cells composing it become, as figs. 26 *a* and 26 *b* show, irregularly arranged on account of mutual pressure, as a result of which some of the cells are even forced out of file so as to fall into the blastocœle. These liberated cells have usually a round shape and of course contain each a distinct nucleus. Some other cells are apparently in the process of being pushed out and have a club-like shape, the narrowed end being still inserted between the cells of the layer to be invaginated. A further symptom of incipient invagination consists in the circumstance that the nucleus in most cells of this portion has no longer a peripheral position, but is situated in the middle or rather nearer to the inner end of the cells. Moreover, the nucleus is frequently met with in the form of the karyokinetic figure which shows that the cells are dividing and increasing in number in the layer to be invaginated. *The cells pushed out into the blastocœle are nothing else than mesoblast cells, so that it may be stated that the mesoblast-formation begins simultaneously with the gastrulation.*

At the beginning of gastrulation we can thus distinguish two parts in the blastoderm wall, *viz.*, the *mesentoblast* and the *ectoblast.* The former corresponds to the whole of the portion to be invaginated, while the ectoblast forms all the remaining portion of the embryo. The mesentoblast is composed of large and irregularly arranged cells, while the ectoblast is of taller cylindrical cells regularly arranged in a single row (figs. 26 *a* and 26 *b*). The mesentoblast, as the name indicates, is destined to give rise to both the entoblastic and mesoblastic elements. The characteristic disposition of its constituent cells indicates its being the source of mesoblast proliferation.

The mesoblast proliferation becomes more and more accentuated in activity as the gastral invagination gradually deepens (see fig. 27), but the mesoblast cells thus formed do not adhere as a lining epithelium to the ectoblast, until what are called the anterior diverticula have been formed on both sides of the blastopore. The blastocœle, in which the mesoblast cells are at first loosely scattered about, is henceforth greatly reduced in extent and finally, as the development of the archenteron progresses, is almost obliterated, especially along the dorsal and lateral portions of the embryo where the ectoblast and the gut come into direct contact with each other (see figs. 29 and 30 *b*).

Figs. 28 *a-c* show three cross sections through different parts of a larva of nearly the same stage as that represented in fig. 6, in which the invagination has become recognizable in the surface view. Fig. 28 *a* passes through the central depression which becomes gradually shallower posteriorly (figs. 28 *b* and 28 *c*). As these figures show, the mesoblast cells are at this stage still being proliferated uniformly from every part of the mesentoblast and do not yet form a lining epithelium to the ectoblast. When the blastopore has taken a triangular shape (fig. 7) and the primary archenteric cavity has somewhat bent itself towards the hind end, the posterior border of the blastopore has travelled a certain distance in an anterior direction. If we examine serial sections of this region, a narrow and shallow groove is detected running for a short distance immediately behind, and from, the meeting point of the lateral blastopore lips. Also at about this stage, a paired invagination, the anterior diverticula of CALDWELL, appears along the side of the anterior portion of the archenteron. These points will become clear from a consideration of figs. 30 *a-c*, which are drawn from serial transverse sections of an embryo slightly older than that shown in fig. 7. In fig. 30 *a*, showing the right-hand side of the blastopore, we notice a lateral infolding (*ant. div.*) of the archenteric wall a short distance inside the blastopore lip. Here the component cells are irregularly arranged and their entire disposition reminds one of the mesentoblastic layer. Indeed some indubitable mesoblast cells are found pressed against the tip of the diverticulum. No doubt the mesoblast is here arising, not by direct cell multiplication, but by the pushing in of the cells of the diverticulum. This is more clearly illustrated in fig. 31, which shows a transverse section through the blastopore of a more advanced larva; here the mesoblast cells almost fill up the blastocœlic cavity on both sides of the blastopore. In fig. 30 *b*, a transverse section just behind the closure of the blastopore, the most anterior portion of the primitive groove before mentioned is cut across. Here the wall of the groove underlying the gut is formed of mutually compressed cells, some of which are evidently migrating into the blastocœle (on the left-hand side in the figure). If the sections are followed further posteriorly, the groove still persists, but no mesoblast cell in the actual immigrating process can be discovered, although there are those which have been previously pushed out and are now floating between the two primary germinal layers at this region. Still more posteriorly the groove entirely disappears and the entoblastic and ectoblastic layers are separated from each other by the comparatively wide blastocœlic cavity (fig. 30 *c*). At this stage, therefore, the greater part of the archenteric wall has ceased to contribute towards the mesoblast-formation; in other words, it has lost its mesentoblastic nature. The mesoblast is now being produced only from two limited regions, *viz.*, anterior diverticula and the ventral groove.

In a slightly more advanced larva, the ventral groove is still present for some distance immediately behind the blastopore, but the layer which forms the groove has entirely ceased to give rise to mesoblast cells (fig. 32, which is taken from a transverse section very near the blastopore). It appears to me that this groove is to be regarded as but the posterior portion of the original mesentoblast, which, owing to the fact that the central depression is eccentrically placed nearer to the anterior end, has to traverse a longer distance before it can be reflected inwards, and thus on its inward course lags behind the anterior and antero-lateral portions. Eventually all the cells of the wall of the groove that are left behind after proliferating the mesoblast cells, are without doubt invaginated and form a part of the entoblast. The groove then entirely disappears. I could not discover any remnant of it in any part of the posterior region where, according to CALDWELL, the ectoblast and the entoblast are said to stand in fusion to give rise afterwards to the anus. In such an advanced stage, the anterior diverticula have also ceased to give off mesoblast cells and have become straightened out, their walls acquiring a normal epithelial character (entoblastic).

From the facts above adduced, it may be concluded that *both the anterior diverticula and the ventral groove, present at a certain developmental stage of the Phoronis embryos, are remnants of the original mesentoblast which at an earlier stage occupied the the whole extent of the gastral invagination. They are, therefore, merely temporary, and destined sooner or later to split into mesoblastic and entoblastic cells.*

As will be seen in figs. 30 *a-c*, the ectoblast and the archenteric walls are brought together into such close contact, especially along the dorsal and lateral regions that scarcely any interspace is left between them. In the embryo given in fig. 8, the cavity of the rudimentary preoral lobe is filled with mesoblast cells produced from the original mesentoblastic layer. So far as I can make out, these show no difference whatever from those proliferated from the anterior diverticula: both are indistinguishably mixed together. Though most of the mesoblast cells in the preoral lobe lie loose during the active period of the diverticula, there are found a few that have already apposed themselves flatty to the ectoblast (see fig. 29), while the cavity behind the blastopore still remains without a mesoblastic lining. This last condition persists till the period when the nephridial invagination makes its appearance. The state of things in question is to be seen in fig. 29, which represents a median sagittal section through an embryo of nearly the same stage as fig. 8.

Soon afterwards the anterior diverticula and the ventral groove entirely disappear and the preoral lobe begins to bend more distinctly downwards. Meanwhile an unpaired ectoblastic invagination appears at the posterior end of the larva, on the ventral side of the blind end of the now greatly elongated gut. It appears at first as a shallow depression (fig. 33, *nep. p.*) of purely ectoblastic nature, having nothing to do with the mesentoblast. It is from this invagination that the future nephridia of Actinotrocha develop and hence I shall call it the nephridial pit, in preference to the name "anal pit" of CALDWELL, who for the first time described this structure. I have very frequently noticed signs of vigorous cell-division in the cells of the pit wall, evidently only for enlargement of the pit itself, since the axis of the karyokinetic spindle is always placed paratangentially to the wall. I have moreover often noticed peculiar ectoblastic cells round in shape and in process of multiplication, situated just outside the edge of the entrance to the pit (fig. 33).

In larvæ of the stage of fig. 9 the nephridial pit can be well seen in surface views. This stage further attracts our special attention on account of several important developmental processes taking place in it. First to be noted is the fact that from the posterior end of the primary gut a small and short evagination protrudes itself touching the ectoblast with its blind posterior end. This hollow protuberance is the rudiment of the intestinal canal of Actinotrocha. In longitudinal section it is shown in fig. 37 (*int.*).

In fig. 34, representing a slightly oblique frontal section of a larva of nearly the same stage as that of fig. 9, we see below the pit-like nephridial sac, which is quite free from the gut. The ectoblastic wall of the preoral lobe is at this stage somewhat uniformly lined with flattened mesoblast cells, while in the cavity behind the blastopore the mesoblast cells are for the most part freely scattered, though a few have already begun to arrange themselves against the ectoblast layer in this region. In fig. 35, a transverse section through the posterior end of a larva of nearly the same stage, the nephridial pit appears as a single flattened sac (*nep. p.*) lying in front of the intestine (*int.*); the ectoblastic wall is internally lined with a few isolated and flattened mesoblast cells. In a slightly more advanced stage, the ectoblast behind the blastopore, and in a less complete degree the gut wall also, shows a similar mesoblastic lining, though a few mesoblast cells still remain free, especially in front of the nephridial sac.

In order to facilitate comparison with the statements of other writers, I will here add a few words on the change of form undergone by the nephridial pit. When in a larva slightly older than that of fig. 9, the preoral lobe and the future intestinal portion of the gut have become considerably elongated, the nephridial pit, which has meanwhile become deeper than before, begins at its inner blind end to divide into two lateral branches. Each of the latter corresponds, as will be fully demonstrated further on, to the nephridial canal of Actinotrocha. Fig. 38, a frontal section of a larva at this stage, shows the bifurcation just alluded to. The relation of the unpaired nephridial sac to the gut will be best understood from the median sagittal section given in fig. 37.

I may here be allowed to put in a short historical review of the mesoblast-formation in the *Phoronis* larva.

Kowalewsky ('67) attributed the origin of the mesoblast to the ectoblast.

Metschnikoff ('82), Foettinger ('82), and E. Schultze ('97) confounded the plasmic corpuscles with the true mesoblast, and none of them was aware of the presence of the anterior diverticula.

Caldwell ('85) made many interesting observations on the mesoblast-formation. According to his view, there exists no mesoblast before the closure of the blastopore lips (lateral), but it arises later from three distinct sources, *viz.*, 1) the anterior paired diverticulum (entoblastic), 2) the posterior paired diverticulum (ectoblastic) and 3) "the primitive streak" connecting the above two structures. Further it has been declared by him that the body-cavities of the larva arise in two different regions. As to the preoral body-cavity, he writes as follows: "*From the time when two or three mesoblast cells are budded off from the diverticula on either side, a cavity is present in each mass thus formed. These cavities are the two halves of the body-cavity* (*preoral*)" (*l.c.*, p. 374). On the other hand with respect to the posterior body-cavity, he states that "*it is formed independently in a paired mass of cells which grow out to the end of the first formed sacs and remain separated from septum*" (*l.c.*, p. 376). Thus he regards the preoral body-cavity as arising after the enterocœlic type. Lastly the author puts forward in his recapitulation the opinion that the blastopore gives rise to both the mouth and the anus.

ROULE ('90) also distinguished two sorts of mesoblast cells in view of their different origin and fate : " Mesenchymes primaires " and " initales mésodermiques." Both are derived from the " protoendoderme" which forms the primary archenteric wall. The latter gives rise to cells grouped together into two compact " bandlettes mésodermiques," which are regarded as homologous with the mesodermal bands of Annelid larvæ. In reality these bands are, as have been pointed out by SCHULTZE, nothing else than the posterior paired diverticula of CALDWELL.

From the account of the mesoblast-formation given in the foregoing lines, it is evident that the first stages of that process are observable from the very beginning of gastrulation (figs. 26 *a* & *b*), and long before the blastopore takes the small triangular shape. On this point my observations stand at variance with CALDWELL'S. Nor can I agree with that author in the opinion that the mesoblast produced from the anterior diverticula (even though consisting of only two or three cells) incloses an enterocœlic cavity. As already described, the cells in question, after being budded off, lie loose in the blastocœle together with preexisting mesoblast cells and without forming a wall to a special cavity of any sort.

As to the ventral groove, METSCHNIKOFF ('82) was the first to refer to this structure and wrote as follows : " *In passender Lage des Embryos kann man eine in Verbindung mit dem Blastoporus befindlichen Furche (longitudinale) wahrnehmen, welche zum Hinterende des Embryos hinzieht und sich nur auf dem Ektoderm beshränkt. Diese Furche erhöht den bilateralen Bauplan des Embryos erscheint indessen als eine vergängliche Bildung, welche man auf späteren Stadium vergebens suchen würde*" (*l.c.*, p. 301). According to CALDWELL, this groove, which he calls " the primitive streak," is produced by a fusion of the blastopore lips; the cells along the fusion line differentiate after multiplication into the epiblast, the hypoblast, and the mesoblast. And the rapid growth of the epiblast in this region soon obliterates the groove, leaving however its posteriormost portion as the "anal pit." But such, as I have tried to show, is not the case, for the so-called primitive streak entirely disappears leaving no trace whatever, long before the nephridial or anal pit makes its appearance. Therefore there exists no direct genetic relation between the primitive streak and the anal pit.

CALDWELL's view that the two nephridial pouches give off the mesoblast, which eventually lines the posterior body-cavity, can not be sustained; for, according to my own observations, that body-cavity with its mesoblastic lining wall is already present before the nephridial pit divides into the two pouches. It is true that the cells floating in the posterior body-cavity are in some sections found aggregated at the blind ends of the pouches as shown in Fig. 38. This is a condition which might mislead one to the conclusion that mesoblast cells are here in process of proliferation. But solid cell accumulation in such a section is to be considered as simply due to the obliteration by compression of the lumen of the nephridial pouches. Fig. 36 taken from an obliquely cut sagittal section through a larva of this stage, shows no wandering cells in front of the pouches (of which only the right one is seen in the figure); in this case there is certainly no doubt about the matter.

Finally as to the anus, CALDWELL mentions "a solid cord of cells" which he considers to be the posterior remnant of the primitive streak. According to him, this acquires a lumen and forms a fine canal leading from the primary gut cavity to the exterior. However, it seems clear to me that this cord is nothing else than an early stage of the intestinal outgrowth independently produced at the posterior end of the gut. Moreover, in *Phoronis ijimai,* the gut cavity does not come into communication with the exterior at so early a developmental stage as CALDWELL observed ; in that species, the anus first opens at a definite stage when the larva bears two pairs of larval tentacles.

E. SCHULTZE ('97) rejects CALDWELL'S views in regard to the anal pit, but regards it as a rudiment of the future ventral pouch of Actinotrocha. This is, however, certainly not true, since the ventral pouch is a thing that has a distinct origin and appears at a much later stage of larval development.

d. FURTHER OBSERVATIONS ON THE DEVELOPMENT OF THE LARVA.

Some authors have recorded that the larva swims about abroad at such a stage of development as is represented in fig. 8. However in *Phoronis ijimai,* the larva lies hidden in the lophophoral loops of the mother until it has acquired at least two pairs of larval tentacles.

In the larva shown in fig. 9, the somewhat prominent preoral lobe hangs over the larval month. Local ectoblastic thickenings occur at two places, *viz.*, at the centre of the upper surface of the preoral lobe and along the mid-ventral line near the posterior end of the body. The former is the future nerve ganglion ; the latter, the rudiment of the first pair of larval tentacles. The nephridial invagination at the posterior end is still shallow.

At a little later stage, the tentacular thickening divides into two more prominent ridges running on each side obliquely anteriorly. The preoral lobe grows rapidly so as to hang down on the ventral side and as a consequence of this an œsophageal canal is formed (fig. 37, *œs*). The œsophageal wall is, therefore, ectoblastic in origin and is composed of strongly ciliated columnar cells. About this period the nephridial invagination becomes completely divided into two lobes at the proximal end, as I have already described (figs. 37 and 38, *nep. p.*). In more advanced larvæ, the pit is split throughout its entire length into two nearly parallel canals, each of which opens independently to the exterior. Figs. 39 *a-c* show three transverse, though not consecutive, sections passing through the posterior region of a larva at such a stage. In the first of these figures, the two cell-masses (*nep. c.*) on either side of the stomach represent the uppermost portion of the nephridial canals. In the second figure, each of the cell-masses encloses an easily distinguishable lumen. The two canals finally open to the exterior each by a small pore (*nep. o.*), as seen in the third figure (only one pore is cut through in the above figure, the section being slightly oblique to the main axis of the larval body). In the above figure we see an ectoblastic cell-mass separating the right and the left nephridial canals (*nep. c*). How is this partition brought about? I think it is caused by re-evagination of the distal unpaired portion of the nephridial pit, as by that process the pit wall forming the above portion is gradually transferred to the body-surface of the larva.

Meanwhile the œsophagus becomes more and more elongated, while the paired tentacular thickenings bulge out each into two perceptible prominences. The latter represent the rudimentary state of two larval tentacles, each of which has internally a cavity continuous with the postoral body-cavity. Fig. 10 represents a larva with two pairs of as yet very short larval tentacles; this is the most advanced developmental stage to be met with in the embryonal masses. Fig. 40 is a median sagittal section of such a highly advanced larva. Here the œsophagus (*œs.*) and the intestine (*int.*), which latter now communicates with the exterior by the small anus (*an.*), are highly developed, so that the three parts of the alimentary tract (œsophagus, stomach, and intestine), may be said to be almost complete. The nerve ganglion (fig. 40, *gl.*) is well differentiated from the ectoblast of the preoral lobe, presenting itself in section as a round, well marked mass principally composed of nerve fibres. I have been unable to ascertain whether a proctodæum is produced at all, and if so, what part of the postgut it gives rise to.

The preoral body-cavity is, at this stage of development, still very incompletely separated from the postoral cavity by a few mesoblast cells (fig. 43, *mes'.*). The nephridial canals (fig. 41, *nep. c.*) are now distinctly separated and removed from each other, and are found in a cross section to be situated laterally to the intestine (*int.*). One on the right-hand side of the above figure is cut through at its external opening, while on the other side the nephridium is represented by a thick mass of a few ectoblastic cells. This lateral shifting of the nephridia becomes more and more pronounced with the advancement of larval development. A slightly advanced state of the nephridia is shown in fig. 42, where the nephridial canals (*nep. c.*) are now seen tolerably long and have a wall composed of a single row of cubical cells. It is often observed that some mesoblast cells connect the canals with the splanchnic walls (see the above figure). These cells seem to be the first indication of the future collar-trunk septum. Besides, a certain number of mesenchymatous cells, which later undoubtedly become the excretory cells of Actinotrocha, is always found attached to the blind ends of the nephridial canals. CALDWELL says that he saw the excretory cells aggregated around the apex of each canal and that they had numerous plasmic processes, giving them a strong resemblance to the perforated cells known in *Echiurus.* It seems, however, highly probable to me that this strange appearance of the excretory cells is an artefact, since, as I shall point out later, the same cells in Actinotrocha are certainly not provided with any such processes.

I have very frequently detected some gigantic mesoblast cells floating freely in the postoral body-cavity of larvæ with one or two pairs of tentacles (fig. 44, *corp.*). They are round and nucleated and contain numerous large yolk-spheres. After repeated examination I have come to regard them as mother-cells of blood corpuscles which are found as corpuscle-massess in the collar cavity of Actinotrocha. This point will again be treated of in detail in the proper place in the following section.

II. The Structure of Actinotrocha.

a. EXTERNAL APPEARANCE.

It can scarcely be doubted that each species of the *Phoronidæ* has a characteristic form of Actinotrocha peculiar to it. Some of the previous observers (*e. g.*, WILSON and MASTERMAN) have mentioned two distinct types of larvæ as occurring in the same locality. Among the larvæ which I observed at Misaki, I was able to distinguish four different types, each of which had a characteristic form and a more or less definite topographical distribution. I will designate these types by the letters *A*, *B*, *C*, and *D*.

TYPE *A* (fig. 13). The larvæ of this type were principally collected in Aburatsubo and belong in all probability to the species *Phoronis ijimai*, which, as I have said, is found in the same locality. The body is comparatively short and thick, measuring about 1.–1.5 *mm.* in total length. The larval tentacles of a full grown larva never exceed 16 in number.

TYPE *B* (fig. 14). This is a larger form than the preceding (about 2–2.5 *mm.* in length). The body and the intestinal canal are long and slender. The full grown larva has about 28 tentacles which are much more slender than those of Type *A*. Peculiar to it is the sensory spot (*so.*) situated just in front of the ganglion (*gl.*). The larvæ were found in greatest abundance near Kitsunesaki, a point at the mouth of the inlet Moroiso.

TYPE *C.* (figs. 15 *a* & *b*). This type is distinguished from all the others by several characteristic points. In size of body it is intermediate between Types *A* and *B* (usually 1.5 *mm.* in length). The body is relatively short and thick. The number of tentacles, so far as I know, ranges from 16 to 24. A pair of flask-shaped glands (*gld.*) is found one on either side of the ganglion (*gl.*). A pair of retractor muscles (*ret'.*) runs longitudinally through the trunk cavity from the tentacular ring to the apex of the anal cone. Compared with the first two types this is much rarer.

TYPE *D* (figs. 12 and 16). This is a rare form of which I have obtained only seven specimens in all. It is enormously large in comparison with the others (4.–5. *mm.* in total length and 1. *mm.* in width). The preoral lobe is disproportionately small, while the trunk is long and thick. The tentacles are remarkably numerous, sometimes reaching 48 in number. In a single living specimen, the skin of the trunk was of a light orange colour; the subdermal circular muscles were especially well developed in the trunk but interrupted at four longitudinal clearly marked zones.

The youngest swimming larva I have ever obtained was of type *A*. It was already supplied with four pairs of tentacles which, however, were still short. The body measured about 0.5 *mm.* in length. The trunk was short and showed a slight characteristic curvature, the concavity being turned toward the dorsal side. The thickly ciliated hood was comparatively large; the ganglion and the perianal ciliated belt were already well developed. In the surface view of this larva during life, I was not able to detect the ventral pouch nor the corpuscle-masses.

At about the stage with five pairs of tentacles, the trunk becomes elongated and straightend out. The nephridia may then be seen in their characteristic bouquet-form, and the ventral pouch appears as a solid ectoblastic thickening. Neither the corpuscle-masses nor the retractor muscles are yet to be seen.

As the larva grows, the number of tentacles increases in pairs proceeding from the ventral side toward the dorsal; hence, the most dorsally situated tentacles are the youngest and the shortest. In larvæ with 12 tentacles and belonging to type *A*, the ventral pouch is deep enough to be plainly visible from outside. We always notice from this stage on a pair of the retractor muscles which extends between the ganglion (*gl.*) and the dorsal inner side of the tentacular circle (*ret.* in figs. 12, 13, 14, and 15).

The larval organisation of types *A* and *B* is nearly completed in the stages with 14–16 tentacles. Let me next give a somewhat detailed description of the external appearance of Actinotrocha in general.

The preoral lobe. This is a structure which looks like a broad hood with its concavity directed downwards. It almost entirely covers the upper anterior part of the collar,* when not influenced by external circumstances. MASTERMAN has made the statement that in its natural attitude the hood has its length disposed parallel to the principal body-axis. However, if the larva be examined in the living state, it will at once be discovered that its normal disposition is horizontal. It becomes turned up only as the result of preservation. Its whole surface is covered with cilia, most strongly developed along the free margin which constitutes the preoral ciliated belt. In the full grown larva, the ganglion (and also the sensory spot in type *B*) has also a set of specially long cilia on the outside. Numerous fine and refractive nerve fibres are seen radiating from the ganglion (*gl.*) to the free margin of the lobe (*pre. bel.*) (figs. 13 and 14).

MASTERMAN has described and figured two ectoblastic structures which are said to be situated on the ventral wall of the hood and which he has named the "oral" and the "pharyngeal" grooves. These he compares, as to their function, to the gill-slits of the Chordata. I can not but think that that writer has here fallen into a very grave error, which might have been avoided, had he examined the structures in question in living specimens. Among the preserved specimens I have frequently noticed those in which the lower or oral wall of the hood was prominently bulged out in front of the mouth. In consequence of that prominence (fig. 16, *prom.*), there was produced on either

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* I adopt this name of Masterman's to denote that portion of the larval body which lies in front of the tentacular circle and behind the preoral lobe.

side of the mouth a transverse groove, which was visible when viewed from the ventral side. I believe it was to grooves of this kind that MASTERMAN assigned the above important significance. In my opinion, they are simply artificial productions due to preservation.

The Collar. The form of the collar as a whole may be compared to a cylinder obliquely truncated at the posterior end. Its posterior border is fringed with a regular row of tentacles, while anteriorly it is joined to the hood by a narrow neck. The number of tentacles (larval) varies according to the different stages of growth and also according to the type to which larvæ belong. They are most numerous in type *D*, most individuals of which bear 40–48 tentacles (figs. 12 and 16). The rudiments of the adult tentacles make their appearance as bud-like ectoblastic thickenings immediately below the base of the larval tentacles. An exception to this rule is found in the case of larvæ belonging to type *D*, in which the adult tentacles are represented by a local ventral thickening of the wall of the larval tentacles at their proximal portion (see fig. 58 *d*, *s. t.*). It is very probable that the number of the larval tentacles corresponds to that of the adult. In type *A*, at any rate, I have ascertained that the full grown larva and the worm just metamorphosed bear the same number of tentacles, namely 16.

The trunk. This portion, which is the shortest of the three regions in early larval stages (fig. 10), comes with growth to occupy the largest part of the larval body and assumes a long cylindrical form. Its anterior boundary is the tentacular circle; the posterior end is girdled with the perianal ciliated belt which serves as the larval locomotory organ.

b. The Internal Structure of Actinotrocha.

1. *Body-Divisions and Body-Cavities.*

I have endeavored to show in the preceding pages, that the body-cavities of Actinotrocha do not arise from the enteric diverticula, as was insisted upon by **Caldwell**, but that they are simply produced by mesoblastic cells applying themselves to and forming the lining of the ectoblastic, and the entoblastic, wall. They may, therefore, be classed under the "pseudocœle" or "schizocœle" of **Hertwig**. Moreover, the body-cavities of Actinotrocha as a whole do not in their genetic relation correspond to those of the adult, as I shall attempt to elucidate in the sequel. During the metamorphosis, the greater part of the former (the preoral cavity) is almost wholly lost, while the other part (the collar-cavity) is transformed into a vascular space, so that what is known by the same name in the adult is of an entirely new origin. Thus we see that the larval body-cavity of Actinotrocha, *i. e.* the trunk cavity, is the only portion that persists among the body-cavities of the adult, in which it is known as the foot or infraseptal cavity. In correlation with this circumstance are observable certain changes in the position of the nephridia and of the vascular system. As described by **Caldwell**, the nephridia of Actinotrocha, which are not provided with an internal opening, lie for the most part in the collar cavity, while after the metamorphosis they are found wholly in the infraseptal cavity of the worm. Moreover, the paired corpuscle masses which are found only in the collar cavity of the larva, are no longer seen in the same cavity of the adult. These changes to a certain extent at least establish the fact that some profound changes in the arrangement of the body-cavities must occur during the metamorphosis. As is acknowledged by all, the supraseptal cavity of *Phoronis* is greatly reduced in size as compared with that of the larva, and contains almost no organ except the blood vessels. The infraseptal cavity is, on the contrary, very wide, and contains many important organs, *e. g.*, the alimentary canal, the sexual organs, and the main part of the vascular system. Thus it becomes necessary to make distinctions between the body-cavities of *Actinotrocha* and those of the adult and to call them respectively by different names. The former may be termed the larval body-cavities, and the latter, the adult body-cavities.

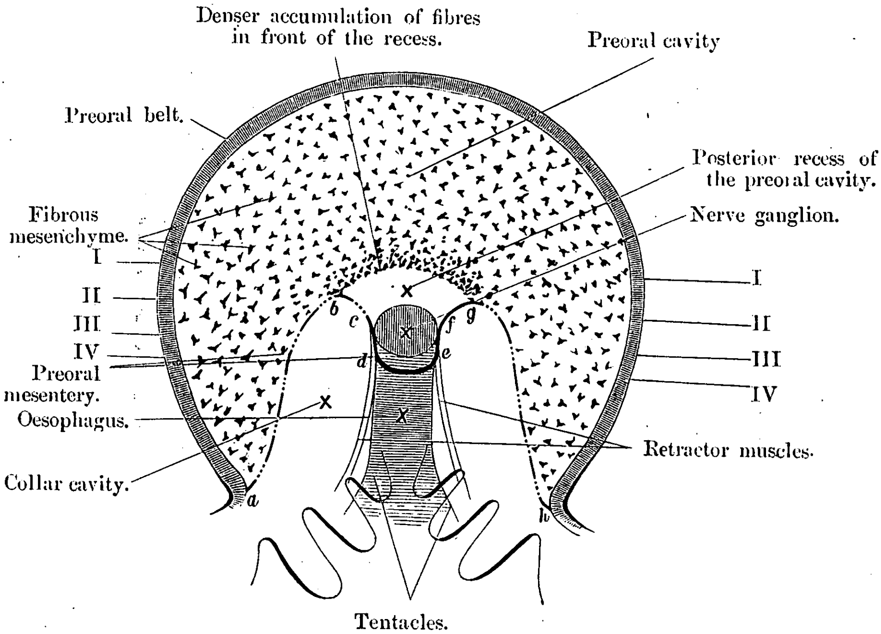
Most previous writers have not taken any particular notice of the relation which exists between the external body-divisions and the body-cavities of *Actinotrocha*, so the words " hood " and " foot " do not denote anything but mere external features. The idea of *segment* was first introduced by CALDWELL; he considers the larval body as divided into three parts: (1) the preoral lobe set in front of the septum, (2) the trunk portion situated behind the septum, and (3) the foot or invaginated pouch. According to this view, the body-cavity is divided by the septum into two contiguous parts, *viz.*, the preoral cavity in front of, and the trunk cavity behind, the septum. MASTERMAN divides the entire body into three portions, *viz.*, the preoral lobe, the collar, and the trunk. These three divisions are not only externally marked by their respective forms, but also by the presence of two transverse septa or mesenteries. Thus we see, the preoral lobe of CALDWELL comprises both the preoral lobe and the collar of MASTERMAN.

Whatever may be the value of MASTERMAN'S Diplochorda hypothesis, I feel inclined to accept with some modifications, his view of the body-divisions. The external appearance of the three portions I have already described in brief. As to the internal body-cavities corresponding to these external portions, I can not agree with MASTERMAN, when he says that they are completely separated from one another; for, as I shall soon show, the septum which lies between the preoral and the collar cavities is always an incomplete formation, at least in all the *Actinotrochæ* which I have observed. Besides, I have been unable to detect the first and third pairs of nephridia, which are said to exist in the preoral, and the trunk cavities (MASTERMAN). Therefore, I can not regard the body-divisions of *Actinotrocha* as "segments" in the sense of that author.

The septa or mesenteries are very delicate in structure and can hardly be recognized in living specimens. I have, therefore, had to study them mostly in sections. I shall hereafter call the two septa the preoral, and the postoral, septa.

Larval Preoral Body-Cavity. The larval preoral body-cavity fills up the interior of the hood, in which there is no entoblastic organ. Innumerable mesenchymatous fibres traverse the cavity (figs. 45, 49, 63 *a*, *m.f.*). A few blood corpuscles are also frequently discovered in this cavity (fig. 49, *corp.*); this fact is, I believe, one of the proofs of the correctness of the view which I now propose to consider.

I have already spoken of the incomplete formation of the preoral septum. Whether this is a mere specific difference or not, remains to me uncertain, as I have had no chance of examining the larvæ investigated by MASTERMAN. In sagittal sections of the larva at any stage of its growth, the septum can constantly be traced so long as the œsophagus is contained in the sections. In figs. 45 and 63 *a*, a slender cellular strand (*mes'.*) behind the ganglion (*gl.*) represents the septum in cross section. It extends between the upper and the lower walls of the hood. Thus it will be seen that the septum completely separates the preoral cavity from the collar cavity just behind the ganglion. But, when we come to sections passing through a more lateral region to either side of the ganglion or of the œsophagus, the upper portion of the septum becomes abruptly indistinct. In fig. 54, which shows a sagittal section through the right-hand side of the œsophagus of a larva of 16 tentacles (type *A*), the septum (*mes'*.) near its ventral attachment is indicated by a comparatively thick layer of cells, while the dorsal portion is divided into fine protoplasmic branches, of which some extend to the upper wall of the hood and others stop short of it. As the relations of this septum are somewhat complicated, I will try to make them clear by referring to a series of cross sections (not continuous) through the hood of a larva of type *D* (figs. 59 *a-d*) and also to the annexed wood-cut. The latter is a diagrammatic representation of the *Actinotrocha* hood and its neighbouring part, as seen from above, *i. e.*, in horizontal projection. The dorsal side is above and the ventral, below. Nearly in the centre is the nerve ganglion. Below it and concealed from sight, is the mouth, from which the œsophagus leads downwards. The little stellate markings, scattered over the greater part of the figure, are supposed to represent mesenchymatous cells, which, with the branched and reticulate fibres arising from them, pervade the preoral body-cavity, except in a small space immediately in front of, and below, the ganglion. This free space I shall call the posterior recess of the preoral cavity. The line *a b c d e f g h*, curved somewhat like the letter *M*, indicates the position of the preoral septum. The part shown in full line represents that portion of the septum which is complete in structure and the part in broken line, that portion of the same which is incomplete. All the space in front of the septum is the preoral cavity, while back of it lies the collar cavity.



If we now study the series of sections (figs. 59 *a*-*d**), the nature of this septum will become clear. Section 59 *a* is the most anterior of the four and passes through the hood at about the plane of the line I-I in tke above wood-cut. The whole of the preoral cavity is filled with the fibres of branching mesenchymatous cells except in the ventral median part (*p.r.*). This is the beginning of the posterior recess of the preoral cavity, which is limited anteriorly by a faint membraneous layer consisting of protoplasmic fibres only. Section 59 *b* is from about the plane of the line II-II. This contains the ganglion (*gl.*) and on each side

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* Unfortunately in the series of sections tissues have undergone considerable disturbance by the action of the killing reagent, but the relations of the layers remain unaltered.

the anterior end of the collar cavity (*col.c.*). Below the ganglion, the posterior recess (*p.r.*) is seen to have a complete wall, that is to say, the posterior septum is fully developed. In the above figure, the two wide spaces lying on both sides of the posterior recess correspond to the two anterior horns of the collar cavity projecting forward (marked *b* and *g* in the wood-cut). And we can certainly see that on the dorsal side as well as laterally there is no distinct partition or continuation of the preoral septum which, according to MASTERMAN, should entirely divide the preoral and the collar cavities at every point. As the figure shows, the dorsal portion of the mesentery (*mes'*) is decomposed into fine protoplasmic processes which join with those of the fibrous mesenchymes dispersed through the preoral cavity. In section, 59 *c*, passing though the middle of the ganglion (the line III-III of the wood-cut), the collar cavities (*col.c.*) are much wider and have become united below the œsophagus (*œs*). The septum (as the wall of the posterior recess, *p.r.*) in this region is a little more definite in form than in the last figure; the posterior recess (*p.r.*) is distinct as before. In section 59 *d* passing through the line IV-IV of the wood-cut, the posterior wall of the recess (*p.r.*) is obliquely cut and appears in the right-hand lower corner as a membraneous slice, the recess being distinctly bounded by the septum (*mes'*.). Outside of it are seen, one on each side, the sections of the retractor muscles (*ret.*), of which more will be said later. The collar cavity (*col.c.*) is now very spacious, but the septum laterally remains in the same condition as before.

From the above descriptions, it will be clear that the preoral septum is complete only in the median portion (indicated by the full line *c d e f* in the wood-cut), while in the more lateral part on each side, it is at the best a loose open reticular membrane, through which the cœlomic fluid of the preoral and the collar cavities is put in free circulation.

A questionable structure has been described from the preoral cavity by **Masterman** under the name "subneural sinus," and is compared to the structure bearing the same name in the Hemichorda. According to him, the subneural sinus is an interstitial space left between the two laminæ composing the preoral septum, just under the ganglion and above the so-called "subneural glaud." Anteriorly and laterally, it is said to be surrounded by the preoral cavity, and posteriorly, by the collar-cavity; its upper and lower walls are claimed to be directly formed of the ectoblast without a peritoneal layer. Further it is said, that the sinus communicates mid-dorsally with the dorsal blood vessel on the œsophagus. After repeated examinations of the larvæ of the four different types, I am convinced that **Masterman's** subneural sinus is identical with what I have called the posterior recess of the preoral cavity. It has nothing to do with the tissue-space in the preoral septum, but is clearly a part of the preoral body-cavity, which is free from the mesenchymatous fibres. Besides, I can not in any way detect the presence of the dorsal vessel on the œsophagus, a vessel which connects the subneural sinus with the dorsal vessel on the stomach. A view similar to mine as above expressed was given by **Harmer** in his paper on Cephalodisus ('97).

Masterman has further given an interesting description of the "proboscis pores," situated on each side of the ganglion. They are compared to the proboscis pores of *Balanoglossus* and are said to fulfill the same function as the collar nephridium of *Actinotrocha*. In the larvæ studied by me, the only things that bear even a remote resemblance to them, are the flask-shaped glands which are seen on the upper face of the preoral lobe of the larva belonging to type *C.* But the position of these glands in relation to the ganglion as well as their histological structure at once reveal their true nature. The internal openings of the organs were described by MASTERMAN as follows: "*Just where the preoral mesoblastic wall slopes away on either side of the sinus there are a pair of thickenings, which traced forwards, show themselves to be the commencement of a pair of internal openings*". (*l.c.*, p. 307). The paired thickenings referred to by him are apparently nothing else than the points of attachment of the retractor muscles in the collar cavity, as will be seen in fig. 59 *d* (*ret.*). Further details respecting these muscles will be given later.

Larval Collar Cavity. The collar-cavity is a comparatively wide space extending between the preoral and the postoral septa. It is produced anteriorly into two horns, embracing between them the posterior recess of the preoral cavity. It is perfectly separated by the postoral septum from the trunk cavity. The postoral septum, or simply the *septum,* as it is more commonly called, is stretched obliquely transversely between the splanchnic and the somatic walls, along a line a little below the tentacular circle (figs. 45, 48, *mes.*). Its dorsal attachment on the splanchnic layer is, as represented in fig. 45 (*mes.*), found at the plane of the junction of the œsophagus with the stomach, while ventrally the attachment lies much further below. In frontal sections of the larva, the septum (fig. 48 *mes.*) is seen on either side of the stomach and its somatic insertion lies just under the tentacles, so that each tentacular cavity is continuous with the larval collar cavity (fig. 45).

The adult collar cavity, or the supraseptal cavity, is already formed in the fully developed larva of every type, as a ring-space running along the inner side of the tentacular circle and above the septum (see figs. 58 *a* and *d*, *s.c.c.*). This, together with several other larval organs in the larval collar cavity, had better be treated at a more suitable place in the sequel.

Masterman has described a dorsal mesentery running along the mid-dorsal line of the œsophagus, and separating dorsally the larval collar cavity into two lateral halves. In the *Actinotrochae* of all the types observed by me and at every stage of the larval growth, no such mesentery is present. It is true that the body walls and the œsophageal walls very frequently come close together, especially in the young larva after preservation, so as to greatly narrow the collar cavity in this region (figs. 49 and 50 *a*). But a mesentery is never to be found. Its absence is quite clear in the large *Actinotrocha* belonging to type *D*, in which the skin and the œsophagus lie well separated by a considerable space (figs. 58 *a* and 58 *b*).

Trunk cavity. The trunk cavity occupies the interior of the third body-division—the trunk. It is completely separated by the postoral septum from the collar cavity, and since the septum is oblique in position, it extends dorsally nearly to the base of the œsophagus. The ventral mesentery extends along the median ventral line of the body wall and of the alimentary canal, and is wholly confined to the trunk cavity. In fig. 45, which shows a median sagittal section of a young larva of type *A*, a portion of this mesentery (*v.mes.*) is represented as a thin cellular membrane extending between the alimentary canal and the ventral pouch (*p.o.*), the latter being still shallow at this stage. The whole extent of the ventral pouch is stretched by the ventral mesentery to the skin as well as to the digestive canal. This relation remains the same as the pouch grows in length and finally winds around the digestive canal. A transverse section through the trunk of a highly advanced larva of type *C*, is given in fig. 57 *a*, in which the much elongated and convoluted pouch is seen cut into several sections (*po.*), connected with one another by the mesentery (*v.mes.*).

Very frequently it happens that the peritoneal mesoblastic epithelium, which lines the perianal ciliated belt, is detached from the ectoblastic wall. This is a purely artificial appearance caused by the killing reagent. It seems probable that MASTERMAN has erroneously considered the space thus formed by splitting to be a vascular space (the "perianal sinus"). The same author states, though with much reserve, that he has discovered a third pair of nephridia in this trunk cavity, which is considered to be a modified part of the body-cavity, and also to be rudiments of the adult nephridia. I can at present say no more than that these are certainly absent in every type of the *Actinotrocha* studied by myself.

2. *Organs of Ectoblastic Origin.*

The epidermis of *Actinotrocha* is represented by a single layer of cubical or cylindrical cells, those of the collar wall and of the upper and the lower walls of the hood being provided with well developed cilia. Besides, there are three specially ciliated regions: the preoral belt, the tentacles, and the perianal belt. The last is the larval locomotory organ; on it the cilia are very long, thick, and somewhat bristle-like when in active motion. At places, where cilia are strongly developed, (*e.g.*, the nerve ganglion, the sensory spot if present, the ciliated belts, *etc.*) the constituent cells are cylindrical, the nucleus generally lying near the basal end. The body wall of the trunk region is very thin and is formed of greatly attenuated cells (especially slender in the advanced larvæ).

Numerous unicellular glands are found in the *Actinotrocha* not only all over the two surfaces of the preoral lobe, but also in the œsophageal wall as well as in the inner ectoblastic wall of the ventral pouch. They are also, though less abundantly, distributed over both the collar wall and the tentacular wall. The glandular cells are all pear-shaped, the nucleus being found always appressed to the base of the cell (figs. 49 and 64 *d*, *m.gl.*). In their staining reactions, the secretory contents of the glands agree with those of mucin. It has been often noticed that living larvæ remain adhereing to the objects they have touched with the hood, and that metamorphosed larvæ behave similarly with the tip of the evaginated pouch.

There exists still another, paired, multicellular gland which is observed only in the larvæ of type *C* (figs. 15, *gld.* and fig. 15 *c*). It is situated on both sides of the median line on the upper surface, and somewhat near, the neck of the preoral lobe. It has the shape of a round flask with a short neck (fig. 15 *c*). The appearance of the section through the body of this gland reminds us of the chorda dorsalis in Vertebrate embryos: it presents to view a mesh-work of protoplasm, a small number of nuclei being found here and there closely pressed against the reticular beams or the nodes of these (figs. 56 *a-c*).* Each of the meshes corresponds to one gland cell. In fig. 56 *b*, which shows an oblique median section of the body of the gland, a comparatively wide round space exists in the centre, surrounded by the gland cells which are arranged more or less radially. This space, when traced upwards, passes into a short and very narrow tubular canal, finally to lead to the exterior by a small aperture (Fig. 56 *c*). Since

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* By an unfortunate oversight, Fig. 56 *b* has had its number omitted in the plate.

the neck portion of the gland is very short, it is difficult to prepare a good longitudinal section of it, in which the canal may be seen opening to the exterior. Fig. 56 *c* represents the terminal part of the emptying canal, which, as can be ascertained by regulating the focus, leads to the external pore. Mr. IIZUKA tells me that similar glands of ectoblastic origin are constantly found on the superior ramus of a parapodium in certain *Polychæta.*

Ventral Pouch. As the ventral pouch is one of the most characteristic structures of *Actinotrocha,* its form and fate have been fully studied by many previous observers. In the 8-armed larva of type *A,* an ectodermal thickening below the tentacular row represents the origin of the pouch. At the 10-armed stage the thickening becomes more conspicuous, but no invagination has as yet taken place. For the first time in the 12-armed stage, the wall at the thickening begins to sink inwards and backwards (fig. 45, *po.*). The invagination is lined with a mesoblastic layer, and, as before noted, is for its whole length suspended by the ventral mesentery, joining it to the somatic and the splanchnic walls. As the growth of the larva advances, the pouch becomes more elongated and bends on itself around the alimentary canal)figs. 48 and 57 *a, po.*). In fully developed larvæ of whatever type, the inner or ectoblastic wall is thrown into small wavy folds (beginning at the distal portion near the pouch pore), while the mesoblastic layer becomes muscular, so that at the end of larval life, it forms a thick muscular sheath whose constituent cells stand vertically to the inner wall. As to the form and position of the pouch pore, I can offer no details in addition to what has been observed by METSCHNIKOFF and many other authorities.

Nervous System. The nervous system of *Actinotrocha,* like that of *Phoronis,* is of a very low development, being represented merely by a local differentiation of the ectoblastic cells into nervous elements. The epidermis over both the ganglion (fig. 14. *gl.*) and the sensory spot (*so.*) is strongly ciliated, so that the organs are easily recognizable in the living larva. The earliest stage in which I found the ganglion was a 4-armed larva of type *A* (fig. 40 *gl.*). In it, the ganglion consisted of only a few ganglion cells and nerve fibres.

Although the ganglion and some nerves directly proceeding from it can be detected with tolerable distinctness in the living specimen on account of their peculiar refractivity, the peripheral nerves are as a general rule so very fine and delicate, that they can not be satisfactorily made out by means of any ordinary process. With fair success I have had recourse to vital staining with methyl-blue. Larvæ of type *B* have been principally employed for this purpose. They are left for about 15-20 minutes in a weak solution of methyl-blue in sea water and immediately afterwards treated with ammonium molybdate. Sometimes, I have made supplementary observations on larvæ lying alive in the methyl-blue solution under the cover glass, but this can be continued for only a short time, since a general overstaining of other tissues soon takes place.

Fig. 60 *a** shows the dorsal view of the anterior half of a larva of type *B*, which was treated in the above way. The nerves are shown in blue. The results obtained as to their distribution differ in many important points from those obtained by MASTERMAN. Whether this difference is due to the technique or is actually existant in the species studied, is difficult to ascertain.

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* As the larva shown in this figure was compressed by the cover-glass, the rim of the hood which appears like its free margin is in reality the line along which the hood was bent and reflected over by pressure. The line drawn close to the peripheral dots in blue represents the true edge of the hood.

MASTERMAN says nothing of the method employed in his investigation, and unfortunately there exists no other study than his with which to compare my results.

As may be gathered from the above-mentioned figure, I can discover no collar nerve ring, nor dorsal or ventral commissure. Besides, in spite of repeated efforts, I have always failed to make out the presence of the so-called perianal nerve ring. The collar ring and the dorsal commisure, if they can be so named, are represented by a small number of parallel fibres, which spring directly out from the posterior corner of the nerve ganglion. In every case examined, they could be traced no further than a short distance from the ganglion. Sometimes, I have been able to discern in sections the main nerves (commonly 3 in number), which run close together and parallel to one another along the mid-dorsal line of the trunk, but they were confined to only a few sections posterior to the first pair of tentacles. On the other hand, a very complex and beautiful system of nerve fibres could be seen on the preoral lobe. The fibres are here exceedingly numerous and fine, radiating from the ganglion on all sides towards the free margin of the preoral lobe. In the median line and anteriorly to the ganglion (*gl.*), the fibres appear as three longitudinal parallel strands on which the unpaired sensory spot (*so.*) is situated not far from the ganglion. After passing through the sensory spot the strands fray out into fine fibres which continue their course towards the free margin of the preoral lobe. The fibres emanating from the ganglion do not all show a regular radial arrangement, but there are some that arising from the lateral edge of the ganglion, soon take an anteriorly directed course. Sometimes there were not wanting, especially near the ganglion, indications of anastomosis between the fibres. However, it seemed to me more probable that these appearances were caused simply by the juxtaposition of intersecting fibres.

The nerve endings in the preoral ciliated belt deserve special notice. In fig. 60 *a*, there is shown a row of small dots along the margin of the band. A portion of the latter more highly magnified is shown in fig. 60 *b*. Here each fibre ends in a small knob which is devoid of any lateral process. At first sight under low magnification, the row of knobs appears like a deeply stained ring. Suspecting that there might exist lateral processes connecting knobs, I have repeatedly made observations and experiments, but without having ever been able to demonstrate such a connection between them.

I can not but think it very strange that post-ganglional nerve fibres, if such really exist in the forms of the collar ring and of the dorsal and the ventral commissures, should not be revealed by the method adopted. The negative result may be considered due to incomplete development of nervous elements in the collar and in the trunk region; but other anatomical relations prove to a certainty that the larvæ investigated were fully grown. As I am not quite sure that my method was not in some respect imperfect, I leave the matter undecided for the present.

According to MASTERMAN, there is an ectodermal depression directed inwards and backwards, just in front of, and under, the ganglion. He calls it the "neuropore," comparing it to the neuropore of *Amphioxus* and even to the medullary canal of Vertebrates. I must say I was much disappointed in failing to detect in the *Actinotrochæ* studied by me this structure of so much theoretical interest. As a matter of fact, it happened very frequently, while observing living larvæ, that the ganglion was retracted deeply inwards by an active contraction of the two retractor muscles in the collar cavity (figs. 13, 14, 15, *ret.*) producing at the same time a deep depression just in front of the ganglion. It is also of almost constant occurrence that the ganglion is withdrawn inwards on the application of reagents, so as to produce a shallow pit or groove in front of, or below, the ganglion (figs. 63 *a*, *gl.*). A quite similar fact is always observed in LOVÉN's larva. From these circumstances I am much inclined to regard the "neuropore" of MASTERMAN not as a really existing structure, but as an artefact.

As to the tentacles, I have at present nothing to add to what is already known about them.

3. *Organs of Entoblastic Origin.*

In the fully grown larvæ the alimentary canal is a long and straight tube; it begins with the mouth which is overhung by the preoral lobe, and ends at the anus in the centre of the anal cone surrounded by the perianal belt (figs. 12-16). Of the whole alimentary tract three parts may be distinguished: the Oesophagus, the Stomach, and the Intestine.

Oesophagus. In the embryological part of this article I have said that the œsophagus of Actinotrocha is of ectoblastic origin, so that the original gastrula mouth is to be sought at the juncture of the œsophagus with the stomach. The œsophagus (Figs. 45, 48, and 49, *œs.*) is a comparatively short and narrow canal with a wall composed of densely ciliated cylindrical cells, among which are scattered numerous unicellular glands (*m.gl.*). Thus the wall does not differ in structure from that of the hood or of the collar.

MASTERMAN has described an unpaired ectodermal invagination situated in front of the mouth and just under the ganglion. It is called the "subneural gland." Here again I am not in a position to confirm his view. In spite of repeated examinations on living specimens, I have been unable to discover any structure which has the slightest resemblance to the subneural gland. To judge from my own observation, the "subneural gland" as well as both the "oral-" and the "pharyngeal grooves" of this author are products of his fixing method. In preserved specimens, it is frequently noticed that the lower wall of the hood is bulged out and downwards in front of the mouth (fig. 16, *prom.*), and, as a result of this, there is brought about on the wall behind the prominence a depression, which appears on sections as a tolerably deep pit (fig. 63 *a*).

Stomach. The stomach forms the largest and widest portion of the alimentary canal. It is especially long in the larvæ of type *D*, in which it extends below nearly to the plane of the perianal belt. The greater part of the stomach wall is composed of cylindrical cells with short cilia whose spherical nucleus is usually situated in the centre of the cell (figs. 45, 48, and 50 *a*, *stom.*). But the anterior portion of the wall along the mid-dorsal line and the posterior portion near the intestine greatly differ in their constituent cells from the remaining parts. They consist exclusively of tall ciliated cells which contain elongated nuclei, and are, in a word, of the æsophageal type (fig. 45). In the full grown larva, the ventro-lateral portions of the stomach wall form two digestive areas placed in the neighbourhood of the septum. Here the cell boundaries are indistinct and the nuclei are imbedded in a common mass of protoplasm, in which remains of various unicellular organisms are enclosed.

From the anterior end of the stomach a pretty wide and *unpaired* diverticulum protrudes itself forwards (fig. 14, *div.*). The position of the organ is wholly *ventral* to the œsophagus (*œs.*), and the form is like that of a sac compressed in the dorso-ventral direction (figs. 45, 49, 50 *a*, and 63 *a*, *div.*). The internal cavity is continuous with the stomach cavity. The roof of the diverticulum in the fresh state generally shows a reddish brown tint. This coloration is due to the superposition of the fundamental brownish colour on the hæmoglobin of the blood corpuscles which, in advanced larvæ, overlie the organ in either one, or two masses. The cells which compose the diverticular wall are tall and slightly curved, and are ciliated on the free ends (fig. 50 *a*, *div.*) In fully grown larvæ of every type, each cell constantly contains a single small round vacuole in its distal end (fig. 61 *b*). The vacuoles can not be stained by most of the staining reagents. I have seen them in the diverticular wall of a highly advanced larva belonging to type *A*, which had already evaginated the ventral pouch ; even in this case, they were found only one in each cell (fig. 61 *b*). The whole of the diverticulum is lined externally with the thin peritoneal layer (see the above figure).

Many previous observers have noticed this organ and have called it by various names :—

J. MULLER ('46)—" Blinddarme " (paired),
GEGENBAUR ('54)—" Haufen der Leberzellen,"
WAGENER ('47)—" Leberblinddarme,"
CLAPARÈDE ('63)—" A dark mass with globules " (after MASTERMAN),
METSCHNIKOFF ('71)—" brown specks,"
WILSON (A.G.) ('81)—" glandular lobes of the stomach,"
MASTERMAN ('97)—" Notochord " (paired),
ROULE ('98)—" Notochord " (unpaired).

Thus it will be seen that while some authors have apparently confounded the organ with the overlying corpuscle masses, others have considered it to be a glandular appendage of the stomach, and still others have regarded it as a skeletal structure. According to **Masterman**, who maintains the last mentioned opinion, the stomach wall is produced, in the antero-lateral region, "into two remarkable diverticula which in the fully developed larva lie as a pair of elongated organs, Notochords, laterally to the œsophagus" ('97, *l.c.*, p. 302). The organs are said to soon undergo a remarkable metamorphosis, *i.e.*, vacuolization. The vacuoles are produced successively one after another at the distal ends of the cells and are arranged alternately in several layers. On account of these facts **Masterman** rejects the view that the organ is of a glandular nature, and holds that it is to be compared in function and structure with the notochord of the Chordata. In 1898 **Roule** published his third paper on *Actinotrocha*, in which he denied that the organ is double in number and lateral in position to the œsophagus, but admitted the vacuolization in the larva of *Phoronis sabatieri* (=*P. psammophila* **Cori**).

I can not at present decide whether the variations in the number of the diverticulum and in the degree of vacuolization are of specific value or not. For the present I must be content with simply noting that the stomach diverticulum in the larvæ studied by me is constantly unpaired and undergoes no farther vacuolization process than the production of one vacuole in each cell.

Intestine. The intestine which leads to the anus is a slender canal whose wall is composed of a layer of somewhat cylindrical, ciliated cells with round nuclei (figs. 45 and 48, *int.*).

4. *Organs of Mesoblastic Origin.*

As the mesoblastic organs have been but little studied in their development, so their structure and fate after metamorphosis are very imperfectly known. Although I have endeavoured to make my study of the organs as exhaustive as possible, some important questions remain yet unsolved. The principal organs to be described in this place are the *muscular elements,* the *vascular system,* and the *nephridia.*

Nephridia. I will treat these under the mesoblastic organs, for, though the nephridial canals are of ectoblastic origin, the organs as a whole bear intimate relations to the mesoblast. Most of the earlier observers overlooked the presence of nephridia in the larva. The first discoverer was Wagener ('48), whose description is, however, very meagre and gives us no exact idea of the organ. Caldwell in his preliminary note ('82–'83) has given a detailed description of the nephridia. According to his view, the nephridial canal at no time during larval life, opens into the body-cavity.

Masterman ('97) has described the excretory system of the larva in detail and has suggested an hypothesis which seems to me to be an extraordinary one. Each of the three "segments" of the larval body, he concludes, is provided with a paired organ which performs the excretory function. The three pairs of organs are called respectively the "proboscis pores," the "collar nephridia," and the "trunk nephridia," Of these, however, the presence of the first and the third is, as I have before pointed out, very doubtful. The second pair, or the collar nephridia, are the organs which I consider to be the nephridia. Masterman's views on the structure of the nephridial canals are in the main similar to those of CALDWELL, except in one important point, *viz.*, that the canals are said to open by means of funnels into the collar cavity.

When a larva of any type is examined in the living state, the proximal ends of the organs are seen, as described by WAGENER, as two bonquet-shaped masses which are formed by a crowding together of the excretory cells (fig. 13, *neph.*). They are placed symmetrically one on each side of the stomach and in front of the postoral septum. Each of them consists of two parts, the nephridial canal and the excretory cells. The former is composed of a layer of cubical cells, and contains a narrow lumen which ends blindly at the internal end and distally leads to the nephridial pore lying on either side of the pouch pore. The greater part of the nephridial canal, together with the excretory cells, rests on the upper surface of the postoral septum. Fig. 50 *b* shows a cross section of the larva, passing through the left nephridial canal near its internal blind end, where the excretory cells adhere. In the above figure, a small cell mass (*nep.c.*) on the right of the figure, shows the cut end of the nephridial canal which is attached to the septum (*mes.*). In the figure, the left canal (*nep.c*) containing a small lumen, is found applied to the somatic walls. If traced a little downwards, these two canals become attached to and imbedded between the two layers of the somatic walls, and are no more to be seen in the collar cavity. Such a state is represented in fig. 50 *c*, in which the two canals (*nep.c.*) are wholly imbedded in the somatic walls on both sides of the stomach (*stm.*). This condition is more distinctly shown in figs. 47 (*a-c*), which are taken from serial longitudinal sections of a larva of type *A* with 12 tentacles. These figures show only one portion of the skin, where the nephridial canal and the somatic attachment of the postoral septum (*mes.*) are situated. In fig. 47 *a*, one portion of the peritoneal layer of the stomach wall is also represented. Now we see in the first two figures of the above series, that the nephridial canal (*nep.c.*) which is here imbedded in the somatic layers, lies distinctly below the septum (*mes.*). So, in the third figure the nephridial pore (*nep.o*) is seen as a small pit in the trunk wall, which is situated considerably below the septum. The infraseptal position of the nephridial pores has also been acknowledged by CALDWELL. Though MASTERMAN has made no direct statement on this point, it may safely be inferred from his figures, that he must have regarded the pores as lying in front of the septum.

Fig. 51 *a* represents a longitudinal section through the middle of the supraseptal portion of the nephridial canal. Here the canal appears as a comparatively long tube with a narrow lumen; it is invested throughout with a thin mesoblastic epithelium. At its upper extremity where the lumen disappears, a certain number of spindle-shaped excretory cells is found aggregated together. In fig. 51 *b*, which is taken from the same series as fig. 51 *a*, the canal has wholly disappeared from the section, leaving only a bunch of the excretory cells (*exc.c.*) adhering to the septum (*mes.*). All of these spindle-shaped cells have their nuclei in the swollen ends. I have never found either among, or in, the neighbourhood of the cell bunch any perforated excretory cells bearing many processes,—cells which are said to have been present in the *Actinotrocha* studied by CALDWELL.

MASTERMAN considers that each bonquet of the excretory cells is composed of a cellular mass traversed by a system of minute funnels; and that these funnels communicate with the main canal of the nephridium as well as with the collar cavity. But I may say with certainty that, at least in the larvæ studied by me, there existed no such funnel-system nor any such free communication between the collar cavity and the nephridial canals. The same negative result was also reached by me in my examination of the just metamorphosed larva of type *A*. Thus in fig. 64*f* which is drawn from a section through the tip of the nephridium, the excretory cells (*exc.c.*) still remain compactly grouped on the blind tip of the canal (*nep.c.*), but are not traversed by any sort of canal-systems.

Muscular System. The muscular system of *Actinotrocha* remains in a low state of development, which may account for the fact that most previous observers have paid no particular attention to it. It was therefore of much interest to discover two pairs of tolerably well developed muscles, which had hitherto remained apparently unknown. They show a strong resemblance to the retractor muscles which have been known in many forms of *Trochophora* larvæ.

Though the longitudinal and circular muscles of the body wall may be observed with tolerable distinctness in the living larva, they are usually very poorly preserved after hardening. They are all subdermal in position and very delicate in structure, so that as a rule they can not be satisfactorily distinguished from the underlying peritoneal layer. However, in certain preparations of the entire larva the circular muscles of the upper and lower walls of the preoral lobe and of the trunk body wall could be detected as fine deeply stained fibres. In the same way the longitudinal muscles of the collar wall, especially in the larva of type *D*, were fairly traceable. The larva of that type also exhibited a peculiar arrangement of the circular muscles of the trunk, in that these formed four, equidistant, longitudinal series around the periphery of the trunk (see fig. 12). In the larva of type *C* I have always found a comparatively thick layer of circular muscles. In fig. 57 *b*, which shows a portion of the trunk wall containing the nephridial canal (*nep.c.*), the muscles are represented as a thin fibrous layer (*cir.m.*) intercepted between the ectoderm and the peritoneal epithelium. The floor of the mouth just opposite the stomach diverticulum, is always associated with a particularly well developed muscular sheet. The mesenchymatous unicellular fibres which traverse the preoral cavity are to be regarded as a kind of primitive muscles. The most highly developed parts of the muscular system of the somatic, and of the splanchnic, walls are to be found in the muscular sheaths of the ventral pouch and of the dorsal wall of the stomach in the advanced larvæ of all types. Each of them is formed of a thick layer of enormously elongated muscular cells which stand vertically to the ectoblastic, or the entoblastic, wall as the case may be (figs. 58 *c* and 63 *e*, *m.sh.*). The sheath of the stomach wall is thickest along the mid-dorsal line of the stomach; it is shown in fig. 58 *c* and fig. 63 *e*, the former figure being taken from a cross section and the latter from a longitudinal section through the dorso-anterior region of the stomach. The muscular sheath (or the external wall) of the ventral pouch is essentially similar to that of the stomach.

The *Retractor Muscles* can be constantly detected in every type of the larva as two slender threads on both sides of the œsophagus (figs. 12, 13, 14, 15, *ret.*). They spring from the hind lateral corners of the ganglion (*gl.*) and run divergently downwards until they insert themselves in the collar walls between the first, and the second, tentacles. In order to obtain a clear idea of the position of these muscles, it is necessary to study them in sections;

the larvæ of types *B* and *D* are best suited for this purpose, as the muscles in these are remarkably large and long. Figs. 63 *a-c* are taken from serial sagittal sections through the right side of the nerve ganglion. In figure *a*, a median section, we see behind the ganglion nothing but the preoral septum (*mes*'.) In the next figure, *b*, the septum is found to have shifted to a more anterior position and its dorsal termination is accompanied by a strong muscle-band (*ret.*). This band corresponds to that portion of the retractor muscle which is nearest to its anterior insertion on the postero-lateral side of the ganglion. The two muscles are shown in fig. 59 *d* (*ret.*), a cross section through the posterior recess (*p.r.*) of the preoral cavity, where they spring directly from the septum (*mes*'.). In fig. 63 *c*, which shows a more lateral region than fig. 63 *b*, the muscle is found to have retreated far backwards, touching with its posterior portion the œsophageal walls (*œs.*). The posterior insertion of the muscles on the somatic walls are best studied in serial cross sections of the larva. Figs. 58 *a* and *b* show two cross sections passing through the mouth (*a*) and through the middle of the œsophogus (*b*). In both figures the muscles (*ret.*) are found on both sides of the œsophagus (*œs.*). A little further down they soon detach themselves from the œsophagus and begin to traverse freely the body-cavity (larval collar cavity), and after that they again apply themselves to the skin on each side between the first and second tentacles (*t*' and *t*'' in fig. 58 *b*).

There is also present another pair of muscles, which can be discovered only in the larvæ of type *C*. They are so very long as to equal the entire length of the trunk (fig. 15 *b*, *ret*'.). They arise on each side from the somatic walls just above the nephridial pore and run straight downwards traversing the trunk cavity, and ending at the terminal portion of the intestine. Fig. 57 *a* represents a transverse section through the middle portion of the trunk where the stomach (*stm.*) joins the intestine (*int.*). There the muscles appear as two small striated masses (*ret.'*) lying on both sides of the intestine. As represented in fig. 57 *b*, which is taken from the portion of the body wall containing the nephridial canal (*nep.c.*), each of the muscles is in its origin traceable to the circular muscle layer (*cir.m.*) which is subdermally interposed between the ectoblast and the peritoneal epithelium. When these two muscles, in their downward course, reach the level of the terminal portion of the intestine, they fuse together into one on the dorsal side of the intestine. I think that the above mentioned muscles are a kind of retractors serving to contract the trunk of the larval body. I can not help thinking that the "Afterbänderung" of WAGENER ('47) is probably simply the posterior portion of the muscles in question in the proximity of the anus.

Vascular system. It is a well known fact that the closed vascular system of *Phoronis* offers one of the greatest obstacles to the idea entertained by some naturalists that the animal is of the Polyzoan type. Many writers are, therefore, much inclined to attribute the simple body organization of *Phoronis* to secondary adaptation, and to erect the animal into a distinct order very closely related to the Chordata. Putting aside for the present all theoretical speculations, it is of great importance in ascertaining the phylogenetic relation of the animal to note that one portion of the larval body-cavities is transformed into a blood vessel, and that the simple and rudimentary vascular system of *Actinotrocha* undergoes a wonderful change and suddenly attains the high organisation seen in the adult during metamorphosis.

KROHN ('50) proved that the "Leberzellen" of WAGENER and GEGENBAUR were really blood corpuscles. However, he did not discover any blood vessel in *Actinotrocha*; he thought that the blood vessels of the metamorphosed worm arose in the corpuscle masses of the larva.

CLAPARÈDE ('63) mentioned a ring-like vascular canal under the tentacular row of the larva, but did not explain its nature.

SCHNEIDER ('62) discovered two vessels in *Actinotrocha,* which ran parallel along the mid-dorsal line of the stomach.

METSCHNIKOFF ('71) described and figured in a larva of 10 tentacles the "feinen Häutchen" situated just above the invagination pouch, which was said to be the "Gefässanlage." Besides, it is stated that he saw a ventral "sinusartigen Schlauch" which covered the greater part of the stomach and communicated anteriorly with the collar cavity. According to his view, this Schlauch should give rise to the ring vessel of the adult. But what are really meant by the "Schlauch" and the "Häutchen" is not clear from his text and figures.

WILSON ('81) confirmed the main points of METSCHNIKOFF's observations, but disproved the presence of a blood vessel along the intestine, and also the free communication between the pseudohæmal space and the perisviceral cavity. According to this author, there are two sorts of corpuscles: the one kind floats in masses in the perivisceral cavity, and the other (the pseudohœmal corpuscles) arise within the cavity of a sinus which is formed in the stomach walls and form the circular ring vessel of the adult.

CALDWELL ('82–'83) gives us a concise description of the vascular system in *Actinotrocha* and in its adult form. He says that the corpuscle masses "*arise from the mesoblast cells in front of the septum,*" and that "*The vessels arise as slits in the splanchnopleure. The adult condition is reached partly by constrictions, partly by out-growth from these. Thus we have at the* *close of the larval life the blood system in the following condition*:

1. *Blood corpuscles aggregated in two or more masses, lying in the body-cavity of the preoral lobe, i. e., in front of the septum.*

2. *A blood vessel formed on the dorsal wall of the stomach, a marked structure of the larva.*

3. *The splanchnopleure, which in the region of the stomach forms a loose sac surrounding the gut.*

4. *Cœcal prolongations of this sac.*

5. *Cœcal prolongations into the rudiments of the adult tentacles*" ('82–'83, *l.c.*, p. 377). Besides, the author insists on the free communication between the splanchnopleuric sac and the body-cavity in front of the septum. Thus it may be understood that CALDWELL detected only one vessel (*dorsal*) in *Actinotrocha* and thought the ring vessel of the adult was produced from the splanchnopleuric sac around the stomach.

MASTERMAN's views ('97) of the vascular system differ greatly from those of all the others above quoted. The subneural sinus is said to communicate posteriorly by a chink with the dorsal vessel on the œsophagus. The dorsal vessel runs down till it communicates with the ventral vessel at the juncture of the stomach and the intestine, by means of a small ring sinus. Anteriorly also the dorsal vessel gives off two branches which, after passing along the inner side of the two notochords, again meet together in the mid-ventral line, forming a post oral ring sinus. From that meeting point originates the ventral vessel which runs down along the whole length of the gut and opens into a large sinus-ring situated just within the perianal belt. Further, the author denied the free communication of the blood vessels with the body-cavity, which had been maintained by METSCHNIKOFF and CALDWELL.

I have already stated my belief that the mother cells of blood corpuscles appear as the gigantic mesoblast cells in the body-cavity of the larva with one or two pairs of tentacles (fig. 44, *corp.*). These cells may be easily distinguished from other wandering mesoblast cells in that they have an enormous size and are loaded with an abundant quantity of large yolk grains. Now in 8–10-armed larvæ of type *A*, not only such peculiar cells but also the so-called corpuscle masses can not be found in any part of the body-cavities. Instead of them the collar cavity and often also the tentacular cavities contain a few large and isolated mesoblast cells which closely resemble in size and structure the blood corpuscles of a highly advanced larva. These cells no longer contain large yolk grains, but enclose numerous fine, refringent granules. Fig. 46 represents two such corpuscles (*corp.*) floating in the tentacular cavity (*t.*) of a larva of 10 tentacles. It can with propriety, I believe, be admitted, that these corpuscles have arisen by repeated division from the gigantic mesoblast cells, whose yolk contents have been gradually used up during the process. If this be not the case, then how is the presence of those isolated corpuscles in the young larvæ to be explained? If, as is imagined by CALDWELL, they are produced by cell-multiplication taking place in certain parts of the splanchnopleuric walls and form the corpuscle masses from the first, why should such freely floating and isolated corpuscles be actually present in the young larvæ in which the masses are not yet discernible? So far as I know, in the larvæ of type *A*, the corpuscle masses do not exist until the animal has so far developed as to possess at least 14 tentacles (fig. 13, *corp.*). At the stages of 14 and 16 tentacles, these masses are present commonly in two pairs, the one covering the stomach diverticulum and the other just in front of the septum and on both sides of the stomach (fig. 13, *corp.*). They appear as pinkish spheres in fresh specimens. They do not constantly adhere to the stomach walls as WILSON and some others have remarked, but are very frequently found floating freely in the collar cavity, showing that there exists no direct connection with the splanchnic walls. This fact may be clearly seen in fig. 53 (*corp.*), where the mass is located at an appreciable distance from the stomach walls (*stm.*). The same state was also observed in a 14-armed larva of the same type. Besides, I have noticed at these stages of growth two sorts of corpuscles in the masses: the one sort is large and somewhat coarsely granular; the other is much smaller and finely granular. On sections it was found that the former sort is imbedded here and there in groups of the latter (see fig. 53, *corp.*). I can not exactly see the significance of this fact unless it be that it shows the developmental process of the blood corpuscles, in which the larger ones give rise to the smaller by division (the karyokinetic figures in the former can be made out with tolerable distinctness by staining with eosin methylblue). The larger cells are essentially identical with the corpuscles of both the younger (12-armed) and the older larvæ (so far as advanced as to be ready for metamorphosis) of the same type. Thus it seems to me probable, though I state this with a certain degree of reserve, that these smaller corpuscles develop into the normal blood corpuscles of the highly advanced larva, for in the latter we no longer find the smaller forms in the corpuscle masses. Fig. 61 *a* represents four corpuscle cells composing a corpuscle mass of a larva of type *A* which has already evaginated the ventral pouch. Of course at such a stage approaching the end of larval life, the number of the corpuscles is actually greatly increased as compared with that in younger stages.

From the above observations it may be concluded that the blood corpuscles of *Actinotrocha* do not arise at the expense of the splanchnic walls, but are produced by a continual division of certain previously differentiated mesoblast cells.

I will next describe the blood vessels which can be seen during larval life, with reference to the formation of the adult collar cavity. I have shown in the foregoing pages that MASTERMAN's subneural sinus is probably nothing but a posterior recess of the preoral body-cavity, and that neither the dorsal vessel nor the dorsal mesentery is present on the œsophagus in any species of *Actinotrocha* I have been able to obtain. I have endeavoured to ascertain the presence of the dorsal and the ventral vessels as well as of the ring-sinuses around the gut, and I am convinced that at no time during larval life any vessels other than the dorsal on the stomach and the cœcal capillaries are present in the larvæ.

In the *A* type-larva of 14 arms, the dorsal vessel, as figs. 50 *b* and 50 *c* will show, is not yet formed and the stomach wall is uniformly lined with a thin mesoblastic layer. This layer thickens later and its constituent cells become muscular, beginning first at the base of the postoral septum and along the mid-dorsal line. When the larva grows to the stage of 16 tentacles, the dorsal vessel is inceptionally formed. It arises as a solid cord of cells interposed between the muscular, and the entoblastic, walls of the stomach. As shown in fig. 52, the vessel in section is represented by a loose mass of mesoblast cells distinctly delimited on all sides from the surrounding parts; but as yet no lumen is visible in it. I have not seen the definite lumen establish itself in this rudiment of the dorsal vessel at any time during the whole larval life of this type, while, on the other hand, in the advanced larvæ of the other three types, it could be readily recognized as such.

Fig. 58 *c* represents a portion of a transverse section through the anterior region of the trunk of a larva belonging to type *D*. The vessel in question here appears as a small canal (*d.v.*) running in the stomach walls (*stm.* and *m.sh.*). As will be seen in the figure, the canal is distinctly lined with an epithelial cell-layer. The dorsal vessel terminates anteriorly just behind the postoral septum, so that the whole course of the vessel is confined to the trunk region. During larval life, the dorsal vessel does not extend so far posteriorly as to become confluent with the cœcal contractile capillaries which are formed at the point of juncture of the stomach and the intestine. Thus we see in Fig. 55 *b*, which is taken from a transverse section through the lower portion of the stomach, that the gut is covered with a thin mesoblastic wall without a trace of the dorsal vessel, but the capillaries (*v.c.*) are here already developed at this period. In the above montioned figure they are found as cell masses protruding into the trunk cavity from the right side of the splanchnic attachment of the ventral mesentery to the gut; one capillary is seen in cross section. The capillaries shown in that figure are certainly in an early state of development, and, when fully developed, they appear like a tuft consisting of tolerably long, blindly ending tubes. Sometimes I have observed that the capillaries are formed not only on one side of the ventral mesentery, but on both sides of it; and that they are not constantly formed on the gut walls, but sometimes on the ventral mesentery. Thus we see in fig. 57 *a* on the ventral mesentery (*v.mes.*) a rosette-like figure (*v.c.*), the rudiments of the capillaries seen under a low power. It is represented highly magnified in fig. 57 *c* (taken from another neighbouring section of the same series). Here are seen signs of cell-multiplication on either side of the ventral mesentery (*v.mes.*), in places not in direct contact with, but separated by a considerable distance from, the gut.

From the facts above stated, we are justified in concluding that the cœcal capillaries are not produced as out-growths of the dorsal vessel, but are formed independently by cell-multiplication taking place in certain parts (near the gut) of the ventral mesentery, and, therefore, that the dorsal vessel and the capillaries have different origins.

Next I will consider the origin of the ring vessel of the adult animal. Although some early authors have frequently referred to the so-called ring vessel of *Actinotrocha*, yet its origin, form, and position have never been satisfactorily elucidated.[*] Nobody has investigated it by means of sections, and the statements which have been made about it do not rest, it seems to me, on actual anatomical studies of *Actinotrocha*, but rather are mere inferences from facts known respecting the metamorphosed larva. Consequently there have been put forth several irreconcible views in regard to the ring vessel. The structure described under that name by CLAPAREDE, SCHNEIDER, METSCHNIKOFF, and CALDWELL does not seem to be even one and the same thing.

In order to make clear the relations of this system of organs, I must first of all describe somewhat minutely the adult collar cavity. In the fully developed larva of every type, the rudiment of that cavity is represented by a circular space on the inner side of the tentacular row just above the septum. The space is in form not a complete ring, but is interrupted at the median

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[*] In passing I should say that MASTERMAN's ring sinuses have nothing to do with the vessel under consideration, because of them the first is stated to be priœsophageal, the second peri-intestinal, and the third perianal in position; and thus they must be something entirely different from the ring vessel of other authors, which is a sinus in the splanchnic walls around the stomach.

dorsal point. The relative position of it with regard to the septum and the tentacles can be most conveniently studied on sagittal sections of the larval body. In fig. 63 *a* the adult collar cavity (*s.c.c.*) is indicated by a vertical club-shaped space just inside the the body walls and above the postoral septum (*mes.*). Fig. 63 *d* shows a portion of the ventral part of a nearly median sagittal section similar to Fig. 63 *a*. Here the cavity (*s.c.c.*) is seen also as a vertical and comparatively wide space situated inside the body walls and below the tentacles (*p.t.*). It can also be made out that the wall of the cavity is formed of a single layer of mesoblastic cells and its ventral wall is in contact with the somatic walls of the adult tentacle (*s.t.*), while the posterior wall is superposed on the anterior side of the septum (*mes.*). In fig. 58 *a* and 58 *b* the two cellular circles (*s.c.c.*) attached to the inner side of the tentacles (*t'.*) represent a somewhat obliquely cut transverse section of the adult collar cavity. The two tentacles belong to the first pair, and the farthest dorsal point reached by the cavities is, therefore, at the bases of these tentacles. When the serial sections are traced posteriorly, these cavities gradually extend more and more ventrally along the body walls and at last join with each other in the median ventral line. In fig. 58 *c* the cavities appear as two narrow spaces (*s.c.c.*) appressed against the ectoblast (*ech*). In fig. 55 *a*, which is taken from a cross section of an *A*-type larva cut nearly parallel with the tentacular row, the cavity (*s.c.c.*) appears as a long slit-like space intervening between the postoral septum (*mes.*) and the body walls. Here the cavity is seen entirely free of the septum, because the section passes through that portion of the collar which lies slightly above the somatic insertion of the septum (compare fig. 63 *d*).

In somewhat younger larvæ of all types, the adult collar cavity is not yet extended dorsally as far as in fig. 58. Thus, in fig. 63 *e* it is represented by a mesoblastic cell-mass (*s.c.c.*) which is placed just under the second tentacle (*t.″*), and encloses no lumen in itself as yet. It is evident therefore that the adult collar cavity extends itself during development from the ventral towards the dorsal side of the body, as is also the case with the tentacles.

As the larva reaches the end of the swimming period, the adult collar cavity in the adult tentacles becomes wider and wider, nearly filling up the interior of the latter, while the larval tentacular cavity is henceforth gradually reduced to a narrow space appressed to the upper roof of the tentacle. Fig. 58 *d* represents a median sagittal section of a larval tentacle of a larva of type *D*. The portion belonging to the adult tentacle (*s.t.*) is characterised by a very thick ectoblastic layer forming the ventral wall of that tentacle. The adult cavity appears as a remarkably wide space (*s.c.c.*) beginning at the somatic insertion of the postoral septum (*mes.*) and ending at the tip of the adult tentacle (*s.t.*) The narrow cellular band (*p.c.c.*) resting upon the adult cavity (*s.c.c.*) corresponds to the larval collar cavity of the tentacle. The larval cavity is clearly seen in cross sections of the tentacle; in fig. 58 *e* it is visible as a small space (*p.c.c.*) inclosed by the adult cavity (*s.c.c.*) except at the median dorsal point. Tracing the cavity (*p.c.c.*) in the above figure to the base of the tentacle, we see that it communicates by a tiny opening with the larval collar cavity. That portion of the tentacle, which is thrown off during the metamorphosis (see fig. 58 *d*) is distinctly different from the persistent portion (the adult tentacle) in that the former has no trace of the adult tentacular cavity (*s.c.c*). I have ascertained after repeated examinations, that the retrogressing larval tentacular cavity becomes after metamorphosis the tentacular vessel of the adult, as will soon be further explained.

Concurrently with the growth of the adult collar cavity the larval cavity reciprocally diminishes in extent, and finally after metamorphosis it is reduced to a narrow cavity surrounding the gut in front of the postoral septum: *this is the ring vessel of the adult.* In fig. 65, which is taken from a transverse section through the tentacular region of a larva (type *A*) just undergoing metamorphosis, a semicircular space (*cir.c.*) on the left side of the gut represents the greatly reduced larval collar cavity or the ring vessel, which is found dorsally attached to the somatic walls and is laterally quite independent of the latter, though ventrally it is fused with, and rests on, the postoral septum (*mes.*). One of the tentacular vessels which proceeds from the ring vessel (*cir.c.*), is denoted in the plate by *t.r.* Fig. 64 *e* also shows a transverse section of the head portion (or a frontal section through the supraseptal portion) of another partly metamorphosed larva represented in fig. 11. Here a comparatively spacious cavity (*cir.c.*) surrounding the gut and filled with the corpuscles, represents the ring vessel from which the tentacular vessels are given off, though this is not shown in the figure. In the above two figures the adult cavity distinctly appears as a narrow space (*s.c.c.*) outside of the ring vessel (*cir.c.*). In these stages of the metamorphosis, the both dorsal ends of the adult collar cavity become continuous with each other so as to form a completely circular space above the postoral septum.

I am still uncertain as to how this rudiment of the ring vessel comes into communication with the dorsal vessel or with other vascular spaces which make their appearance during metamorphosis, for it is almost impossible to obtain larvæ of intermediate stages in which this vascular communication is just becoming established. But from observations on metamorphosing larvæ I have obtained certain suggestions respecting this process.

III. Metamorphosis.

As to the external changes of the larval body accompanying metamorphosis, I have scarcely anything to add to the exact and detailed descriptions given by METSCHNIKOFF and WILSON. I will, therefore, confine myself mainly to some anatomical points which have been less studied by previous observers. My observations of the metamorphosis were mostly made with the larvæ of types *A* and *B* which were most abundant in the neighbourhood of the Station. Sometimes I have observed under the microscope the whole course of the phenomenon, the duration of the so-called critical moment being usually not more than 15–25 minutes.

Among the the material obtained with the surface-net we often find larvæ which carry about the partly evaginated pouch, but these individuals can not be said to be undergoing metamorphosis in the strict sense of the term, for they may continue the free swimming life for several days after capture. Besides, they do not show any remarkable change in the internal organs. In them the corpuscles are still in masses, the nephridia preserve their original form and position, while the alimentary canal is of the ordinary form and length.

When the metamorphosis takes place, the partly evaginated pouch protrudes suddenly outwards to its full extent and the alimentary canal is thrown into convulsive contractions. Meanwhile the latter, especially its œsophageal and intestinal portions, is drawn out into a long tube, and at the next moment the junction of the stomach and the intestine is first of all pushed into the now spacious pouch. Thus, the anus and tentacular region are brought into close approximation on the dorsal line of the trunk; the digestive canal folds back on itself into an U-shaped tube, as we find it in the adult. The larval tentacles and the preoral lobe are cast off and digested in the stomach; the perianal ciliated belt atrophies *in situ*. It is during this moment that the corpuscular masses break away and their elements are scattered in the blood vessels, some of which are then being formed. It is highly interesting to observe the breaking up of the corpuscular masses, and the motion of the corpuscles in consequence of the rhythmical contraction and expansion of the dorsal vessel and of the cœcal capillaries. The deep pinkish colour of the corpuscles makes it easy to observe their progress into the vessels. Ordinarily after about twenty minutes the vascular system of the worm is completely formed and the circulation characteristic of *Phoronis* can be noticed. The skin of the foot which now forms the principal part of the body, becomes more opaque on account of the secretory products from the innumerable unicellular glands.

To understand the details relating to the metamorphosis we must examine the animal in sections. Figs. 64 (*a–d*) show a discontinuous series of cross sections of the larva represented in fig. 11, which is approximately at the critical moment of metamorphosis. I will briefly sketch with the aid of these figures the general internal change during metamorphosis.

Entoblastic Organs. When the ventral pouch is fully evaginated and the stomach is pushed into the pouch, the œsophageal portion elongates downwards enormously, so that the stomach diverticulum descends far below the postoral septum, that is to say, into the infraseptal cavity (fig. 64 *c*, *div.*). The vacuoles in the cell of the diverticular wall disappear at this period, and the diverticulum itself is immediately afterwards wholly obliterated, probably as the result of a histological atrophy. The stomach wall does not essentially differ in structure from that of the larva.

Mesoblastic Organs. Among the mesoblastic organs the vascular system undergoes most noteworthy changes. MASTERMAN has maintained that this forms a completely closed canal system even in the free swimming stage of the larva. So far as I have been able to ascertain, the closing up of the vessels into a continuous system occurs after the critical moment of metamorphosis. Having already described my own observations respecting the origin of the ring vessel of the adult, I will now describe other vessels which arise during metamorphosis.

Fig. 64 *a* shows a cross section of the foot near the posterior extremity where the alimentary canal is bent upon itself. Here we see the cut ends of three contractile capillaries (*v.c.*) and three sinuses (*s.s.*) in the stomach walls. A comparatively wide space (*d.v.*) is found intercepted between the two limbs of the alimentary canal. This space corresponds to the most posterior portion of the dorsal vessel, which, if traced further posteriorly, shows itself to be continuous with both the capillaries (*v.c.*) and the sinuses (*s.s.*). A short distance more anteriorly, the dorsal vessel divides into two parts each of which attaches itself to a limb of the alimentary canal; still more anteriorly the branch on the intestine disappears. In that portion of the stomach which lies close to the œsophageal tract, the dorsal vessel becomes enveloped with a thick muscular sheath which we have before seen in *Actinotrocha* (fig. 64 *b*, *d.v.*). The vascular sinuses gradually tend to unite into one common space lying on the ventro-lateral side of the gut (fig. 64 *b*, *s.s.*; fig. 64 *c*, *v.v.*). At the place where the degenerating stomach diverticulum still persists, the sinuses completely blend together into one large blood vessel corresponding to the ventral vessel of MASTERMAN (fig. 64 *c*, *v.v.*). This vessel acquires a definite form at a more anterior region of the œsophagus, becoming lined on its sides with a thin mesoblastic wall (fig. 64 *d*, *v.v.*). At about this level the dorsal vessel (*d.v.*) on the œsophagus becomes a small canal, such as we know it to be in the adult animal. According to my observations, the large ventral vessel opens at this stage not by two branches, as is the case in the adult, but by one directly into the ring vessel. To my great regret, however, I have not been able to study microscopically the larvæ in which the communication between the ring vessel and the dorsal or ventral vessel was in the process of being established.

Now we see that the dorsal vessel of *Actinotrocha* corresponds to the afferent, and the ventral vessel to the efferent vessel of the adult. The sinuses around the stomach, which have newly arisen during metamorphosis, develop into the complicated organ of the adult.

Nephridia. At the stage when the metamorphosis takes place, the nephridia do not show any important alteration in form and structure from those of the swimming larva; the excretory cells (*exc.c*) are found still attached to the blind end of the nephridial canal (fig. 64 *f*, *nep.c.*) and the external nephridial pores open behind the septum (fig. 64 *e*, *nep. o.*). In fig. 64 *e* we notice only that the nephridia as a whole have shifted to a more dorsal position than that occupied in the preceding stages (compare with fig. 50 *c*). This shifting of position becomes more and more marked as the metamorphosis advances, so that when the process is nearly finished, the nephridia on both sides come close to the so-called anal ridge on the dorsal side. Thus we see in fig. 66, which shows a transverse section through the nephridial region of a completely metamorphosed larva of type *A*, that the two nephridial canals (*nep.c.*) are situated very close to the intestine (*int.*) which lies in the dorsal median line, and that one of them (the right in the figure) opens to the exterior. By examining serial sections it was found that the excretory cells were entirely absent on the nephridial canals, and that the latter were of an inconsiderable length, ending blindly at the inner extremities. It seems very probable, as was pointed out by CALDWELL, that the excretory cells of the larval nephridia are thrown off into the body-cavity; it is also probable that that portion of the nephridial canal, which lies in the collar cavity of *Actinotrocha*, is obliterated, since, as we see in fig. 66, the inner end of the canal (the right in the figure) lies wholly outside of the septum (*mes.*), that is to say, in the trunk walls, as is known to be the case in the adult. Thus we may assume that the formation of the infraseptal nephridial funnels of the adult is due to secondary outgrowths of the infraseptal portion of the atrophied, larval nephridial canals.

Ectoblastic Organs. As CALDWELL has correctly observed, the larval tentacles and the greater part of the preoral lobe are torn off and are digested in the stomach. I have very often met with the remains of these organs in the interior of the latter (*R*, fig. 64 *a*). Although I have said, in agreement with CALDWELL, that the greater part of the preoral lobe is cast off, yet I can not agree with the view entertained by several authors, that the epistome of the adult developes from the remnant of the preoral lobe of the larva. Because, according to my studies, the nerve ganglion of the larva, which nearly marks the posterior limit of the lobe, can not at all be discerned in the larva at such a stage as is represented in fig. 11. It is no doubt thrown off together with the other parts of the preoral lobe. Besides, it is equally true that the epistome can not be found in the neighbourhood of the larval mouth at such a stage, while, on the other hand, in the worm after metamorphosis the rudimentary organ is seen as a small bud on the dorsal median margin of the mouth.

As before noted, the ring nerve of the adult is not yet formed in the swimming larva. This nerve and the so-called brain of the adult are of new formation; and the complicated nervous system which had developed only in the preoral lobe, suffers the same degeneration as that larval organ. In fig. 64 the ring nerve (*r.n.*)* is seen in section, just exterior to the septum (*mes.*). It consists of a thick layer of very fine nerve fibres.

IV. Supplementary Notes.

J. Müller ('46), the first discoverer of *Actinotrocha*, described the animal as an adult worm under the name *Actinotrocha branchiata.* The ventral pouch was considered by him to be the sexual organ. Doubts were afterwards thrown on his views by Krohn ('54); and they were finally refuted by Schneider ('62), who maintained that *Actinotrocha* is the larval form of a certain Gephyrea. This idea was confirmed by Kowalewsky ('67) who ascertained that *Actinotrocha* is the free swimming larva of *Phoronis.* Since this renowned discovery of Kowalewsky numerous papers on the anatomy and development of *Phoronis* have been published by many celebrated naturalists. But the singular fact is that the life history of the animal has not been subjected

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[*] Unhappily the letter *r* is misprinted in the plate as *v*.

to a detailed study. The metamorphosis of *Actinotrocha* is, of course, one of the most curious phenomena in the animal ontogeny. But the question which interested me almost to the same extent was: how do the free swimming larvæ come to establish colonies at such fixed and limited spots as are found in the Aburatsubo inlet? Accordingly I made several visits to the Misaki Station solely for the purpose of obtaining clues to the elucidation of this point. The results obtained are, I think, worth mentioning.

As I have before stated, the breeding season of *Phoronis ijimai* ranges through about one half of the year, say, from November to June or July, during which months the swimming larvæ, though few in number, are constantly found in Aburatsubo. They, are, however, most abundant from the middle of July to the middle of August. Among these larvæ some are very young, having apparently just swum out of their birth-place; but the majority of them are fully grown.

On July 16th., 1898, I visited the place where *Phoronis ijimai* flourishes, to see if it was still in possession of embryonal masses. But these could no longer be found in the tentacular coils of the mother animals which were, however, in the normal state.

On the 22nd. of the same month, I went again to the same place, and every thing was in the same condition as before.

On August 6th. I visited the place for the third time and to my astonishment I discovered that the animals had all died off. Several deformed colonies were brought back to the Laboratory and were kept in an ærated aquarium. On a close examination of these colonies after a few hours, nearly all of the chitinous tubes were found empty, only a few containing the putrefying remains of the animal body. When I reexamined the colonies in the aquarium on the next morning, I found that some younger animals had attached themselves to the tip or inner-side of the tubes of the departed generation; no doubt they had been hiding themselves somewhere in the colonial masses on the previous day. Most of these young worms measured 1–2 *c.m.* in length.

I had the same experience in the summer of 1899. On August 2nd. I visited the place and dived under the ledge of rock where the colonies had formerly flourished; but I could obtain nothing but some decaying masses of the tubes which emitted a disgusting odour.

Judging from these facts, it seems to me not improbable that *Phoronis* annually changes its generation.

As to the formation of colonies of *Phoronis*, it may be supposed that the putrescent remains or a certain fluid secreted by the adult act on the larvæ as a chemotropic reagent. But this can scarcely be admitted as taking place in the wide and open sea. I think, on the contrary, that this phenomenon is not to be attributed to such a complex cause, but is to be regarded merely as an accidental matter. The colonies of *Phoronis ijimai* form a compact and rigid mass together with some Ascidians and Molluscan shells, and adhere very tightly to the rocks; so that, when once the animals form a colony in a suitable place, it may well be assumed that they become gradually luxuriant. But this is not really the case in Aburatsubo where the colony has remained almost the same in size for several years. I think, what takes place must be somewhat as follows: the places where the *Phoronis* colonies are established year after year, must naturally be well adapted to the life conditions of the worm, and when a large number of larvæ is metamorphosed, as must be the case, during the above mentioned months, those larvæ that happen to attach themselves to the tubes of the already formed colonies, flourish and attain full growth; on the other hand, if the larvæ become attached at some unfavorable places, they must soon be washed off by waves and many of them must perish before they can find other suitable places. To this wasteful death of the larvæ which have lost the opportunity of finding suitable localities to grow on, must be due the fact that they remain comparatively stationary in the number of colonies and in their distribution.

Specific Position of Phoronis ijimai OKA. According to CORI's table, there are 7 known species of *Phoronis.* But I can not refrain from entertaining serious doubts as to the correctness of the present mode of classifying the *Phoronidæ* in general. Most of the systematic data have hitherto been taken from the external characters of the animals, such, for instance, as the colour and size of the body, the number of tentacles, the general form of the colony, etc. The question now is whether these external characters are constant and can be depended upon for systematic use. Is there not a necessity for taking internal anatomical points into our consideration? According to my observations, *Phoronis* annually changes its generation and about one half of the year belongs to the growing period. Specimens collected during this growing season must necessarily differ from the adult in the breeding season in the number of tentacles, in the length and size of the body, *etc.* Wide discrepancies are therefore found between OKA's observation and mine on the same species, *viz.*, *Phoronis ijimai.* I have no doubt that OKA made use of only the younger individuals as will be obvious from the following comparison:

	Body length.	Number of tentacles.	Length of tentacles.
Oka	40 *mm.*	150 (aver.)	2 *mm.*
Ikeda	60–100 *mm.*	200–210 (aver.)	5 *mm.*

I think it possible that the distinction made between other species may rest on a similarly unsound basis.

Moreover there is another no-less important point to be considered. Soon after the report on *Phoronis buski* by Mc'Intosh ('88) was issued, Blaxland Benham published his paper ('88) on the anatomy of *Phoronis australis,* in which he ascertained and rectified many important points which had been till then but incompletely known. Among these the following two are the most remarkable.

(1) Afferent and efferent blood vessels open respectively into the recipient and the distributing vessels which run parallel and form so-called ring vessel. Each tentacular vessel is connected at its basal end not only with the recipient but also with the distributing vessel.

(2) Each nephridial tube communicates internally with the infraseptal cavities by means of *two* funnels. One, the smaller, opens into the lateral chamber, while the second is considerably larger and opens into the rectal chamber.

It is stated by Benham, that "Mr. Caldwell dealt only with the larger of the two funnels, in his 'preliminary note,' but he informed me by letter that he became aware of the existence of the second funnel, shortly after the publication of his paper." How is it now with the ring vessel in *P. kowalewsky*? Caldwell says nothing about it. Cori, on the contrary, denied the above characters for *P. psammophila.*

In *Phoronis ijimai* I have ascertained that these two structures are the same in every point as in *P. australis.* Fig. 62 *a* shows a dorsal portion of a transverse section through the septum; two nephridial canals (*nep.c.*) are seen one on each side of the intestine (*int.*) and are partly imbedded in the chondroid tissue of the septum (*mes.*). These canals open by funnels (*f.*) into the body-cavity (rectal chamber) which separates the intestine (*int.*) from the œsophagus (*œs.*). These funnels correspond to the larger funnels of BENHAM, so that in a longitudinal section they appear as long ciliated cell-masses running longitudinally along the lateral mesenteries. If we trace the funnels a little downwards, we find another kind of funnels on the opposite sides of the lateral mesenteries, opening into the lateral chambers. They are represented in fig. 62 *b* (*f.'*), which is taken from the right nephridium. They are short indeed and are apt to be overlooked.

Again as to the ring vessels, they are found always as two concentric loops (the recipient and the distributing) standing side by side, and, as was described by BENHAM, each tentacular vessel receives two small branches respectively from the two vessels. Besides, in *Phoronis hippocrepia*[*] which is known from Ilfracombe, I have ascertained that the above cited structures (the nephridia and the ring vessels) are indubitably present without any modification.

If these structures can not really be found in *Phoronis psammophila,* similar specific anatomical deviations must exist in other little studied species, for instance, in *P. ovalis, P. gracilis, P. buski,* and so on. From these facts and from the variability of the external characters, I am at present unable to discover points by which *Phoronis australis, P. hippocrepia,* and our *Phoronis* can be differentially diagnosed.

Tokyo Imperial University,
Science College.

October, 1899.

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[*] For the opportunity of investigating this species, I am much indebted to Prof. Yasuda of the Second Higher School who kindly gave me a small portion of a colony.

Postscript.

While the manuscript of the present article was undergoing revision, I was much pleased to read ROULE's elaborate work on the development of the *Phoronidæ.*[*] For the sake of brevity I will not here discuss the author's theoretical considerations, but will offer some remarks with regard only to his investigations relating to developmental facts. Some of his results differ considerably from mine and from those of previous observers. And the differences are such as do not seem to be due merely to different conditions in the species investigated (*Phoronis sabatiēri*).

According to ROULE's observations, the first four planes of segmentation are all vertical and radial, the fifth being the first that is horinzontal, thus giving rise to 16 blastomeres; and the egg is composed of two sorts of larger and smaller cells from the first cleavage. This differs considerably from the account given in the preceding pages, which is in agreement with the studies of FOETTINGER and MASTERMAN (1900). It is, however, very difficult to decide which of the two opinions is correct or whether both are correct. I am at present rather inclined to the latter view. As to ROULE's belief in the peculiar unequal segmentation, which is said to return soon after to the equal, I fear that the eggs dealt with by the author may have been somewhat premature. I have often observed premature eggs undergoing a remarkable unequal segmentation when mixed in water with spermatozoa.

With respect to the nature of *plasmic corpuscles*, ROULE's view is certainly identical with that of CALDWELL, though he does

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[*] Etude sur le development embryonaire des Phoronidiens.—Ann. d. Sci. Nat. Zool., T.XI, No. 1-6, 1900.

not refer to the latter author. In examining Roule's figures (31 and 32), certain nucleated cells are seen dispersed in the blastocœlic cavity. They are said to be the inner ends of some elongated blastomeres. In the embryos of *Phoronis ijimai,* similar processes are indeed discovered protruded from some blastomeres, but they nerver contain the nucleus in their distal or inner ends: the nucleus belonging to these elongated blastomeres is situated also peripherally as in other normal blastomeres. The plasmic corpuscles which have, as I have described in the present paper, arisen from subsequent fragmentation of certain elongated blastomeres, exist very distinctly as separate bodies dispersed in the blastocœlic cavity.

In his present paper Roule reiterates his former views about the dual origin of the mesenchyme-cells. Upon this point I have already given my own ideas, and here I have to add only the following remark:

Though Roule, like Schultze ('97) has regarded, the nephridial pit as the origin of the ventral pouch of *Actinotrocha,* the subsequent development distinctly shows that the two structures are entirely independent of each other in their origin.

I can not refrain from doubting the correctness of Roule's observation that the larva studied by him possessed at no time during the swimming life any septal membrane in the body-cavities. They are structures which in the other forms of *Actinotrocha* have been so accurately demonstrated by previous observers. The technique employed by the author may perhaps be found to be faulty in this respect. The fine threads denoted by "brides mésentériques" in his figures (57, 75, and 97) seem to have arisen from the pieces of the otherwise continuous postral septum broken by the knife-blade in microtomizing.

ROULE has described the nephridia of the larva in a somewhat peculiar way. According to him, the organs lie considerably anteriorly as they are found on both sides of the stomach diverticulum, and are said to be constructed of cells forming a syncytial mass which is attached to the somatic walls. No lumen and no leading canal have been detected in these masses. But, if judged from the author's text and figures, it seems to me highly probable that his so-called nephridia correspond to the corpuscle masses of other writers. Having overlooked the postoral septum and the true corpuscle masses, he seems to have come to mistake the latter for nephridia. Thus, so far as I can understand his description, he did not notice the change in position of the organs with regard to the postoral septum during metamorphosis.

As to the number and the position of the stomach diverticulum, the larva of *Phoronis sabatieri* is described to be in the same condition as that of *P. ijimai*. But the vacuolization process of the organ is in the former species more complex than in the latter, though it is simpler than in the larvæ studied by MASTERMAN. When these three cases are considered together, it may be concluded that they indicate specific variations.

ROULE's "cordon dorsal," which is considered to show the rudimentary state of the rectum of the adult, has not been described by any previous author, and also could not be detected in any of the types of the larvæ studied by me. If this "cordon dorsal" be reconstructed from ROULE's text and figures, it seems to me almost without doubt that the structure referred to corresponds to the dorsal vessel on the stomach. When ROULE's figure (75) and mine (58 *c*) are compared, both of which show a cross section through the tentacular region at a similar level, it will be noticed that the "cordon dorsal" and the "vaisseau dorsal" in the former figure correspond respectively to the dorsal vessel and the trunk cavity (anterior portion) in the latter. Again, in regard to the alimentary canal, Roule states that the end portion of the intestine atrophies, but according to the observations of Caldwell and myself, no portion of the larval alimentary canal, except the stomach diverticulum, undergoes histolysis during metamorphosis, the entire tract growing gradually in length.

Roule's views as to the origin of the blood vessels greatly differ from those of Caldwell, Masterman and myself. According to Roule, the vascular spaces and the body-cavities are ontogenetically the same thing, and the former is formed from a coalescence of the irregular lacunal spaces of the latter. Though I agree with him in considering the ring vessel of the adult as a derivative of the body-cavity (collar) of *Actinotrocha*, yet I can not accept his view attributing other vessels to the same process. Besides, we see in his figures (82, 83, 86, 87, 88, etc.) that the same vessel (dorsal) is placed sometimes between the afferent, and the efferent, branches of the intestine, and sometimes on the somatic walls. Can such a peculiar disposition of the blood vessels against the skin be verified in the adult animal?

It may be known from Roule's contributions, that the larval development is more accelerated in *P. sabatieri* than in other species. So that the ventral pouch and some other organs are in that species already well developed even in a larva of 6 tentacles. But this seems of not much significance, since we know that even in the same type of the larvæ the progress of larval organisation does not always keep pace with the increase in the number of the tentacles.

Finally I can but refer here to MASTERMAN's new work (1900),* which I was able to read only a short time ago. His views differ so radically from those of previous authors and from my own, that I cannot fully discuss so weighty a matter in a brief postscript.

May, 1901.

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* On the Diplochorda, III, the early development and anatomy of Phoronis Buskii, *Mc'I.* Quat. Jour. Micr. Sci., Vol. 43, 1900.

* * *

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Postscript.

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List of Abbreviations.

an., anus.
ant.div., anterior diverticulum.
a.v., afferent vessel.
bl., blastopore.
bl.c., blastocœlic pore.
cir.c., ring vessel, *i.e.*, the collar cavity.
col., collar of larva.
col.c., collar cavity.
corp., blood corpuscles and corpuscle mass.
dig.a., digestive area.
div., stomach diverticulum.
d.v., dorsal vessel.
ect., ectoblast.
exc.c., excretory cells.
f. and *f'*. nephridial funnels.
f.n., female pronucleus.
gl., nerve ganglion.
gld., gland.
int., intestine.
m., mouth.
mes., postoral septum.
mes'., preoral septum.
Mes., mesoblast cells.
m.gl., mucous gland.
m.f., mesenchymatous fibres.
m.n., male pronucleus.
m.sh., muscular sheath of the stomach.
nep.c., nephridial canal.
neph., nephridium.
nep.o., nephridial pore.
nep.p., nephridial pit.
n.f., nerve fibre.
œs., œsophagus.
o.po., pouch pore.
p.b., polar globule.
p.c.c., larval collar cavity.
per., peritoneal epithelium.
per.bel., perianal belt.
pl.co., plasmic corpuscle.
po., ventral pouch.
pre.bel., preoral belt.
pre.c., preoral body-cavity.
pre.l., preoral lobe.
p.r., posterior recess of preoral cavity.
p.t., larval tentacle.
ret., retractor muscle in the collar.
ret'., retractor muscle in the trunk.
s.c.c., adult collar cavity.
s.o., sense organ.
s.s., sinus space.
s.t., adult tentacle.
stm., stomach.
t'., *t''.*, *t'''.*;......first, second, third larval tentacles.
tr., trunk.
tr.c., trunk cavity.
t.v., tentacular vessel.
v.c., vascular cœca or capillaries.
v.gr., ventral groove.
v.mes. ventral mesentery.
v.v., ventral vessel.

PLATE XXV.

Explanation of Figures.

Plate XXV.

Fig. 1.—Egg with two blastomeres. × 4 B (Zeiss).

Fig. 2.—Egg with three (*a*) and four blastomeres (*b*). × 4 B.

Fig. 3.—Egg with eight blastomeres, side view. × 4 B.

Fig. 4.—Egg with thirty two blastomeres, seen from the future ventral side. × 4 B.

Fig. 5.—Young morula, ventral view. × 2 D.

Fig. 6.—Ventral view of an advanced blastula in which the gastral invagination has become visible from the outside. × 2 D.

Fig. 7.—Ventral view of a gastrula, in which the blastopore has taken a triangular form. × 2 D.

Fig. 8.—Side view of an advanced gastrula, in which the blastopore has become a transverse slit. × 2 D.

Fig. 9.—Young *Actinotrocha* in which the nephridial pit is visible from the outside. × 2 D.

Fig. 10.—Larva of four tentacles (side view). × 2 D.

Fig. 11.—Metamorphosing larva of type *A*, sketched from a preserved specimen. × 4 A.

Fig. 12.—Larva of type *D*, sketched from a living specimen. Greatly magnified.

Fig. 13.—Larva (of 14 tentacles) of type *A*, in the living state. Greatly magnified.

Fig. 14.—Highly advanced larva of type *B*, bearing 28 tentacles, ventral view. Greatly magnified.

Fig. 15.—Larva of type *C*, bearing 20 tentacles. *a* represents a dorsal view, *b* a ventral view, and *c* the multicellular gland on the hood. Greatly magnified.

Fig. 16.—Larva of type *D*, bearing 48 tentacles, after preservation. Greatly magnified.

Fig 12

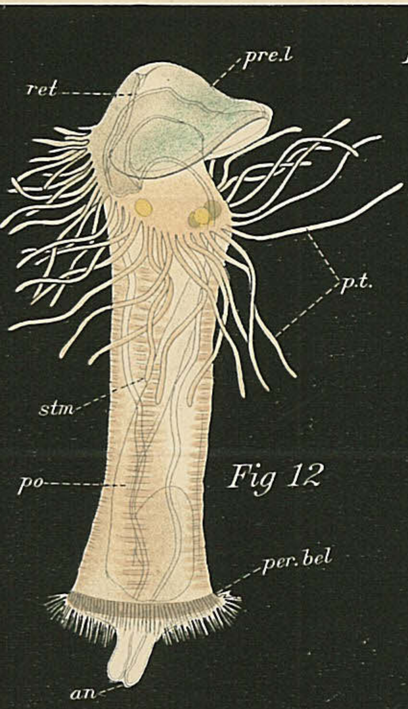


Fig. 11

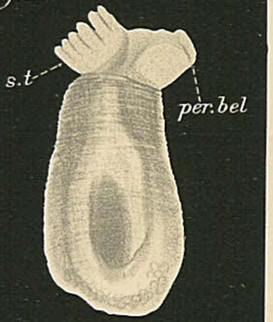
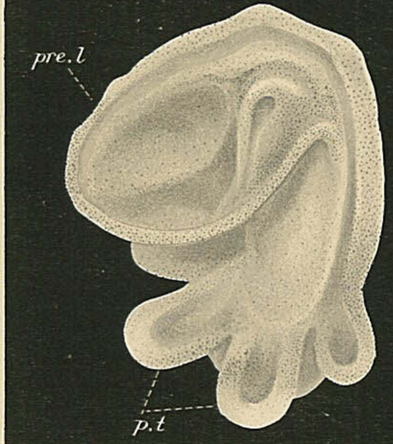


Fig. 7

Fig. 10



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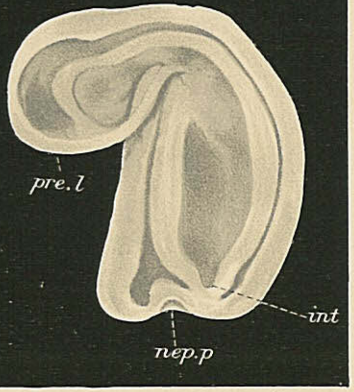
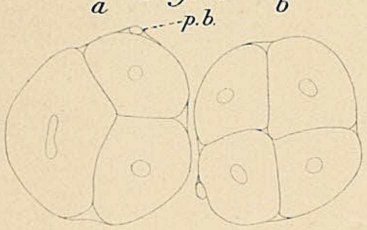


Fig. 2



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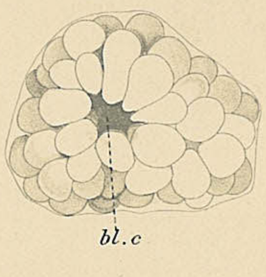

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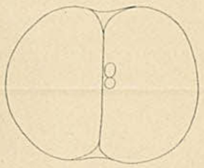

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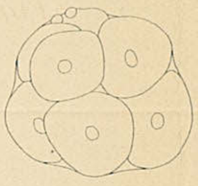

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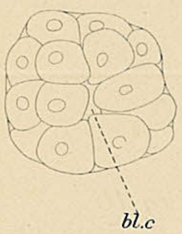

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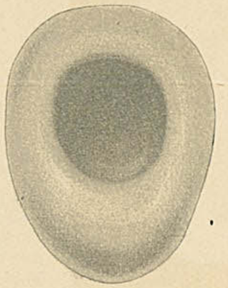

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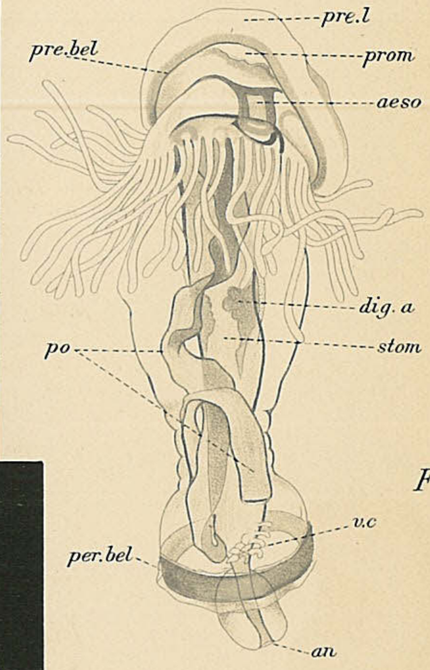

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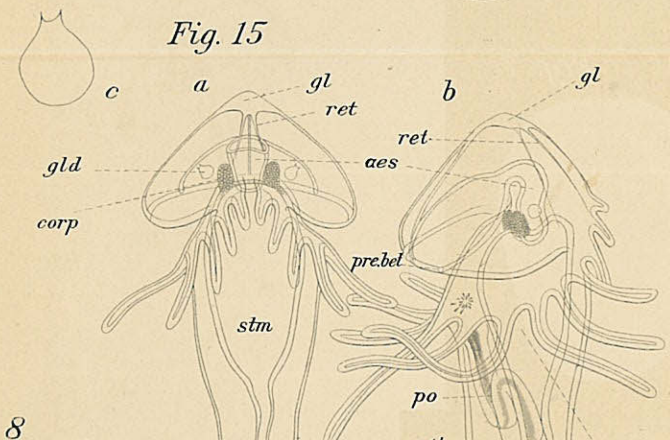

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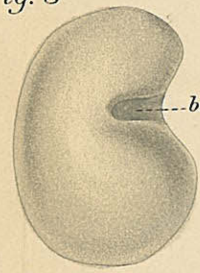

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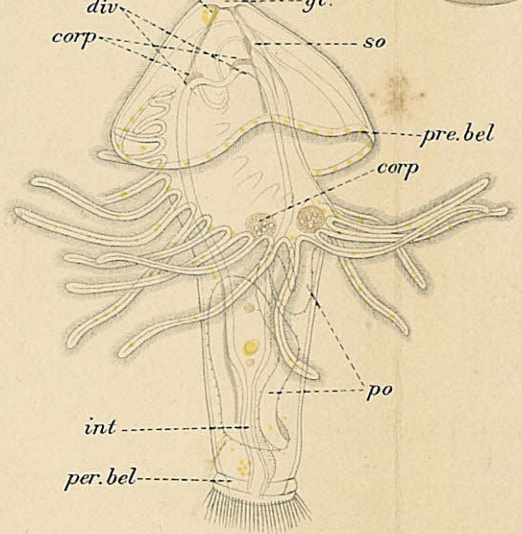

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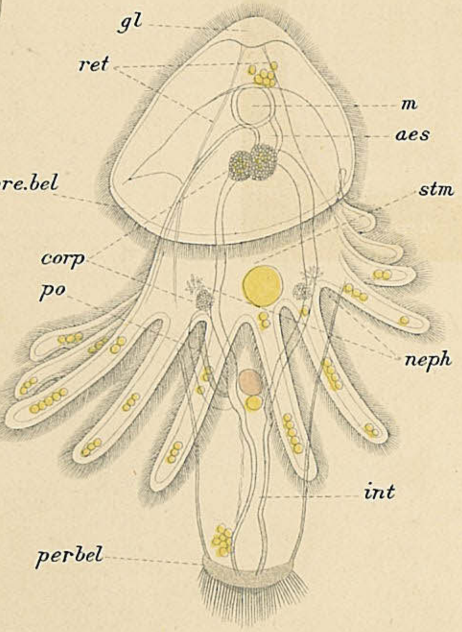

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PLATE XXVI.

Plate XXVI.

Fig. 17.—Primary oocyte, showing the karyokinetic figure for the first polar globule. × 2 imm. $^1/_{12}$.

Fig. 18.—Section through the equatorial plane of the karyokinetic figure for the first polar globule; the egg was preserved with sublimate solution (Winkel *oc.* 3. × *ob.* 8).

Fig. 19.—After emission of two polar globules (Winkel *oc.* 3 × *ob.* 8).

Fig. 20.—Section showing one stage of fertilization, when the two pronuclei (*m.n.* and *f.n.*) stand side by side. × 2 imm. $^1/_{12}$ (Zeiss).

Fig. 21.—Median section of fertilized egg, in which is found a karyokinetic figure for the first cleavage. × 2 imm. $^1/_{12}$.

Fig. 22.—Median section of a young morula. × 2 F.

Fig. 23.—Median section of a young morula, showing the blastocœlic pore (*bl.c.*). × 2 F.

Fig. 24.—Median section of a young blastula, in which one blastoderm cell is seen giving off plasmic corpuscle (*pl.co.*). × 2 F.

Fig. 25.—Median sagittal section of an advanced blastula; two plasmic corpuscles are detected in the segmentation cavity. × 2 F.

Fig. 26 (*a*, *b*).—Sagittal sections of a highly developed blastula, in which the invagination has just begun; *a* shows a median section. × 2 imm. $^1/_{12}$.

Fig. 27.—Median sagittal section of a gastrula in which the invagination is deeper. × 2 imm. $^1/_{12}$.

Figs. 28 (*a*-*c*).—Three transverse sections of an advanced gastrula; *a* through the central depression, *b* behind the central depression, and *c* near the posterior end of the embryo. × 2 F.

Fig. 29.—Median sagittal section of an embryo at nearly the same stage as in Fig. 8. × 2 imm. $^1/_{12}$.

Figs. 30 (*a*-*c*).—Transverse sections of an embryo at nearly the same stage as the preceding; *a* shows a portion (left) of a section through the blastopore, *b* just behind the blastopore and through the ventral groove (*v.gr.*), *c* near the posterior end. × 2 F, *a* with the tube drawn out.

Fig. 31.—Transverse section through the blastopore of a larva, in which the anterior diverticula (*ant.div.*) are well developed. × 2 F.

Fig. 18

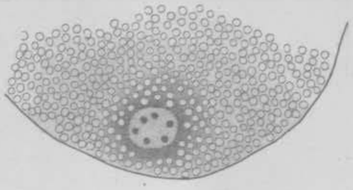
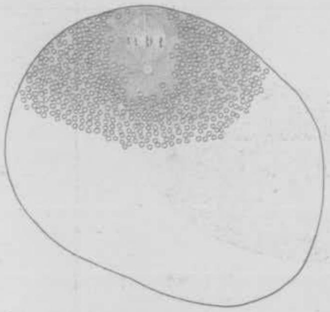


Fig. 17



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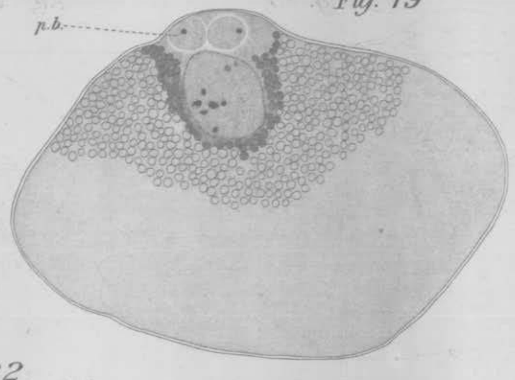
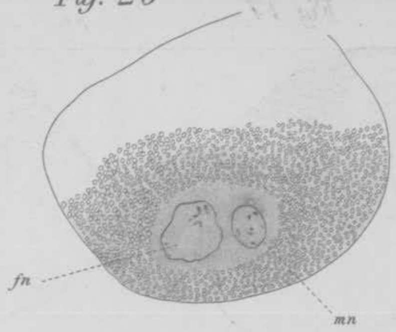


Fig. 20



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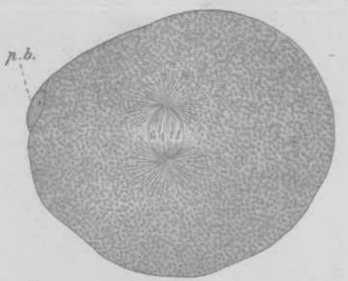
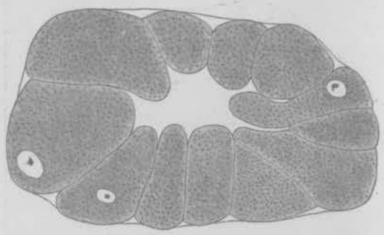


Fig. 22



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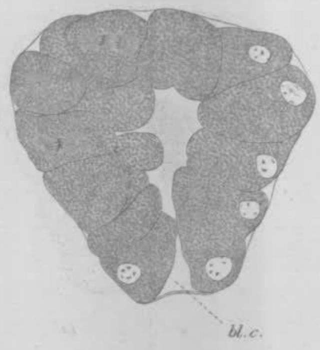

Fig. 26,a

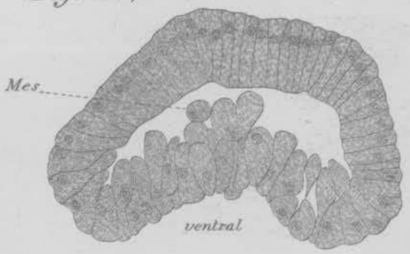

Fig. 24

Fig. 25

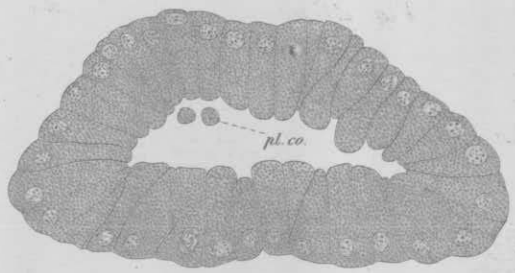

Fig. 26,b

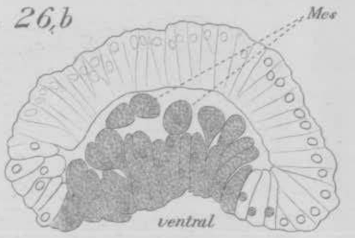

Fig. 30,a

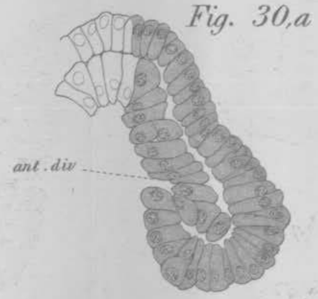

Fig. 27

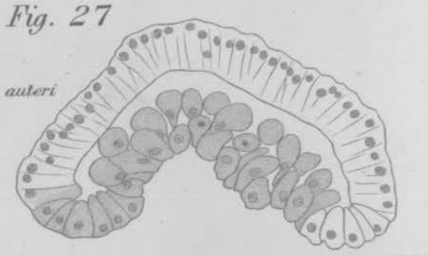

Fig. 28,b

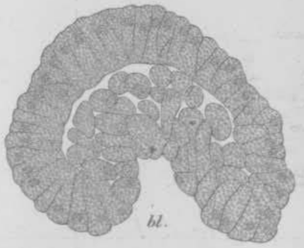

Fig. 29

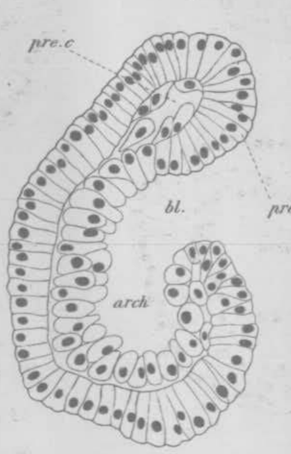

Fig. 30,c

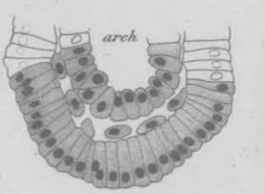

Fig. 30,b

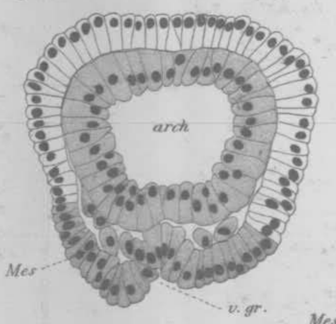

Fig. 28,a

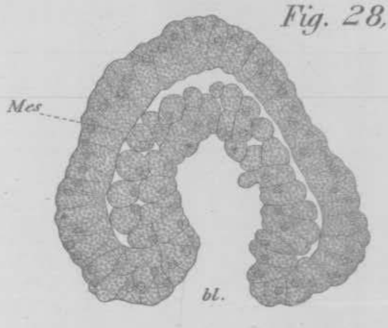

Fig. 28,c

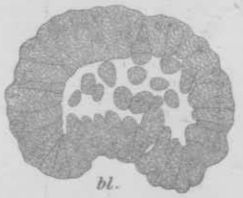

Fig. 31

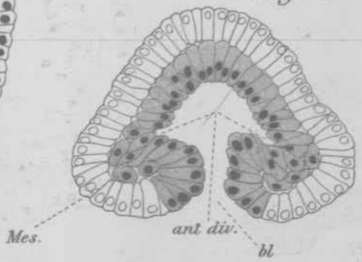

PLATE XXVII.

Plate XXVII.

Fig. 32.—Transverse section through the middle portion of a larva in which the ventral groove has ceased to give off mesoblast cells. × 2 F, with the tube drawn out.

Fig. 33. Slightly oblique sagittal section of a larva in which the nephridial pit (*nep.p.*) has made its first appearance. × 2 imm. $^1/_{12}$.

Fig. 34.—Oblique frontal section of a larva nearly at the same stage as in Fig. 9. × 2 F.

Fig. 35.—Transverse section through the nephridial pouch (*nep.p.*) as yet unpaired. × 2 F.

Fig. 36.—Oblique sagittal section of the nephridial pouch (*nep.p.*) partly divided. × 2 F.

Fig. 37 and Fig. 38.—Show respectively sagittal and frontal sections of larvæ, in which the proximal end of the nephridial pit is about to divide into two. × 2 F.

Figs. 39 (*a-c*).—Three transverse sections of the two nephridial canals, each of which has respectively an internal opening. × 2 F.

Fig. 40.—Sagittal section of a larva at the stage represented in Fig. 10. × 2 F.

Fig. 41.—Frontal section of a larva at the same stage as the preceding, in which the two nephridial pores have separated widely from each other. × 2 F.

Fig. 42.—Oblique frontal section through the nephridial region of a larva a little younger than the preceding. × 2 F.

Fig. 43.—Transverse section through the upper portion of the œsophagus of a larva of four tentacles. × 2 F.

Fig. 44.—Large mesoblast cells which are found in the body-cavity of the larva of two or four tentacles. × 2 F.

(*All the figures from fig.* 45 *to fig.* 55 *are drawn from larvæ of type A.*)

Fig. 45.—Median sagittal section of a larva of 12 tentacles. × 2 D.

Fig. 46.—Portion of a longitudinal section of a larval tentacle of 10-armed larva; two blood corpuscles are represented in the tentacular cavity (*corp.*). × 2 F.

Figs. 47 (*a-c*).—Three longitudinal sections of the nephridium of a larva of 12 tentacles. × 2 F.

Fig. 48.—Median frontal section of a larva of 16 tentacles. × 2 D.

Fig. 32

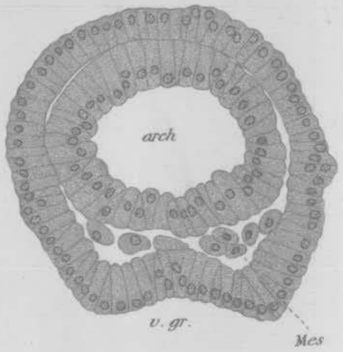

Fig. 33

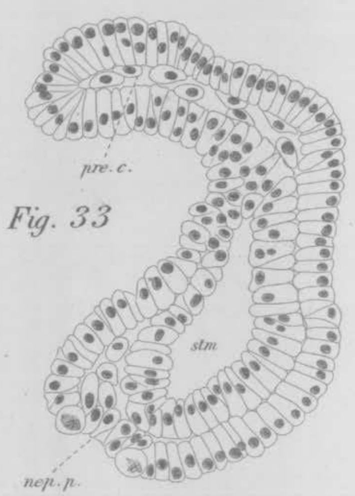

Fig. 36

Fig. 35

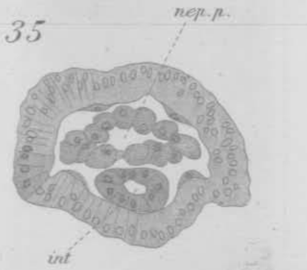

Fig. 37

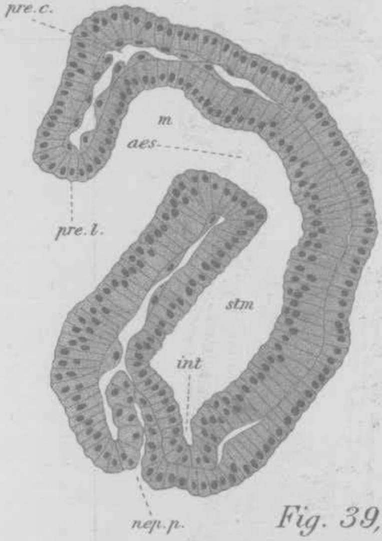

Fig. 38

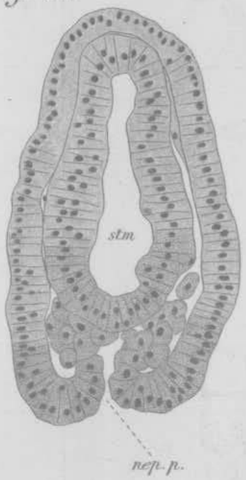

Fig. 34

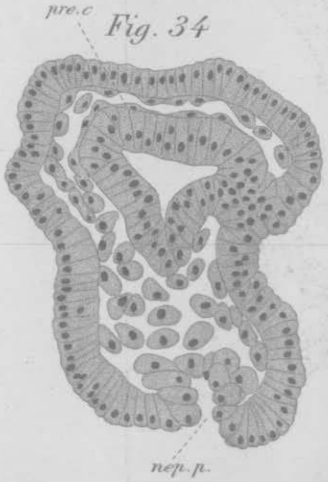

Fig. 40

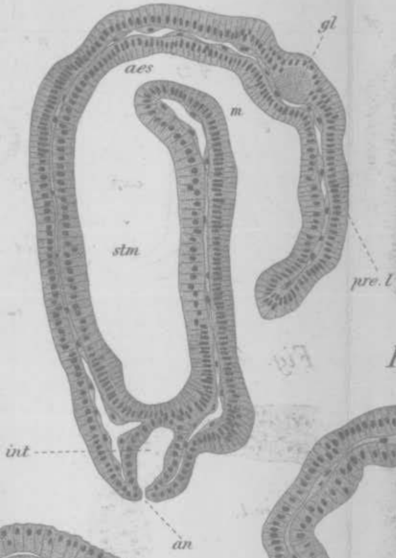

Fig. 39, a

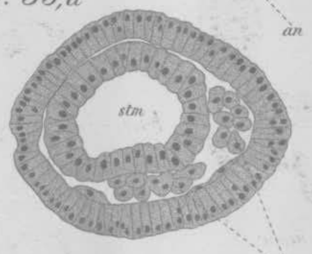

Fig. 39, b

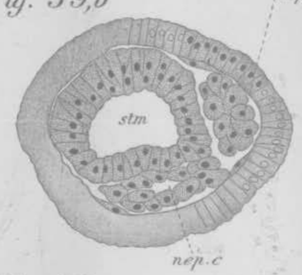

Fig. 39, c

Fig. 44

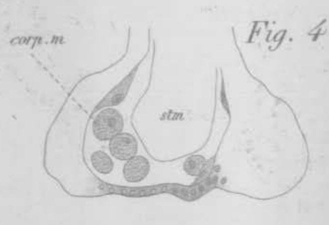

Fig. 46

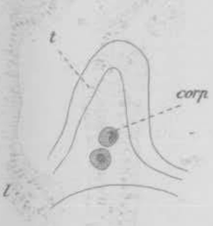

Fig. 41

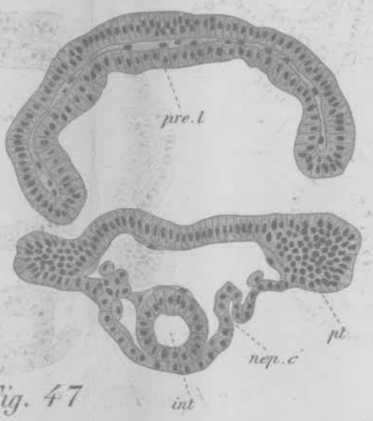

Fig. 47

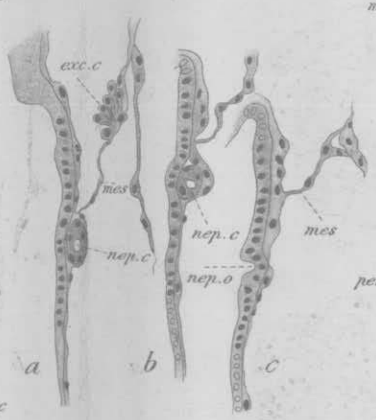

Fig. 42

Fig. 48

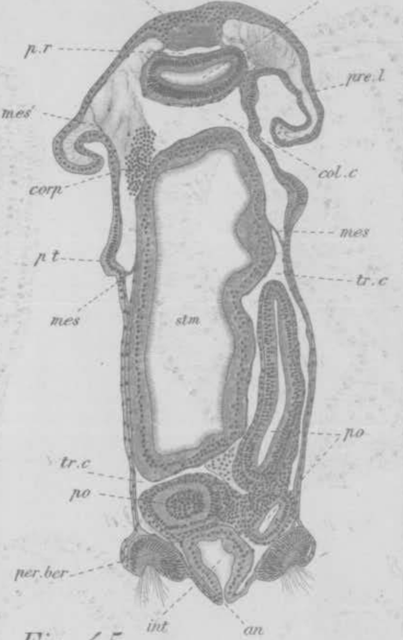

Fig. 45

Fig. 43

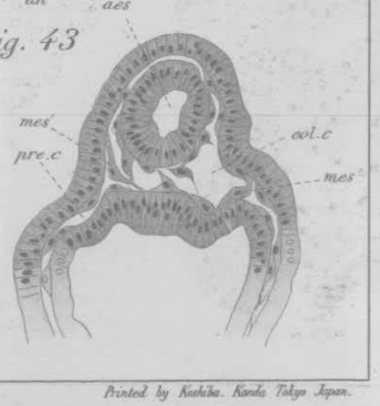

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PLATE XXVIII.

Plate XXVIII.

Fig. 49.—Anterior portion of a median sagittal section of a larva of 14 tentacles. × 2 F.

Figs. 50 (*a-c*).—Three transverse sections of a larva of 14 tentacles; *a* through the stomach diverticulum (*div.*), *b* obliquely through the postoral septum (*mes.*), and *c* above the pouch pore. × 2 D.

Figs. 51 (*a*, *b*).—Longitudinal sections of the nephridium of a larva of 16 tentacles. × 2 F.

Fig. 52.—Transverse section through the junction of the stomach and the œsophagus of a larva of 16 tentacles, showing the rudiment of the dorsal vessel (*d.v.*) × 2 imm. $^1/_{12}$.

Figs. 53.—Section of the above larva, through the corpuscular mass which floats in the collar cavity. × 2 F.

Fig. 54.—Sagittal section through the right side of the œsophagus of a larva of 16 tentacles. × 2 F.

Figs. 55 (*a* and *b*).—Cross sections of a larva of 16 tentacles. In the figure *a* is represented the right half of the section which passes through the stomach (*stm.*) and the adult collar cavity (*s.c.c.*); *b* through the junction of the stomach and the intestine, whereto the contractile cœca (*v.c.*) are attached. × 2 F.

(*Figs.* 56-57 *are drawn from larvæ belonging to type C.*)

Figs. 56 (*a-c*).—Cross sections of the hood, where the multicellular gland is represented in different planes. × 2 D. Letter *b* omitted.

Figs. 57 (*a-c*).—Cross sections through the trunk of a larva of 22 tentacles; *a* under the magnification of 2 × D; *b* a portion of the trunk walls containing the nephridial canal, magnified 2 × F with the tube out; *c* shows a portion of the ventral mesentery near the gut, magnified 2 × F.

Fig. 49.

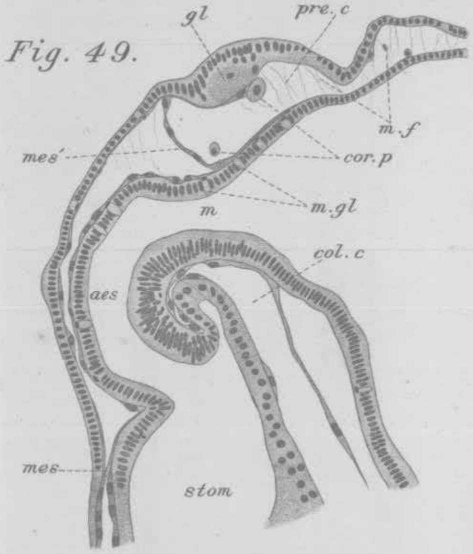

Fig. 51

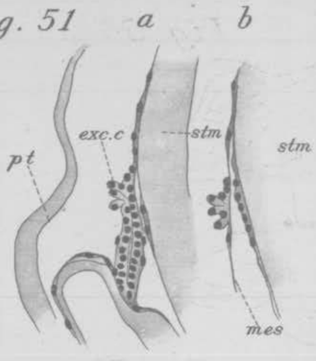

Fig. 52

Fig. 56 a

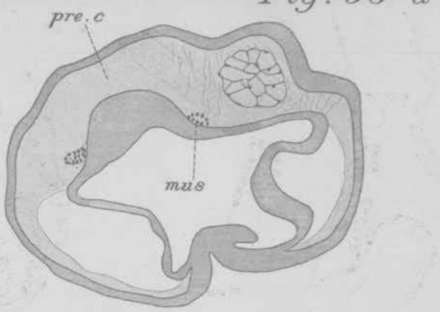

Fig. 56, c

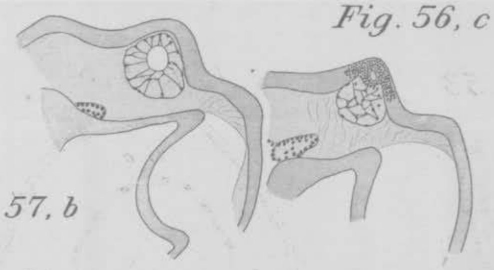

Fig. 53

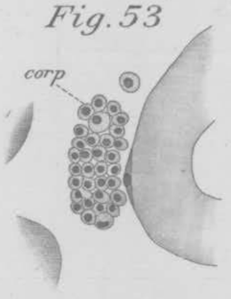

Fig. 55, b

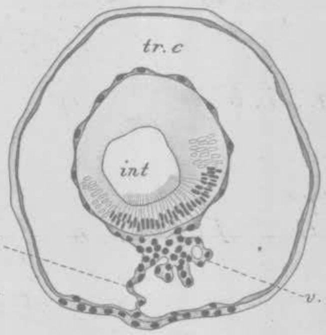

Fig. 57, b

Fig. 50, a

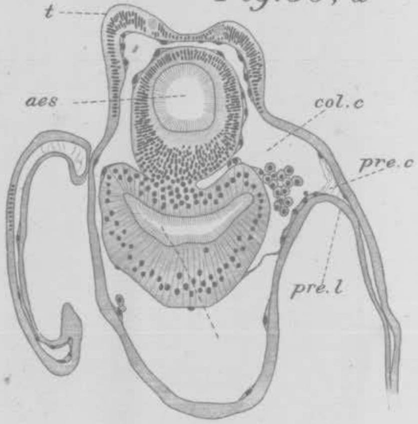

Fig. 50, c

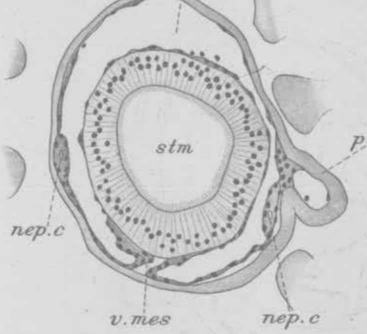

Fig. 57, a

Fig. 55, a

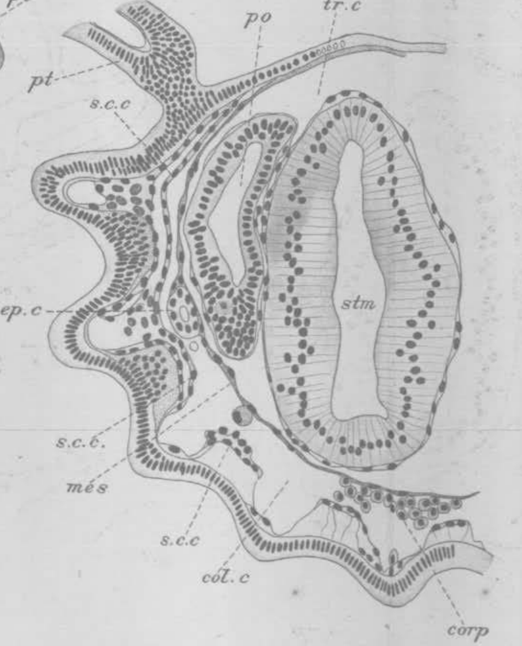

Fig. 50, b

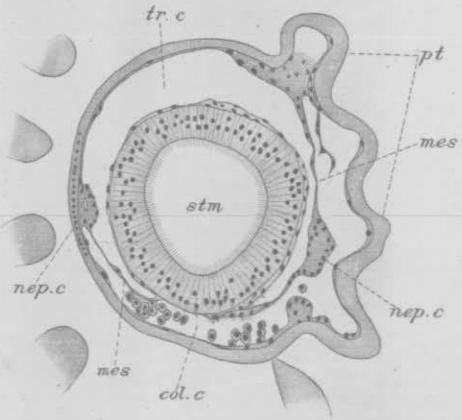

Fig. 54

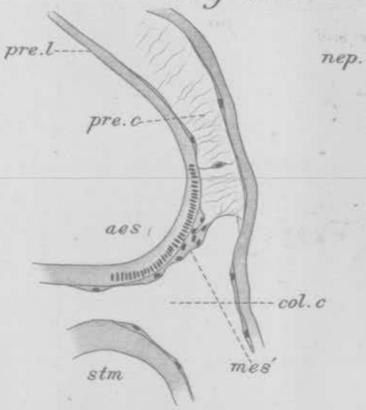

Fig. 57, c

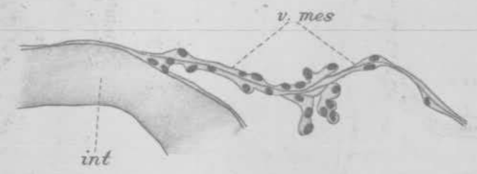

PLATE XXIX.

Plate XXIX.

(*Figs.* 58–60 *are drawn from larvæ belonging to type D*).

Fig. 58 (*a-e*).—These figures show different parts of a larva of 44 tentacles. Figure *a* shows a cross section through the mouth, *b* through the middle portion of the œsophagus (*œs.*); magnified × 2 B; *c* a portion of a cross section of the stomach wall (dorsal) and the trunk walls, × 2 F. *d* and *e* show respectively a longitudinal and a transverse section of a tentacle (the former magnified × 2 D, the latter × 4 D).

Figs. 59 (*a-d*).—Four cross sections taken from a series, not consecutive, and their respective planes of section are given in the text with reference to the woodcut (p. 542). Unfortunately in these series, the tissues have undergone a great disturbance by the killing reagent, but the relations of the layers remain essentially correct, and those spaces which have been produced from the mutual splitting of the layers are denoted by "artefact." × 2 D.

Figs. 60 (*a* and *b*).—Represent the nervous system of *Actinotrocha* (of type *B*), revealed by vital staining with methyl blue and ammonium molybdate. In *a*, as the larva was pressed by the coverglass, the rim which appears like the free margin of the hood is not that edge at all, but represents the line along which the hood was bent by pressure; the line drawn near the peripheral blue dots is the true edge of the hood. *b* shows a portion of the free margin of the hood, where the nerve fibres end. *a* × 4 B, *b* × 2 F.

Figs. 61 (*a* and *b*).—Are taken from serial sections of a *A*-type larva which bears the evaginated pouch ; *a* shows four blood corpuscles, *b* one portion of the wall of the stomach diverticulum. × 2 F.

Figs. 62 (*a* and *b*).—Taken from serial transverse sections through the nephridial region of the adult. *b* shows the inner and of the left nephridial canal where the smaller funnel (*f'*) is attached.

Fig. 58,a

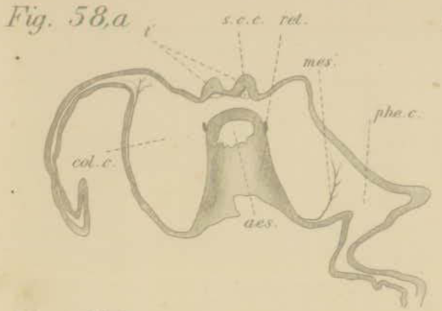

Fig. 59,d

Fig. 60,a

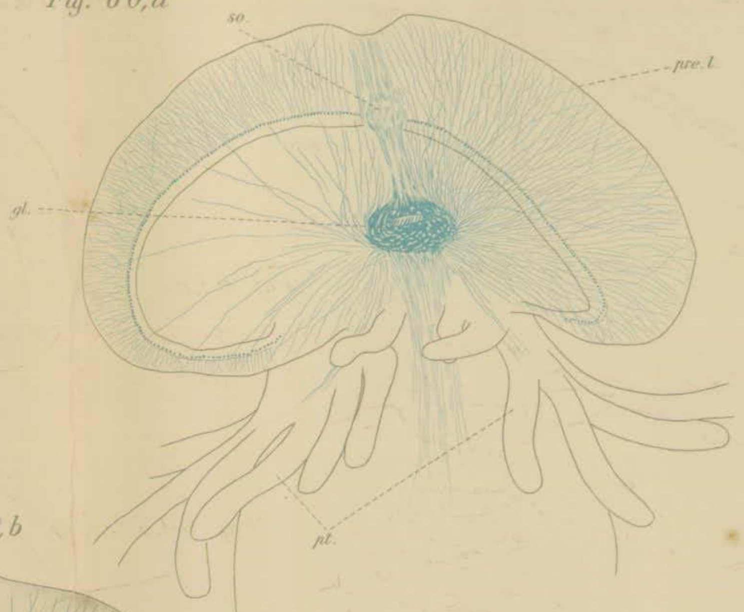

Fig. 58,b

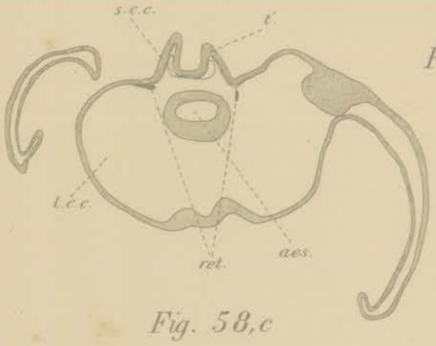

Fig. 61,a

Fig. 59,c

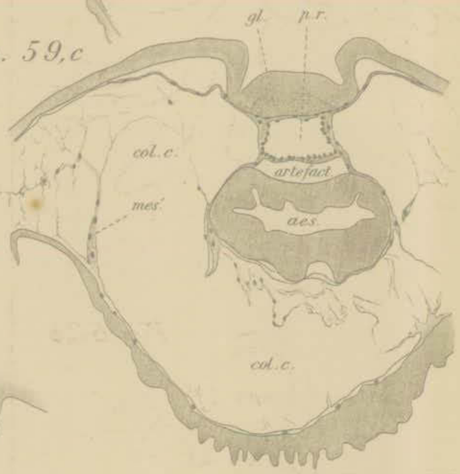

Fig. 60,b

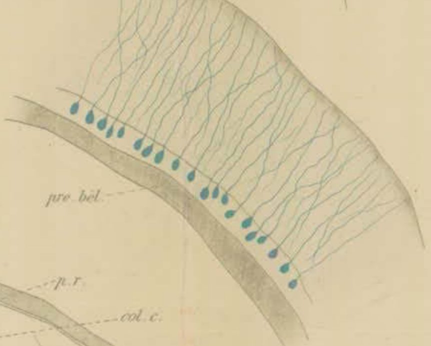

Fig. 58,c

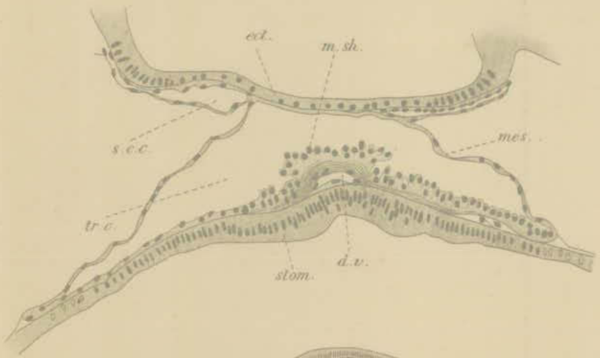

Fig. 62,b

Fig. 59,b

Fig. 58,d

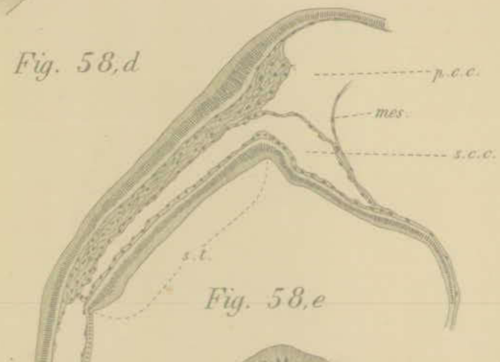

Fig. 62,a

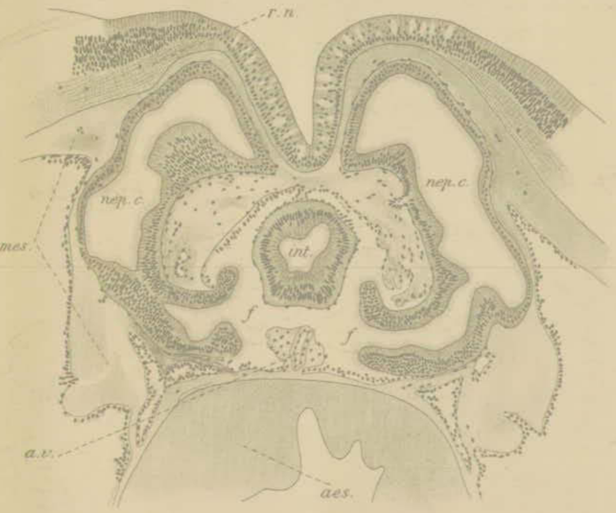

Fig. 58,e

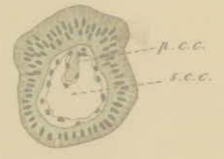

Fig. 61,b

Fig. 59,a

PLATE XXX.

Plate XXX.

Figs. 63 (*a*-*e*).—Are taken from several parts of serial sagittal sections of *B*-type larva of 28 tentacles.

a. median section through the hood and the collar. × 2 D.

b. more magnified figure of the nerve ganglion (*gl.*) and the posterior recess of the preoral cavity (*p.r.*) in the preceding figure. × 2 F.

c. taken from a section lateral to the œsophagus and to the right of that of the figure *a*. × 2 F.

d. ventral portion of the collar-trunk walls, where the septum (*mes.*) and the adult collar cavity (*s.c.c.*) are cut through. × 2 F.

e. portion of a section which passes through the second tentacle (t''). × 2 F.

Figs. 64 (*a*-*f*).—Transverse sections taken from serial sections of a metamorphosing larva of type *A* represented in fig. 11. The respective explanations of them are introduced in the text (p. 584). From *a* to *c* magnified as × 2 D, and from *d* to *f* as × 2 F.

Fig. 65.—Portion (right side) of a transverse section through the tentacular region of a metamorphosing larva of type *A*, slightly younger than that of fig. 11. × 2 F.

Fig. 66.—Transverse section through the nephridial region of wholly metamorphosed larva of type *A*. × 2 F.

Fig. 65

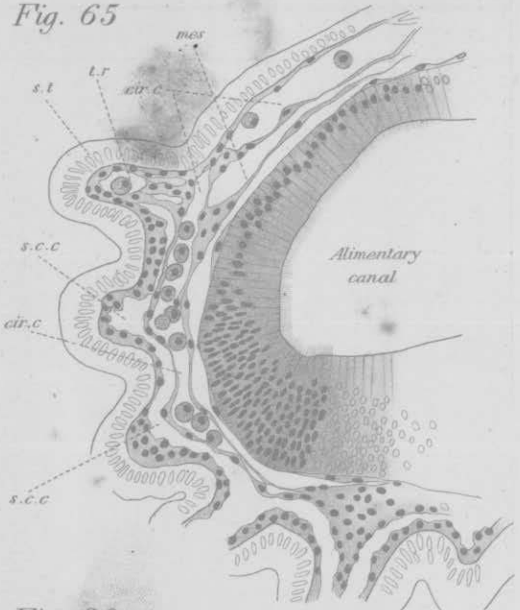

Fig. 63,a

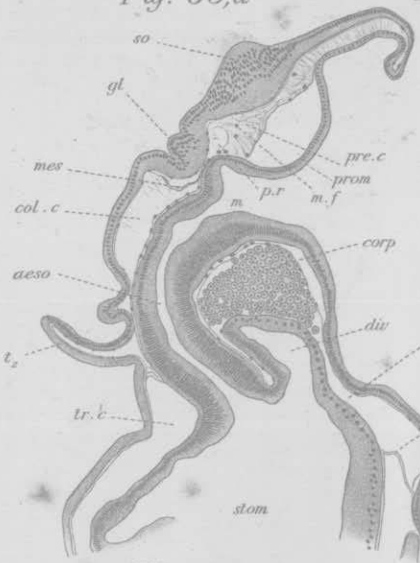

Fig. 63,b

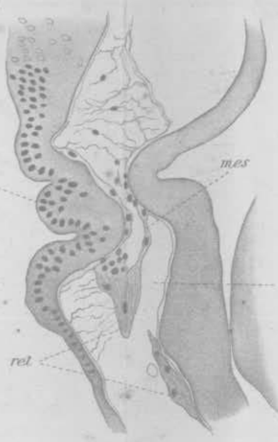

Fig. 63,c

Fig. 66

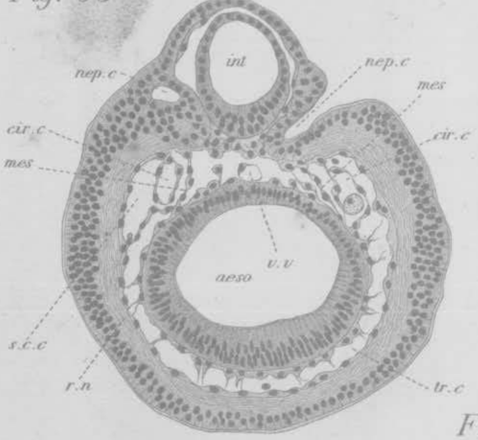

Fig. 64,b

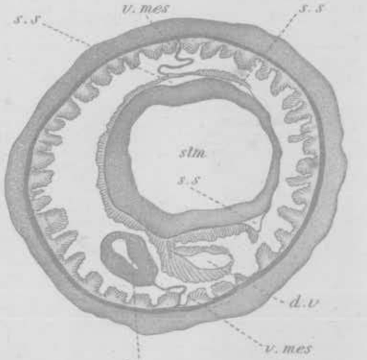

Fig. 64,f

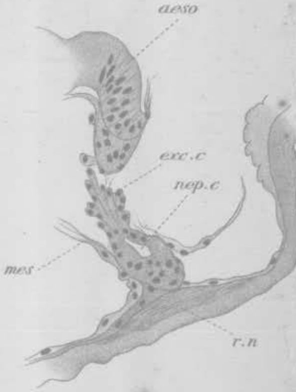

Fig. 63,d

Fig. 63,e

Fig. 64,a

Fig. 64,d

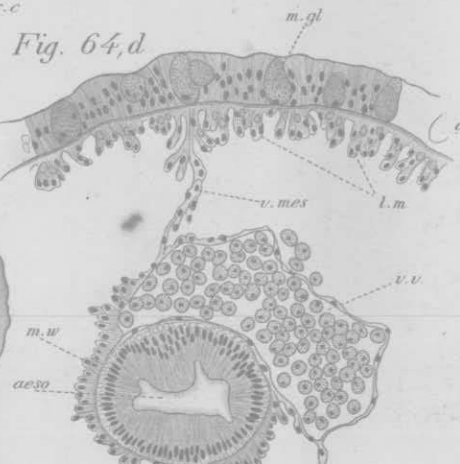

Fig. 64,c

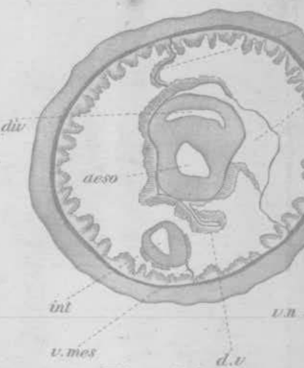

Fig. 64,e