IV. *On the Mitraria Larva of* Owenia fusiformis *Delle Chiaje.*

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[Plates 29–32.]

Contents.

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1. INTRODUCTION.

This work is the outcome partly of a suggestion made by Dr. E. J. ALLEN that the Mitraria larva might prove a fruitful subject for investigation, and partly from an interest in this creature aroused during student days when collecting at Port Erin and Millport. These hopes have been amply fulfilled, and as a result this paper is offered as a contribution towards the biology of one of the strangest of sea creatures.

The chief interest centres around the metamorphosis, cataclysmic in its rapidity and astonishing in the profound changes it brings about within a few seconds. In this respect a few other larvæ resemble it, the most familiar of them being the Actinotrocha, and more especially the endo-larva of *Polygordius lacteus*, so fully and carefully described by WOLTERECK (1902). Throughout the following pages frequent comparison will be made with this *Polygordius* larva, and it should be understood that unless otherwise stated, a reference to *Polygordius* means to the endo-larva of *P. lacteus*.

This paper is by no means an exhaustive account. The cleavage and very early stages have not been studied, and, as will become apparent later, these stages probably hold the key to the explanation of several puzzling facts. The study starts with the fully formed trochosphere and finishes with the young worm a few weeks after metamorphosis. Nor has it been possible during this period of the life-history to fill in all the details; size alone has been a great handicap when studying the smaller structures. The larva at its maximum size is very small—a mere half-millimetre across the comparatively large prototroch—and only an eighth the bulk of *Polygordius*. Failure to ascertain details is possibly as much due to inability to see with the highest powers as to faulty technique.

The terminology used for the parts and the spatial relations of the larval organs is based on WOLTERECK'S 1902 paper. Terms of position are used as far as possible in their true morphological sense, but the larva is so bent that it is difficult to adhere consistently to this rule. It is believed, however, that the reader will have no difficulty in understanding what is meant in each instance. In general a line joining the anus to the mouth runs in an anterior direction, as does one passing from the anus to the head, although the two are at right angles to one another. It is obvious which is the dorsal and which the ventral surface of the gut in spite of the flexure. That part of the prototroch in front of the mouth is, however, considered to be ventral, and the opposite side, behind the stomach, dorsal. The larval chæta-sacs are dorsal to the anus. The head and episphere are said to be above the prototroch ; the hyposphere, chæta-sacs, etc., below it. If the reader imagines the anus to be carried back well below the prototroch in the normal trochosphere fashion, any difficulties in interpreting these terms of position will vanish.

Few measurements of size are given in the text : the majority are presented in the description of the plates, where they are readily accessible if required.

It is with real pleasure that I have to thank Dr. E. J. ALLEN for his continued interest and encouragement throughout the research. My thanks are also due to many members of the staff of the Plymouth Laboratory for assistance or suggestions at various times, to Professor J. H. ASHWORTH and Miss S. M. MARSHALL for the loan of mounted specimens, to Professor H. GRAHAM CANNON for hints on technique, and to Mr. M. BURTON, of the British Museum (Natural History), who very kindly sent me a typewritten copy of FEWKE's paper (1888), together with careful tracings of his drawings, as I was not able to consult the original paper myself. Mr. F. A. POTTS kindly looked up a reference (GROBBEN, 1905) for me in the Balfour Library, Cambridge, this reference having been kindly supplied by Professor F. HEMPELMANN, of Leipzig. Finally, my most grateful thanks are due to my wife for assistance in various ways, particularly in preparing the manuscript for the press.

2. HISTORICAL.

Several species of Mitraria larvæ have previously been figured and described, but there is nowhere an accurate and detailed account of any one, and in most cases their identity is still unknown. JOHANNES MÜLLER, in 1851, was the first to give the name " Mitraria " to a larva he found at Marseilles and Trieste. He described, but did not figure it in his paper published that year. Three years later (1854), however, he re-described this larva and gave figures of it. It was a creature with finely spined, needle-like chætæ, and was probably closely allied to, if not the same species as, *Owenia fusiformis*. He also described and figured in the same paper another Mitraria, from Messina, with club-shaped bristles in addition to needle-like ones, both kinds bearing teeth. He mentioned also, but did not figure, a third species with spindle shaped bristles, from Marseilles. All his larvæ were apparently early stages, and, although he thought that they might be Annelids, he was puzzled by the nearness of the anus to the mouth. His suggestion that should Mitraria develop into a worm the anus must eventually be far removed from the mouth is, of course, borne out by subsequent work, although it occurs in a way he could scarcely suspect. CLAPARÈDE, in September, 1859, collected a Mitraria in Lamlash Bay, and briefly described it (1860). It was evidently the since frequently observed autumnal form of the Clyde (see p. 322). He did not use the term "Mitraria," and was apparently unaware of MÜLLER's work. He, too, thought it belonged to some Annelid. SCHNEIDER (1869), nine years later, obtained at Nizza a Mitraria which metamorphosed in a dish while he searched vainly for another. He thought the worm produced was a Gephyrean with a terminal anus: his figure of it has some resemblance to an abnormally metamorphosed *Owenia* worm. He noticed the cast-off provisional bristles, and some fragments of tissue which were probably bits of the prototroch. His incorrect theory of the metamorphosis is really no more remarkable than the actual truth. He had, however, been forestalled in his observation of the metamorphosis by KOVALEWSKY, who in 1867 had established the fact that Mitraria changes into a Polychæte, but had unfortunately only communicated his results verbally to METSCHNIKOFF. The latter refers to them in his paper published in 1871, a paper that has remained the most important account of the development of Mitraria up to the present time. He described and figured two species obtained at Spezia in May and June: both had needle-shaped bristles, and the first he considered to be identical with MÜLLER's Trieste larva. He got late stages of it, but none metamorphosed. The prototroch was not sinuous, as was the case with his second species. The latter metamorphosed, and, judging from his description and figures, was very probably *Owenia fusiformis*. His account is fairly accurate as far as it goes, and his figures quite good, especially when it is considered that he lacked the optical refinements and section-cutting methods of to-day. He saw the invagination of the trunk rudiment between anus and mouth, although he goes a little astray in believing the trunk in the latest stages to be merely bent on its ventral surface: he apparently did not realise that the anterior part was actually turned inside out. He saw the invagination to form the buccal organ, and he watched the metamorphosis. He does not give many details of the latter, but he saw the body straighten out, the prototroch crumple, and the long bristles fall off. Apart from this his account is rather vague. He mentions that the young worm soon secretes a tube for itself, which becomes covered with a layer of sand. He kept his little worms for about fourteen days, and considered that they belonged to the Clymenideæ (Maldanidæ). His figure of the young worm, however, resembles *Owenia fusiformis* so closely that there is little doubt but that that was the actual species with which he was dealing. It is true that his fig. 12A of the hook shows one tooth only instead of two, text-fig. 25B, C, but as it is so very small, and in certain positions a one-toothed appearance is common, one cannot attach much weight to this discrepancy.

Figures based on his have found their way into several text-books (BALFOUR, 1885; KORSCHELT and HEIDER, 1895; SEDGWICK, 1898).

A very interesting American record was made by FEWKES in 1888. In summer townettings from the Bay of Fundy he found a larva which he considered to be closely allied to Mitraria as described by METSCHNIKOFF, but which differed from it in some respects. He stated that "no external opening of the stomach through an intestine was observed." This was, however, probably an observational error. His figures are reminiscent of a young Mitraria of *Owenia* before the prototroch has begun to arch up. A dark "spherical" body above the larval chæta-sacs—of which a pair was present—was possibly an early stage of the trunk invagination. The bristles were capillary. The species is probably not *Owenia fusiformis*, however, as there were masses of reddish pigment in the prototroch. He recorded that this larva "occurred in countless multitudes in July, and later decreased in numbers and was collected far into August." He only obtained the one stage. In the course of his paper he mentioned that he had obtained specimens of "a true Mitraria" at the Bermudas and at Santa Cruz, but these were not figured or described. He argued that Mitraria represents the common ancestral form of Brachiopods, Bryozoa and Chætopods, with certain secondary adaptations superimposed upon it.

HÄCKER, in 1897, gave a brief summary of the literature up to date (he missed FEWKES' work), and, accepting METSCHNIKOFF's idea of their affinity, definitely placed this summary under the heading "Familie Maldanidæ." He did the same thing the following year in his description (1898, *a*) of various Polychæte larvæ collected during the "National" Plankton-Expedition, although he thought that they might subsequently prove to be closely related to the Hermellidæ. The development of the latter is, however, quite different (see WILSON, 1929). Two of the figures accompanying HÄCKER's paper are reproduced in outline in a smaller paper (1898, *b*), published the same year. While one of his species had needle-shaped bristles only, the other four had flattened club-shaped ones in addition, and one of these latter appears to be identical with MÜLLER's Messina larva.

To WATSON (1901) belongs the credit of having first obtained Mitraria larvæ by fertilisation from the egg. In July, 1898, some adult *O. fusiformis* worms spawned in his aquarium, and the eggs developed as far as the trochosphere stage, when they unfortunately died. Although his figures are mere outline sketches, they were sufficient to fix the identity of one type of Mitraria at least. His figures of the earlier stages (*loc. cit.*, figs. 14, 15 and 16, Plate 25) appear to be based on abnormal individuals, but the remaining two, figs. 17 and 18, showing the trochosphere, are quite normal.

Since WATSON's paper a few scattered references to Mitraria have appeared. BROWNE (1905) and MARSHALL (1925) recorded its appearance in the Clyde, and CHADWICK (1924) figured one from Port Erin. CHADWICK's larva and two I myself collected at Port Erin in 1925 appear to belong to *Owenia fusiformis*. HEMPELMANN (1931) has recently repeated an original figure from GROBBEN (1905) of a Mitraria with club-shaped bristles, resembling somewhat those of MÜLLER's Messina larva. Both GROBBEN and HEMPELMANN were under the impression that Maldanids possess Mitraria larvæ.

In 1904 CIALONA described some very curious Mitraria larvæ from Messina. He figured the early stages of two species, both very similar, and differing mainly in the shape of the bristles. Of one of these he described and figured the development as far as the juvenile worm. The early stage is figured as a medusa-like bell with cilia round the edge. Hanging from the apex inside the bell is a conical projection from the end of which a tuft of long bristles arises. Some of these are clavate, others capillary. Further development consists in the elongation and segmentation of the projection to form the worm body, the bristles becoming arranged along its sides. Tentacles appear at the end of the projection where the mouth is situated. The worm breaks away from the bell, and the long provisional bristles subsequently fall off. CIALONA did not identify the worm produced, but his figure of it strongly resembles a Terebellid. No Terebellid or, indeed, any other Annelid is known to have a development like this, which is very difficult to bring into line with that of other Polychætes.

Through the kindness of Professor J. H. ASHWORTH, I have been able to examine a mounted Mitraria larva which was collected at Messina and sent to him by CIALONA. In structure it differs markedly from either of those the latter described. The clavate bristles are intermediate in shape between those of his two species. The larva itself is very like the autumnal Clyde form, being a trifle larger. It differs from the Clyde species in that the clavate bristles of the latter taper a little more gradually at their extremities, and, indeed, resemble fairly closely those of CIALONA's first species. This Messina larva shows the same type of development as that described for *Owenia* in this paper, and which is also followed by the autumnal Clyde form. It is at a stage at which the body ingrowth between mouth and anus is strongly developed a little while before the segmental mesoderm blocks close round the intestine. The bristles arise from distinct larval chæta-sacs, and it is so similar to the drawing of the Clyde species, text-fig. 50, that a separate sketch of it would reveal no specific difference. It could not possibly develop in the way CIALONA described for his larva. Moreover, I believe I can distinguish the early stages of thread glands in this mount, and these would stamp it as definitely belonging to the Oweniidæ.

The clavate bristles of the larva, whose development CIALONA recounted, are not figured in any great detail, but their general shape nevertheless recalls that of the bristles of MÜLLER's Messina larva. MÜLLER, however, figured the normal Mitraria organisation, and there seems to be no question of his larva subsequently developing in the curious way that CIALONA described.

3. TECHNIQUE.

During the course of this investigation several thousands of larvæ have been examined alive, several hundreds prepared as whole mounts, and about five hundred sectioned in various planes. A few of the preparations were useless, but the majority gave information on one or more points.

Methods of rearing are dealt with in the next section. Various fixatives were tried, including 2 per cent. osmic acid, Flemming without acetic, hot corrosive sublimate and cold Bouin. Finally, by far the best preparations were obtained by using Bouin's fluid at a temperature of 60° C. This temperature was found by experiment; it fixes the larvæ in an almost natural state of expansion. If used colder it allows them to contract, when it becomes almost impossible to interpret the sections; used considerably hotter, it distends them unnaturally, and, although larvæ so fixed are very useful in some respects, they have to be studied with care. It may be noted in passing that this temperature is too high for *Polygordius* larvæ, as it causes the body folds to be forced out until an exo-larva is simulated. Insufficient *Polygordius* were obtained to find the optimum temperature for them, but it appears to be rather less than 30° C. The hot fixative is used by squirting it suddenly on to larvæ swimming in a small quantity of sea-water. After being killed in this way they were removed to fresh cold Bouin, where they were allowed to remain for one to several hours. They were then washed free from the fixative with 70 per cent. alcohol and stored in 90 per cent. alcohol. The tissues were always fixed perfectly by this method. In handling the larvæ, MURRAY's open-tube pyroxylin method (1924) was found most useful, but they were always removed from the tube by pulling out the pyroxylin plug under alcohol with forceps, and not by dissolving the plug as he described. Very few specimens were ever damaged.

Larvæ to be sectioned were embedded in clove-oil celloidin and orientated by a method slightly modified from NEWTH's second method (1919). This gave very exact orientation in the majority of blocks. Late stages were cut in at least five different planes: (i) sagittal, (ii) frontal of head and transverse of larval tissues, (iii) frontal of trunk, (iv) transverse of head and frontal of larval tissues, (v) transverse of trunk.

Various technical difficulties were overcome, one of the chief being that the 60° C. paraffin wax recently supplied had to have a slight admixture of wax of a lower melting-point before it would set without cracking, and even then it did so only when cooled in water at a temperature between 75° and 80° F. In colder water it still cracked, and in warmer crystallised. The suppliers admitted this defect in the quality of the wax, and the above hint may be useful to other workers.

Sections were cut mainly at 8μ, although some were cut at 4μ and a few as thick as 24μ. These latter were very useful in gaining knowledge of the gross anatomy: they occupied a position intermediate between whole mounts and ordinary sections. The latter were flattened in ether vapour, or in extreme cases of crumpling in acetone vapour. Various stains were employed, Heidenhain's iron-alum hæmatoxylin, Delafield's hæmatoxylin and Mallory's triple stain being the most generally useful. Mayer's mucicarmine and muchæmatein were used to identify mucus. Larvæ mounted whole were mostly stained with alum-carmine.

The drawings of the sections were all made with the aid of a squared net micrometer in the eye-piece, drawing in the first place on to squared paper. The representation of the cytology is necessarily diagrammatic, but the histology is recorded as faithfully as possible. Each drawing has been carefully checked from similar sections of other individuals, and occasionally one or two details of the original drawing slightly altered after studying sections which showed those particular details with greater clarity than did the original section.

Using the same micrometer scale, drawings were made of the larvæ while alive and perfectly free to swim about in cavity slides. These drawings form the basis of the figures on the first three plates.

4. METHOD OF MAKING ARTIFICIAL FERTILISATIONS AND REARING.

The writer is indebted to a paper by ELWES (1910) for the hint that *Owenia fusiformis* is common in the Tor Abbey sands at Torquay. It is certainly the most numerous Annelid in the fine clean sand below the low water mark of neap tides. The worms stood the journey back to Plymouth without casualties even on a hot day, and in the laboratory lived exceptionally well under circulation.

Although mature worms occasionally were found as early as the middle of March, the best fertilisations were always obtained in June and July, and it was evident from the state of those collected that the worms at Torquay mostly spawned some time in June during the three years 1928–30. Worms collected in May nearly always contained reproductive products, while those collected at the end of June or in July were mainly empty. Evidently the spawning season is quite short, and it may be that the majority spawn more or less simultaneously. The poor tides of June prevented closer observation on this point.

Worms removed from their tubes sometimes spawned in dishes in the laboratory. The eggs or sperm always issued in two streams from two pores near the anus, as was first described by WATSON (1901). In mature specimens, both male and female, the posterior segments are swollen and white with the contained genital products. After spawning the tail has a translucent appearance and so spent worms were easily distinguished.

When making fertilisations a number of clean finger-bowls, capacity 500 cc., two-thirds filled with filtered* sea-water from the Eddystone region (" outside sea-water ") were used. Worms were removed from their tubes, washed in sea-water and laid on a glass tray. The posterior segments were then slit open with needles to release the eggs or sperm. Examination with a microscope revealed the state of maturity, and mature products were washed off into separate finger-bowls. The eggs were well washed by decanting with filtered sea-water, and usually kept an hour or two before sperm was added. When first shed, they were often irregular in shape, with a conspicuous germinal vesicle, but they rounded up on standing and the germinal vesicle disappeared. Eggs were about 100μ in diameter, and were closely invested in a membrane $1-2\mu$ thick.

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* A Berkefeld filter was used.

White by reflected light, they were dense and finely granular and greenish-grey in colour by transmitted light. After fertilisation the membrane separated, as a fertilisation membrane, from the egg surface for a distance of about 3 μ. The first polar body appeared a short while after fertilisation.

An hour or two after fertilisation the eggs were again washed in filtered sea-water to get rid of excess sperm and then the bowls were allowed to stand. Two days later all healthy larvæ would be swimming at the top, and these could be decanted off from dead eggs and unhealthy larvæ on the bottom. The larvæ so obtained were transferred to plunger-jars (Browne, 1898) or finger-bowls containing filtered outside sea-water. A few drops of a *Nitzschia* culture (kindly supplied by Dr. E. J. Allen) were added as food. The jars had to be carefully regulated as to the amount of light they received by tying brown paper round them. In too strong a light the *Nitzschia* would produce too thick a culture in the jar ; in too weak a light it would not grow fast enough.

The larvæ proved to be delicate, and in only three experiments out of many was complete success attained in rearing the majority through the metamorphosis, although on several occasions they would develop for some time before most of them became abnormal and died off, leaving only a few to metamorphose. They were sensitive to rapid changes of temperature, such as were produced by a cold night after a spell of warm weather. But those cultures which were successful gave abundant material, particularly a fine rearing in July, 1930.

5. General Account of the Development up to Metamorphosis.

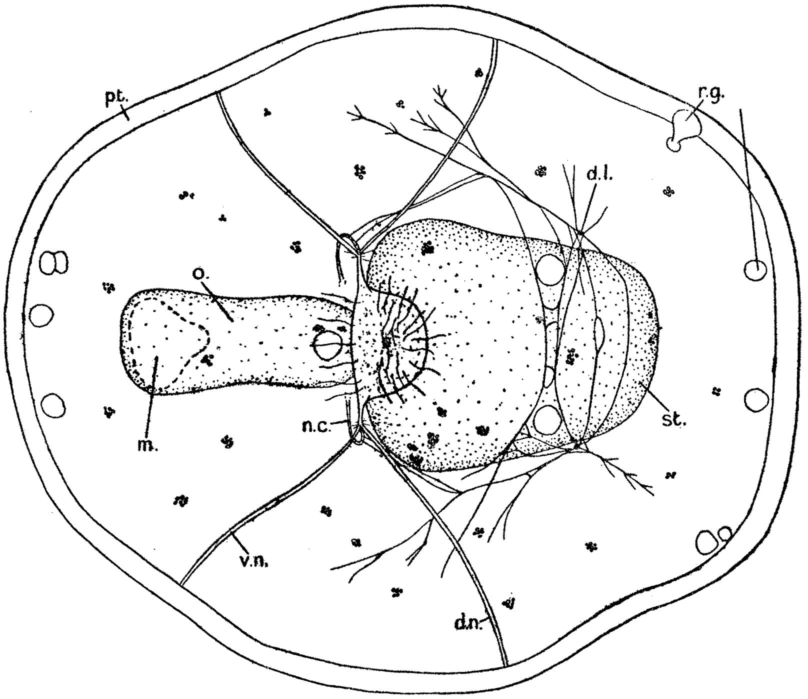
Twenty-four hours after fertilisation the characteristic Mitraria shape is already indicated in the developing embryo, figs. 1 and 2, Plate 29. Above a broad thick zone of cells—the prototroch rim—completely surrounding the embryo arises the conical episphere, and at its summit are the cells destined to form the head. Below, a less regular cone—the hyposphere—bears the chæta-sacs, the recently formed mouth and the anus. Two very short bristles protrude from each chæta-sac. The general outline of the gut is indicated. Cilia are beginning to grow out from a yellow pigmented area on the prototroch rim : they project through the thin transparent fertilisation membrane, which forms the larval cuticle. A few hours later they become much stronger and the larva begins to swim, slowly at first, but soon acquiring power and speed. The bristles lengthen, the larva swells out by enlargement of the blastocœl, and at the same time the epi- and hyposphere walls become thinner ; the head becomes clearly indicated, acquiring fine sensory cilia. The larva begins to feed.

Two to three days from fertilisation the full trochosphere stage is reached, figs. 3 and 4, Plate 29. As it is from this stage that the development has been studied in detail, a full general description will be useful.

The pronounced prototroch rim is roughly elliptical in shape and pale yellow in colour. A band of brownish pigment specks marks the zone from which the comparatively long (approximately 37μ) fine cilia arise, not in one or more rows, as in some other Polychætes, *e.g.*, *Sabellaria*, *Polydora*, but in a general broad band. The thin transparent episphere connects the rim with the head ; its peculiar refringent spheres and other structures are dealt with below (p. 244). The head is a hemispherical plate, text-fig. 1, bearing sensory cilia, yellowish and with brown pigment specks. On each side a circumœsophageal nerve commissure (*n.c.*) leaves it and runs down through the blastocœl to the hyposphere alongside the anus. From its under surface muscle fibres form a pair of retractor muscles, and likewise pass down one on each side of the gut text-fig. 27A and B.

The surface of the larva below the prototroch, fig. 4, Plate 29, in normal extension is almost flat. Immediately below the prototroch rim is a broad ciliated area, the metatroch, which completely surrounds the larva and passes into the mouth on each side. Below or internal to this is the thin, non-ciliated hyposphere tissue lying posterior to the mouth, bearing the anus and the larval chæta-sacs. An area of thin, non-ciliated tissue is situated anterior to the mouth, and is slightly raised up above the general surface. This area is not represented in *Polygordius*. The mouth is guarded by a pronounced pale yellow anterior lip bearing long fine cilia, and by two similarly tinted crescent-shaped lips at each posterior lateral corner. The last are strongly ciliated, and from their outer extremities there continues a row of cilia in single file to form the lower border of the metatroch. Some of these details are more clearly seen in drawings of later stages, figs. 8 and 10, Plate 30. The mouth leads into a strongly ciliated œsophagus, which opens into a globular stomach. The latter passes into a short intestine opening at the anus. The gut is tinted a very pale yellow, but in later stages the stomach and intestine are often darker and in some cases, after feeding on green algæ or flagellates, may be greenish. Owing to the strong ventral flexure of the gut the anus is brought close to the mouth. Just dorsal to the anus are the larval chæta-sacs ; as will be shown below (p. 249), each is divided into two compartments. They are yellowish in colour, with brown specks scattered over them. In early larvæ a few long and very fine cilia are present on the wrinkled skin close to the anus, but these disappear after a time. At this stage four bristles of differing lengths are present on either side, but others soon appear. From the dorsal border of each sac a muscle fibre runs up on either side of the stomach to be attached to the episphere about half-way between the head and the prototroch. These are the dorsal levators. To them the nephridia are secured, text-fig. 12 and fig. 6, Plate 29. The nephridial ducts open by minute pores on either side of the anus. From the ventral part of each chæta-sac two muscle fibres take their origin : one is the main fibre of the retractor (see p. 278), the other runs over the interior hyposphere surface to the ventro-lateral corner of the prototroch. This muscle, the ventro-lateral hyposphere muscle, is much branched, especially in later stages, figs. 8 and 10, Plate 30. A similar branched muscle runs from close to the base of the nephridium to the dorso-lateral corner of the prototroch on either side. This, the dorso-lateral hyposphere muscle, is also branched, but it does not arise from the chæta-sacs. Its main fibre passes below the base of the nephridium towards the main retractor, whose fibre it accompanies upwards for a short distance, finally leaving it to cross the blastocœl to be attached to the ventral surface of the œsophagus as the œsophageal muscle, text-fig. 33. The dorso-lateral hyposphere muscles are connected by a transverse fibre behind the chæta-sacs.

Besides the muscles already mentioned, the blastocœl is traversed by certain finer contractile fibrils. The dorsal levators are attached to the episphere by several fibrils, and these are connected by a network of fine fibrils across the dorsal part of the stomach, text-fig. 1. Other branched fibrils radiate out to be attached at various points to the episphere. One or two fibrils on each side run to the circumœsophageal nerve commissures. The exact distribution of all these fibrils varies with different individuals, and between different sides of the same individual, but the general plan is as stated. Moreover exceedingly fine mesenchyme filaments, not specifically contractile, cross the blastocœl in all directions from the gut and other internal structures to the larval wall.



TEXT-FIG. 1.—Sketch from life of upper aspect of a Mitraria about 12 days old. Not to scale. Cilia of prototroch omitted. *d.l.*, position of attachment of dorsal levator to episphere. The lines radiating from this point are contractile fibrils. Some of these are attached laterally to the episphere, some to the circumœsophageal nerve cord and some strands cross the upper surface of the stomach to join the fibril complex on the other side; *d.n.*, dorsal nervure; *m.*, mouth; *n.c.*, circumœsophageal nerve commissure; *o.*, œsophagus; *pt.*, rim of prototroch; *r.g.*, refringent globules. One has worked through the episphere and is projecting to the exterior; *v.n.*, ventral nervure.

These filaments have frequently been observed in other larval forms. They are not illustrated in the figures in this paper, as it is impossible to convey any idea of their excessive fineness. Their addition to the illustrations would only make them confusing.

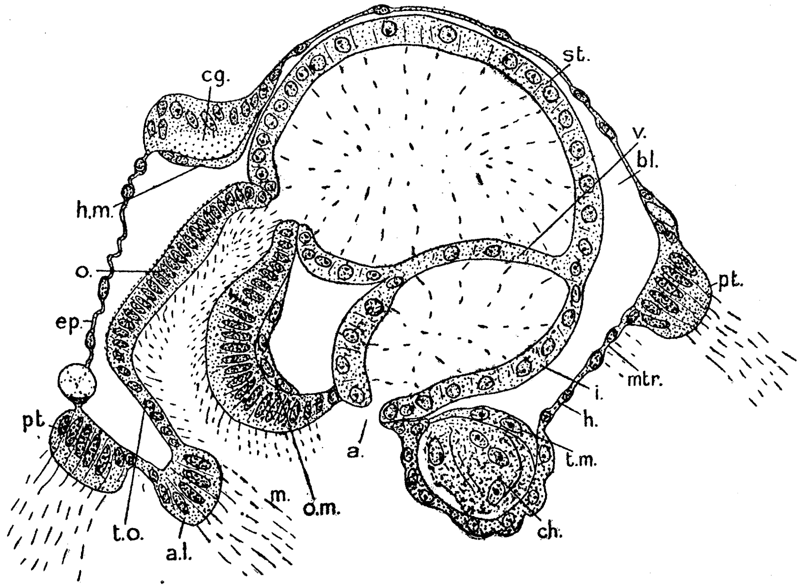
The larvæ swim steadily and with moderate speed—compared with other Polychæte larvæ—in the direction of the head, the bristles trailing behind. The position assumed is more or less upright—the position in which they are drawn—but they incline over to the horizontal when they swim from one side of the bowl to the other. The habit of healthy larvæ is to swim up away from the bottom and they therefore tend to congregate just below the surface film in still water. When irritated, as by bumping into some foreign object, they contract violently, and erect their bristles to point in all directions. In contraction the head is indented by the retractors and the prototroch pulled in by the four hyposphere muscles, there being no circular muscle underlying the prototroch, as in *Polygordius*. The action is very similar to that of *Sabellaria* larvæ, and probably serves the same ends (see WILSON, 1929), although in this case experiments with young fish, etc., have not been tried. Feeding seems to be brought about by the broad metatroch acting as a food-collecting area. The larvæ are so small, and so difficult to keep properly expanded while trapped upside-down in cavity slides, that the usual carmine particles were not satisfactory for experimental feeding, and *Nitzschia*, although eaten in the plunger jars, appeared merely to irritate confined larvæ. On several occasions, however, fine particles of debris were observed to be swept along the metatroch into the mouth, there to be either ejected or swallowed. This was very striking in late larvæ, where they had to pass around several folds of the prototroch before reaching the mouth. Perhaps the metatroch only acts efficiently as a food-collecting organ when the larva is swimming forwards, and when observation is well-nigh impossible.

For a long time after attaining the trochosphere stage, development is very slow. As in *Sabellaria*, the formation of the provisional bristles is hurried until a considerable number are present, and more are continually being added as the older ones grow longer. In the last larval stage there are about twenty-eight of them altogether, but some are quite short. For about a third of its length from the base each bristle is plain; it then bears fine teeth arranged spirally, text-fig. 51A, as far as the tip, which ends in a fine point. The larval tissues slowly increase in size, and the gut keeps pace with them. The prototroch after some days begins to arch up in front and behind, and at the sides. The down-turned portions form ventro-lateral and dorso-lateral lappets to which the hyposphere muscles are attached. When the latter contract the lappets are pulled inwards towards one another below the hyposphere, and the front, back and sides of the prototroch are strongly arched up. It may be that the action of these muscles stimulates and promotes arching of the prototroch rim. Fig. 5, Plate 29, shows the condition twelve days after fertilisation. Close inspection of living larvæ reveals a slight thickening of the hyposphere wall immediately anterior to the anus and a tendency for this region to invaginate. The invagination deepens slowly while the prototroch rapidly increases in size and sinuosity. Five days later, so rapid has been the growth that the prototroch, gut and larval tissues have nearly reached their full size, fig. 6, Plate 29. The prototroch is strongly arched at the four places already mentioned, and, in addition, a small indentation has appeared half-way up each side of the ventral arch. When the hyposphere muscles contract the four lappets are pulled in below the hyposphere until they touch or almost touch one another, and the bristles are spread out between the lappets in the regions of the arches. The invagination anterior to the anus stands up stiffly, and is yellow or pale brown in colour. It now grows quickly and from below, fig. 8, Plate 30, its crescentic slit-like external opening can be seen. With further growth this slit gradually extends round the anus until the horns of the crescent meet dorsally and the intestine becomes surrounded by the ingrown tissues. This ingrowth is, in fact, the trunk rudiment, and it is already divided into several segments long before the intestine is enclosed within it. From the anterior border of the crescentic slit to the posterior border of the mouth an area of thickened hyposphere tissue can be seen, and in this a little later a second invagination takes place to form the buccal organ. This can be seen in lateral view in fig. 7, Plate 29, of a larva twenty-one days old. The trunk rudiment is much larger than four days ago, but it has not yet completely surrounded the intestine. The large thread glands, so characteristic of the adult, are seen developing in some of the segments. The head, too, is growing fast, and possesses a reddish-brown eye, formed from a cluster of oily-looking globules on either side. Another two or three days, and the intestine is enclosed within the trunk, but only for a short distance dorsally, fig. 9, Plate 30. The trunk now exhibits contractile movements, some of the muscles of the body wall having been formed. The thread glands are prominent, and adult bristles are distinctly seen. Since the previous stage, the prototroch and larval tissues have grown little, if at all, having reached their maximum development. The part of the trunk dorsal to the intestine grows very quickly, and in a day or two is equal in length to the ventral part. A little more general growth of the trunk and head tissues, and the larva is ready to metamorphose, four weeks after fertilisation, fig. 10, Plate 30, and fig. 11, Plate 31. It can now be seen that the curious way in which the trunk developed has resulted in a most unusual disposition of the parts ; in effect, the first five segments—all except the first being chætigerous and possessing large thread glands—are turned inside out, and drawn back over the posterior segments, much as the top of a stocking can be turned inside out and drawn back over the foot. The head is a large, saddle-shaped structure, widely separated from the body, and only connected with it by the circumœsophageal nerve commissures and retractor muscles, and, of course, by the tissues of the blastocœl wall. The stereogram, fig. 15, Plate 32, should make the general relations of the parts clear, but before the metamorphosis is described the structure and development of the tissues must be studied in more detail.

6. Histology of the Larval Tissues.

(i) *The Episphere.*

The whole larva is covered externally by a thin layer of cuticle. In the very earliest stages this is the fertilisation membrane, which is never actually thrown off. It is evidently added to later by the underlying cells, or its substance is gradually incorporated in the cuticle secreted by those cells. The episphere wall below the cuticle consists of a single layer of flattened cells united by their edges in the form of a pavement epithelium. In the early stages the nuclei are conspicuous and dark-staining, and form bulges in the thin tissue, text-fig. 2. Division stages are frequent. Later on, when the prototroch has reached the limit of its growth, the nuclei stain scarcely at all, as if very little chromatin remains. They also become irregular, and many of them finally take on an irregular ring-shaped form, as do the episphere nuclei of *Polygordius* (Woltereck, 1902). Divisions no longer take place.



Text-fig. 2.—Median sagittal section of a Mitraria about 2 days old. × 756. *a.*, anus; *a.l.*, anterior lip of mouth; *bl.*, blastocœl; *c.g.*, cerebral ganglion; *ch.*, larval chæta-sacs; *ep.*, episphere; *h.*, hyposphere; *h.m.*, mesoderm on under surface of head; *i.*, intestine; *mtr.*, metatroch; *o.m.*, œsophageal mesenchyme or mesoderm; *pt.*, prototroch; *st.*, stomach; *t.m.*, membrane separating cœlomic cavity of larval chæta-sacs from blastocœl; *t.o.*, thin larval part of œsophagus; *v.*, valve between stomach and intestine.

During life neither the nuclei nor the cell limits can be seen. Small oily-looking globules are scattered over the episphere, and after the very early stages have been passed there are definite clusters of these, text-fig. 3A. Most striking are the large refringent spheres which are present throughout larval life, Plates 29–31. The majority are scattered just above the prototroch, but there is almost invariably one present a little distance in front of the head, and another on each side close to where the dorsal levators are attached to the episphere. Roughly, but not strictly spherical, they vary in size (average diameter, 12–15μ), and smaller ones are seen which may be early stages in the formation of larger ones. Close inspection shows each sphere to enclose a number of smaller globules or bubbles, see text-fig. 3A, *r.g.*, so that a delicate foam-like appearance is produced. Sections reveal the presence of a flattened curved nucleus associated with each sphere, text-figs. 2 and 4B. The nature of these spheres is unknown. They remain colourless in osmic acid, but neutral red (used *intra vitam*) stains them a deep red and resolves them into morula-like clusters of large globules. Nile blue used in the same way stains them heliotrope, and stains some of the component globules darker than others. With methylene blue they are scarcely stained at all. In healthy living larvæ fresh from the plunger jar the spheres are attached to the under surface of the episphere, and so project into the blastocœl, but it has been frequently noticed, in larvæ which had been confined in a cavity slide for some time, that a few of the spheres work through until they form projections on the outside surface of the episphere, text-fig. 1, *r.g.*, to be finally cast off.

The special part of the episphere immediately above the prototroch is described below in connection with that organ (p. 247).

An important feature of the episphere are the two pairs of nervures, which can be seen in most larvæ under favourable conditions. In early stages they spring from the point at which the circumœsophageal nerve commissure leaves the episphere and enters the blastocœl, text-fig. 1, *v.n.* and *d.n.* The ventral pair crosses the episphere to reach the prototroch at the places which, in late stages, form the ventro-lateral lappets. The second pair similarly runs to the prototroch, to reach it at points which, in late stages, lie in the middle of the upper part of the lateral arches. In the later stages the nervures on each side unite just below the edge of the lateral head pocket, which is then present, text-fig. 30, but their further course inwards cannot be followed. The nervures appear as lines brighter than the general episphere surface; no definite branches from them have been observed. They do not stain with any of the stains used, and are rarely visible in preparations. It is an open question as to whether they are nervous or not.

The episphere also presents short, bright, wavy lines of an unknown nature, which run mainly parallel to the prototroch.

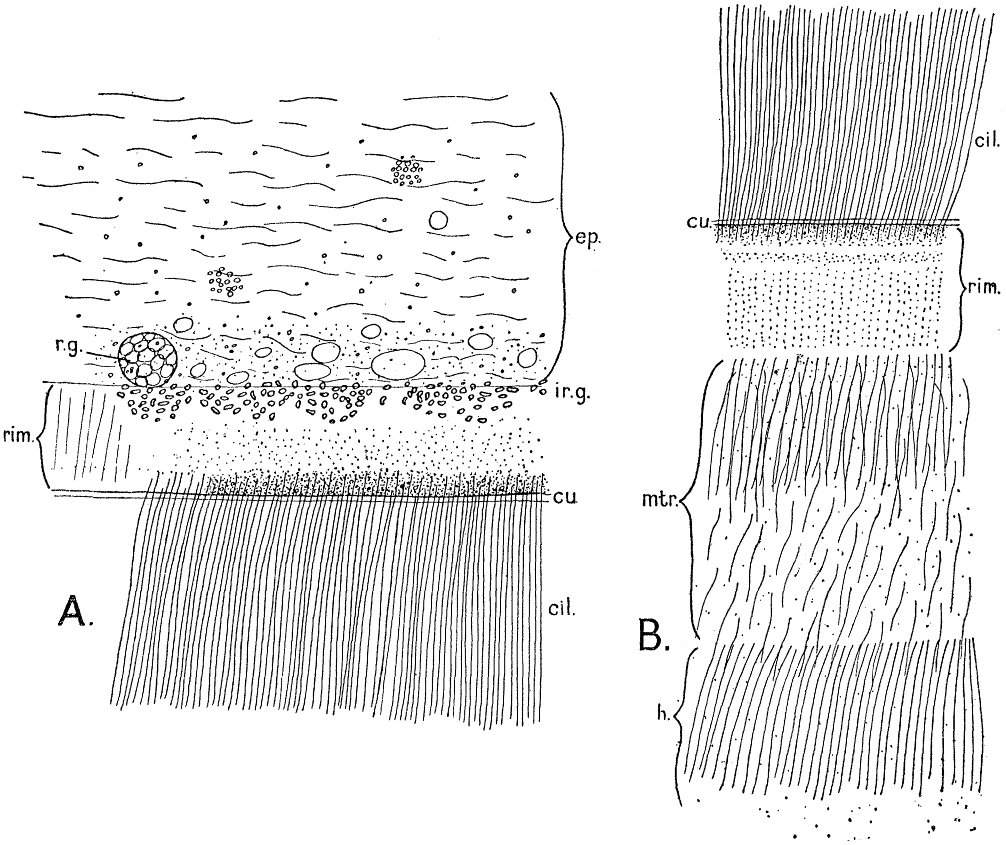
In *Polygordius* the episphere is a much thicker and more complicated structure, and is provided with a regular network of muscle fibres and nerve fibres on its under surface. Such fibres have not been seen in Mitraria.

(ii) *The Hyposphere.*

This, like the episphere, is a thin pavement epithelium, only it is much thinner. Nuclei are normal during the early stages, but, like those of the episphere, when the prototroch has reached its full size, they remain almost entirely unstained and appear to retain even less chromatin. It does not possess any special cells or nervures, but over its internal floor there run the special muscles to the four lappets which have already been described (p. 242). The epithelium of the raised, non-ciliated area anterior to the mouth is similar in character to the general hyposphere tissue.

(iii) *The Region of the Prototroch and the Metatroch.*

After the very early trochosphere stages have been passed, the prototroch during life presents a very characteristic appearance. Viewed from above, text-fig. 3A, there is seen on the outermost edge of the thick rim a broad band of yellowish-brown globules and granules on a pale yellow ground. From this region the long fine cilia (*cil.*) of the prototroch arise and penetrate the overlying cuticle (*cu.*). It can be seen that they spring from the ends of tall columnar cells—the limits of which are just visible—which form the pronounced rim of the prototroch. Just above the cilia there is an area of minute yellow granules, and above these a zone of prominent, irregularly shaped globules (*ir. g.*), which, when seen under low powers, give a dark upper border to the prototroch rim. Each globule is pale greenish-yellow with a dark border, the latter being due to

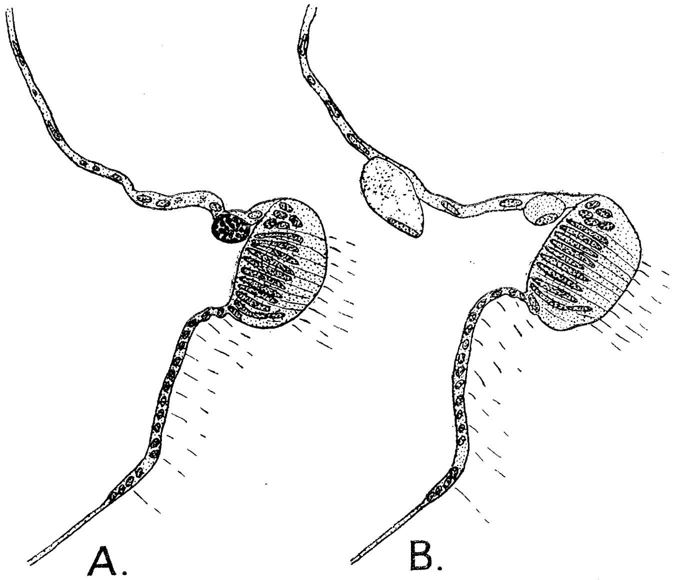


Text-fig. 3.—Sketches of prototroch from life. A, from above. B, from below. *cil.*, prototrochal cilia ; *cu.*, cuticle ; *ir. g.*, zone of irregular globules showing marked Brownian movement ; *r.g.*, refringent globule ; *rim*, rim of prototroch. In A the cell boundaries are indicated on the left.

refraction of the light passing through. They exhibit Brownian movement very markedly. Above these, again, there is an area of episphere tissue containing large, roughly spherical masses, with an occasional large refringent sphere. The episphere surface exhibits bright wavy markings parallel to the prototroch, and small pale yellow globules are scattered over it. Here and there a cluster of globules is to be seen.

Viewed from below, text-fig. 3B, there is less diversity. Below the ciliated band (*cil.*) there is first a narrow zone of yellow globules, and then a broader one of similar globules arranged in parallel rows. A broad area of cilia, the metatroch (*mtr.*), follows with a definite line of cilia on each side. Over this area a few yellow-brown globules are scattered, and similar ones are distributed over the hyposphere surface below the metatroch.

Transverse sections of the region under discussion show, text-fig. 4, the columnar cells from which the cilia spring. Their nuclei are elongated. The upper part of the rim has smaller irregularly-placed nuclei. The episphere wall where it joins the rim is thicker than it is higher up. The roughly spherical masses seen during life show a variety of structure; certain ones contain large granules, which stain very darkly with Delafield's hæmatoxylin, text-fig. 4A; others do not show any special structure, text-fig. 4B. Here and there large refringent spheres appear in the sections, text-fig. 4B, and see p. 245. The functions of all these cells are very obscure.



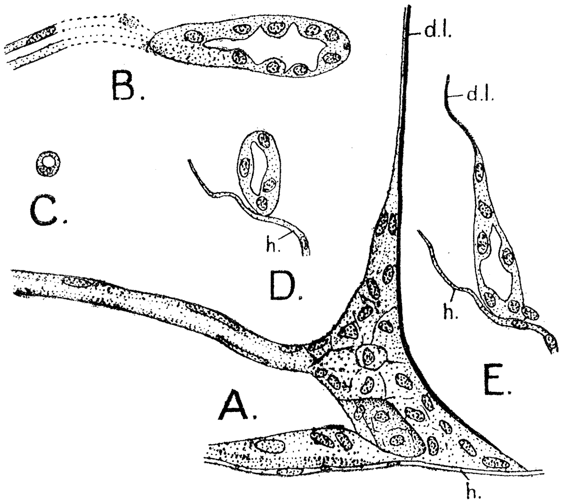
TEXT-FIG. 4.—Adjacent transverse sections of the prototroch and adjoining tissues of a Mitraria ready to metamorphose. × 756. Cilia diagrammatic and cut short; cannot be seen at all clearly in the actual sections.

Below the rim the metatroch area contains numerous small nuclei, and is in marked contrast to the thinner and sparsely nucleated hyposphere wall below it. Its lower margin is formed by a narrow band of nuclei, which are separated from the main part of the metatroch by a narrow non-nucleated gap. From this band, possibly, springs the inner row of cilia in single file, continuous with the posterior lateral lips.

(iv) *The Nephridia.*

Mitraria possesses a single pair of nephridia. They are attached to the dorsal levator muscles, and by long narrow bases to the hyposphere wall on either side of the chæta-sacs. They are hollow structures, text-fig. 5, and each discharges to the exterior by a tubular duct. In the early stages the nephridiopores are situated in the hyposphere wall on each side of the anus, but on growth of the trunk they become embodied in the first or buccal organ segment and discharge through it—close to its posterior border—into the lumen of the trunk invagination, text-figs. 12 and 15.

The cells forming the wall of the nephridium are seen during life to be granular, and to contain a number of clear refringent bodies, large and small, very like oily globules in appearance. The duct also contains a number of small but similar bodies. Long, excessively fine flagella can under very favourable conditions be seen in the lumen, and projecting down the duct, where they occasionally produce a flickering movement. The detailed structure has, however, defied observation, although many days have been spent in fruitless efforts to make out the attachments of the long fine flagella, and as to whether there is anything approaching the nature of solenocytes. Unfortunately, with the highest powers and best lighting conditions, little can be seen, and the flagella are almost invisible except when they are beating. Observation is rendered all the more difficult by the constant twitchings of the muscle fibre to which the nephridium is attached, and by the awkward position of the nephridium itself, so that it can never be observed without other structures lying above or below it. In teased preparations the nephridium shrivels up. It is still a question whether the flagella arise from the bottom of short canals contained in the main bodies of their cells—the cells which form



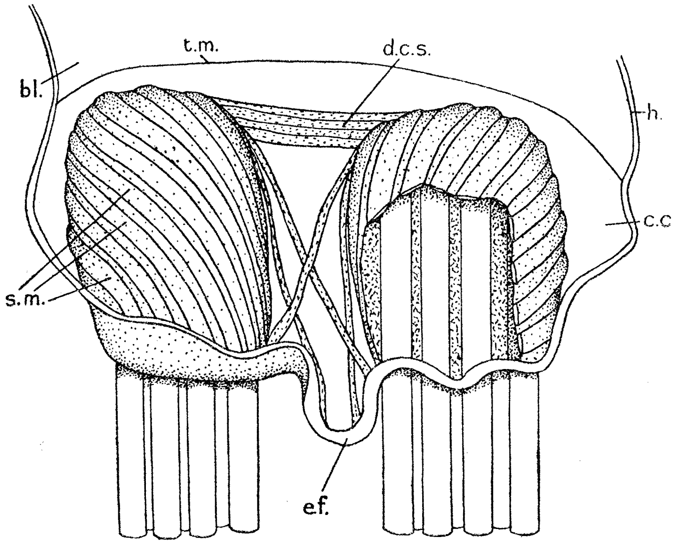
TEXT-FIG. 5.—Sections of nephridia. × 756. A. Practically the whole nephridium and duct in lateral view. A portion of the wall is cut away showing hollow interior. B. Nephridium and portion of duct in horizontal section. C, D, E. Transverse sections of the same nephridium and duct. This nephridium is smaller than that in A and B. *d.l.*, dorsal levator.

the walls of the nephridium—or whether they project freely at once into the cavity and down the duct. The latter appears to be the more likely condition; there are here certainly nothing like the solenocytes found in the *Polygordius* endo-larva, which are seen easily with quite moderate magnification.

(v) *The Larval Chæta-Sacs.*

The long provisional chætæ spring from what appears at first sight to be a single pair of chæta-sacs. On closer inspection each sac is seen to consist of two parts: a larger ventral and a smaller dorsal, Plates 29–31. Both parts are, however, enclosed in a common muscular sheath, and it will be convenient to refer to the whole structure as consisting of one larval chæta-sac. The cavity in which the sacs are contained is cut off from the blastocœl of the larva by a membrane. In the very early stages the dividing membrane is relatively thick, text-fig. 2, *t.m.*, but it becomes drawn out until it is excessively thin and extremely difficult to detect, and in some preparations it is quite invisible. In the last larval stages it can nearly always be made out, and is occasionally clearly visible, but it is never more than a fraction of a micron thick, text-figs. 18 and 24, *t.m.* As will be shown later, this cavity enclosing the chæta-sacs comes to communicate with the common cœlomic cavity of the first and second chætigerous segments of the worm trunk, and, indeed, after metamorphosis, forms part of that cavity in the young worm. There is thus no necessity to regard it other than as a cœlom from its earliest stage.

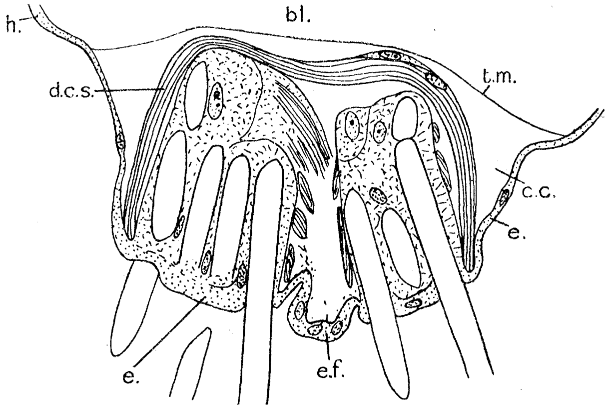
Text-fig. 6 represents diagrammatically the muscular system by which the chæta-sacs



TEXT-FIG. 6.—Diagram of larval chæta-sacs cut open to show structure and viewed from the region of the mouth. × 756. Bristles cut short. Larva ready to metamorphose. *c.c.*, cœlom; *d.c.s.*, divaricator muscle of chæta-sacs; *e.f.*, ectodermal fold; *s.m.*, spiral muscles.

and their contained bristles are moved. Each sac (consisting of ventral and dorsal parts) is surrounded by a layer of muscles (*s.m.*) running spirally up from the base. Each rather strap-like muscle completes a turn equal to a third to a half of the total circumference of the sac. On the right hand side the direction is anti-clockwise, and on the left clockwise when viewed from below. So far as can be ascertained, each muscle is attached at one end to the bottom of the chæta-sac and at the other, its top end, to the base of a bristle or to the tissues immediately associated with it. The bristles themselves appear to be pivoted where they pass out to the exterior. Assuming that they are free to move elsewhere, it will be seen that contraction of the spiral muscles will cause them to be spread by depressing and pulling around the base of each. The chæta-sac tissue in which they are embedded has a spongy or vacuolar structure, and permits this movement to take place.

When the bristles are to be spread the openings of the chæta-sacs are turned laterally outwards away from one another. A fold of skin between the sacs (*e.f.*) makes this possible. The movement is brought about by contraction of a strong muscle (consisting of several separate fibres), which runs across the tops of the sacs dorsally with its ends inserted in the bases of the dorso-lateral parts of the sacs, text-fig. 7, *d.c.s.* The movement may also partly be brought about by the contraction of certain muscle fibres which run from near the tops of the sacs on the inner sides to the fold of skin, text-fig. 6. The function of these muscles is, however, by no means certain.



TEXT-FIG. 7.—Transverse section of larval chæta-sacs of a Mitraria ready to metamorphose. × 756. *e.*, ectoderm.

It seems that the sacs are brought back into the position of rest by the cross muscle fibres shown in text-fig. 6. Running from the top of each sac to near the base of the opposite sac, they will, during divarication of the sac openings, be stretched, and their contraction will then restore the sacs to their normal position.

The exact position of attachment of the main retractor fibre and the ventro-lateral hyposphere muscles to the tops of the sacs, as mentioned on p. 240, is uncertain, and therefore no attempt has been made to insert them in text-fig. 6.

The histology of the chæta-sacs is difficult to follow. The bristles appear to be inserted in ectodermal pockets, but these are rarely clear. Near the base of each bristle there is a nucleus with a large, darkly-staining nucleolus, text-fig. 14.

(vi) *The Alimentary Canal.*

Mouth and Oesophagus.—The anterior lip and the two posterior lateral lips are composed of columnar ciliated cells with elongate nuclei, text-figs. 2, 24 and 31, reminiscent of those forming the prototroch. The mouth leads into the œsophagus, which first runs upwards and then bends back to join the stomach. The first part is thin-walled dorsally, text-figs. 2 and 24, and this thin portion (*t.o.*) extends round on to the sides, but the remainder of the œsophagus and all the ventral wall is thick. This thin portion is purely larval, and breaks away at metamorphosis (see p. 288), the adult mouth being formed where the thick-walled part begins. The thin-walled portion is less densely nucleated than the thick-walled. The latter consists of tall, very narrow cells with elongate nuclei, which stain so deeply and are so crowded that they give the œsophagus a very striking appearance in the sections. The œsophagus is strongly ciliated, and the main beat of the cilia is generally in the direction of the stomach. The lumen, except near the junction with the stomach, is not circular in cross section, but is roughly triangular, text-fig. 32. The thick-walled portion is surrounded from the very early stages by circular muscle fibres, formed probably by larval mesenchyme cells. In the early stages the nuclei of this mesenchyme are situated entirely on the ventral wall, text-figs. 2 and 27A, *o.m.*, and tend to congregate in two lateral groups close to the attachments of the œsophageal muscles; later some may be found all round the œsophagus. The muscles assist swallowing by producing peristaltic contractions.

The entrance into the stomach is guarded by a valve formed by a cone of cilia, figs. 3, 5 and 6, Plate 29. This has a central passage, which is nearly always visible, and which widens to allow food particles to pass.

Stomach and Intestine.—The wall of the rather globular stomach, text-figs. 2 and 31, consists of a single layer of relatively large cells, the boundaries of which are fairly clear in preparations. The nuclei generally possess a conspicuous nucleolus. Very long fine cilia arise from the wall and keep up a constant flickering motion, so stirring up the contents. Scattered over the anterior dorsal portion of the stomach are a number of special cells with dark staining, slightly granular cytoplasm, and a clear vacuole-like space towards the lumen. They are shown in sections in text-figs. 24 and 31, *d.c.*, but seen from above they are larger than the surrounding cells and are irregular or amœboid in shape. They are probably the cells mentioned by WOLTERECK as having been seen by him in Mitraria (1902, p. 37). Their function is not known, but perhaps they secrete a digestive enzyme. In life, clusters of greenish globules are seen on the anterior part of the stomach, and may be connected with these cells as they are not seen on other parts of the gut. Small, oily-looking globules are scattered over the whole stomach.

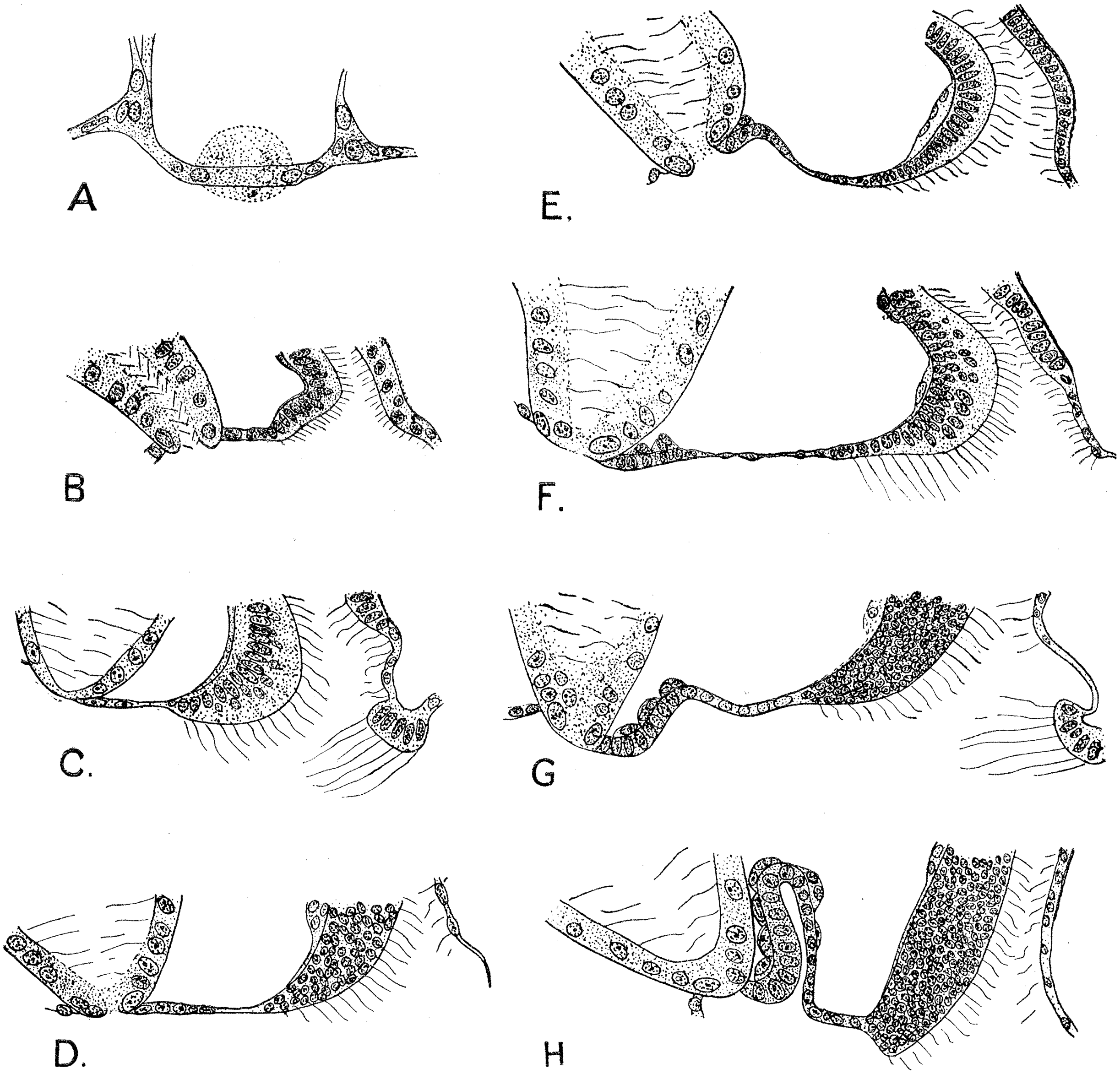
The stomach is separated from the intestine by a valve, text-fig. 2, *v*. This differs from the similar valve of *Polygordius*, which is a definite flap. In nearly all the specimens sectioned there is no communication between stomach and intestine, but in a few there is a passage dorsally between the valve and gut wall in the same position as the permanent passage in *Polygordius*, text-fig. 19C. In these instances the larva happened to be fixed while food was being transferred from the stomach to the intestine. After the transfer has taken place the valve seals up with the gut wall again. In some specimens sealing (or unsealing) was in process when they were fixed, and the attachment to the gut wall is thin. The valve is ciliated on both sides with the long fine cilia characteristic of both stomach and intestine. The cells of the latter closely resemble those of the stomach, but no special cells of any kind are visible.

The state of distension of stomach and intestine varies. Sometimes the stomach may be strongly distended, and the intestine collapsed ; at other times the latter may be swollen with contained fluid, and the stomach smaller than usual. The thickness of the walls varies with the state of distension. No muscle fibres are connected with them, but the stomach is usually constricted slightly between the dorsal levators.

7. Early Development of the Worm Trunk.

When the under surface of a living larva which has just begun to swim is examined with a high power, the hyposphere wall immediately in front of the anus is seen to be thickened in a slightly crescentic line running transversely between the attachments of the nerve commissures. Sagittal sections show that this tissue is one or two cells long, text-fig. 8B and C, while frontal sections show it to be five or six cells broad, text-fig. 8A. The cell on each side immediately in contact with the commissure base is not as tall as the other cells in the middle. Immediately in front of this band of cells—which, as will be seen later, is the primitive ectoderm of the trunk—there is a small area of thin tissue, devoid of nuclei near the middle line of the larva, but with nuclei at the sides ; it connects the primitive ectoderm to the densely nucleated posterior border of the mouth, text-fig. 8C, and on either side merges into the general hyposphere wall. This thin tissue is barely distinguishable at the earliest stage, when the ectoderm band is only one cell long, text-fig. 8B, but by the time that it is two cells long it is quite distinct.

The transverse ectoderm band slowly increases in length, and to a certain extent in width, and about five days later is five cells long, text-fig. 8D, and eight or nine cells wide. The cells are largest near the anus and smallest next to the thin area. In living larvæ this thickened area is conspicuous, and in normal extension causes a slight invagination to form as a transversely elongated pocket, text-fig. 8E. When the larva expands fully this invagination disappears owing to the tissues being pulled out flat, text-fig. 8D ; in contraction it becomes deeper. About this time mesoderm appears in the form of two cells situated side by side in the angle between the endoderm of the intestine and the developing body ectoderm, text-fig. 8E. The origin of these cells is obscure, as no division stages giving rise to them have been observed, and careful



TEXT-FIG. 8.—A. Portion of a frontal section of a Mitraria just beginning to swim to show the trunk ectodermal cells lying between the bases of the nerve commissures anterior to the anus. The section has cut away a thin slice of intestinal wall. As the ectodermal strip is crescentic in shape the central nuclei are practically shorn away. × 756. B–H. Sagittal sections of early stages in the development of the worm trunk. × 756. In each case the anus is on the left of the figure and the mouth on the right. In most the section is taken in or almost in the median sagittal plane, but G and H are a little to one side. Full explanations of these figures will be found in the text.

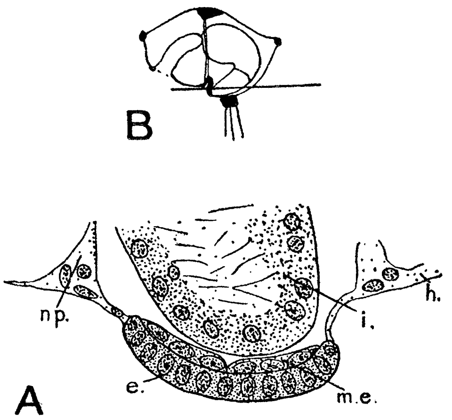
search of earlier stages has not revealed any cells which can be distinguished as their mother cells. For a discussion of this problem, see p. 314.

For some few days growth of the trunk is very slow, although during this period the larval tissues, especially the prototroch, and the gut are growing rapidly. In these circumstances it is not altogether surprising that no division stages have been seen in the mesoderm during the few days subsequent to its first appearance. Nuclei would divide at comparatively long intervals, and then probably quickly, so that the chances of observing them in sections are remote. Later, when development speeds up, they are frequently seen. Division stages have occasionally been seen in the early ectoderm, text-fig. 8E.

The two mesoderm cells presumably divide and give rise in the manner of teloblasts to the paired mesodermal blocks—each pair indicating one segment—which now appear. First a pair of cells is seen in front of the teloblasts, and then each of these gives rise to a little block of tissue containing two, three or four nuclei, text-fig. 8F. Cell boundaries cannot be made out. Behind these a similar pair of blocks makes its appearance, text-fig. 8G, and soon the teloblast nuclei themselves number four, forming, as far as can be seen, paired blocks of two nuclei each. These continue to give rise to further segments.

By the time that three segments, including the teloblasts, are clearly indicated the mesoderm has been developing for five days, and the larva is about 12 days old. These times vary somewhat with different individuals and with the temperature. The prototroch is about half-grown, and still growing fast, and the trunk now begins to grow rapidly as well. Division stages are frequently seen in both ectoderm and mesoderm. When three segments only are present the anteriormost occupies the top of the invagination, text-fig. 8G, but as further segments form they pass over the top and down the anterior wall of the ingrowth, followed by those behind them. This is brought about not by the mesoderm blocks sliding along over the ectoderm, which actually keeps pace in its growth with the mesoderm, but by the position of the fold moving back towards the anus as the whole invagination becomes larger. At the same time, the invagination deepens and the external opening, viewed from below, takes the form of a crescent partially surrounding the anus (fig. 8, Plate 30, shows a rather later stage). At first, as already noted, the invagination is flattened out during full expansion of the larva, but as it deepens this occurs less readily, and when five or six segments have formed it no longer takes place, although a certain amount of sliding of the anterior wall over the posterior continues for some time.

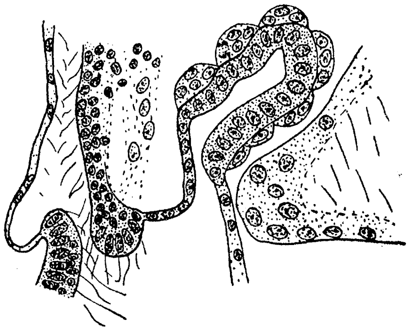
In the stage with six segments the first two are on the anterior wall of the invagination, the third occupies the summit, text-fig. 8H. The paired mesoderm blocks of the first two segments have become separated in the middle line, particularly the first pair which come into contact with the tissues at the bases of the nerve commissures. Each block contains seven or eight nuclei, those following rather less, the teloblast blocks still having two nuclei each. The invagination has increased in width owing to growth of the ectoderm, which now numbers 12 to 14 cells from side to side. Text-fig. 9A, shows in transverse section the trunk blastema in a strongly-expanded larva. It is cut at the level of the paired mesoderm blocks immediately above the teloblasts. The position is shown in text-fig. 9B. This increase in width is probably responsible for the separation of the anterior mesodermal blocks. These later join up again in the middle line.



Text-fig. 9.—A. Transverse section of worm rudiment just above the anus at a stage when about six segments are present. × 756. B. Diagram to show plane of A. *m.e.*, segmental mesoderm block of left side; *np.*, portion of nephridium.

Meanwhile changes have been taking place in the thin hyposphere wall between the ectoderm and the posterior border of the mouth. At first this area is usually devoid of nuclei in the middle region, text-fig. 8C and D; but these gradually appear, text-fig. 8E–G, becoming more and more numerous until, by the time six segments are present the whole area has become crowded with them, text-fig. 8H. It seems that these nuclei are derived from the few scattered nuclei which occurred at the sides of the thin area during the early stages. This now crowded tissue joins the ectoderm to the posterior border of the mouth, and subsequently invaginates to form the buccal organ (see below, p. 265).

8. Later Development of the Worm Trunk.



Text-fig. 10.—Sagittal section to one side of median plane of an early stage of the development of the trunk invagination. × 756. Mouth on left side of figure. Larva much contracted.

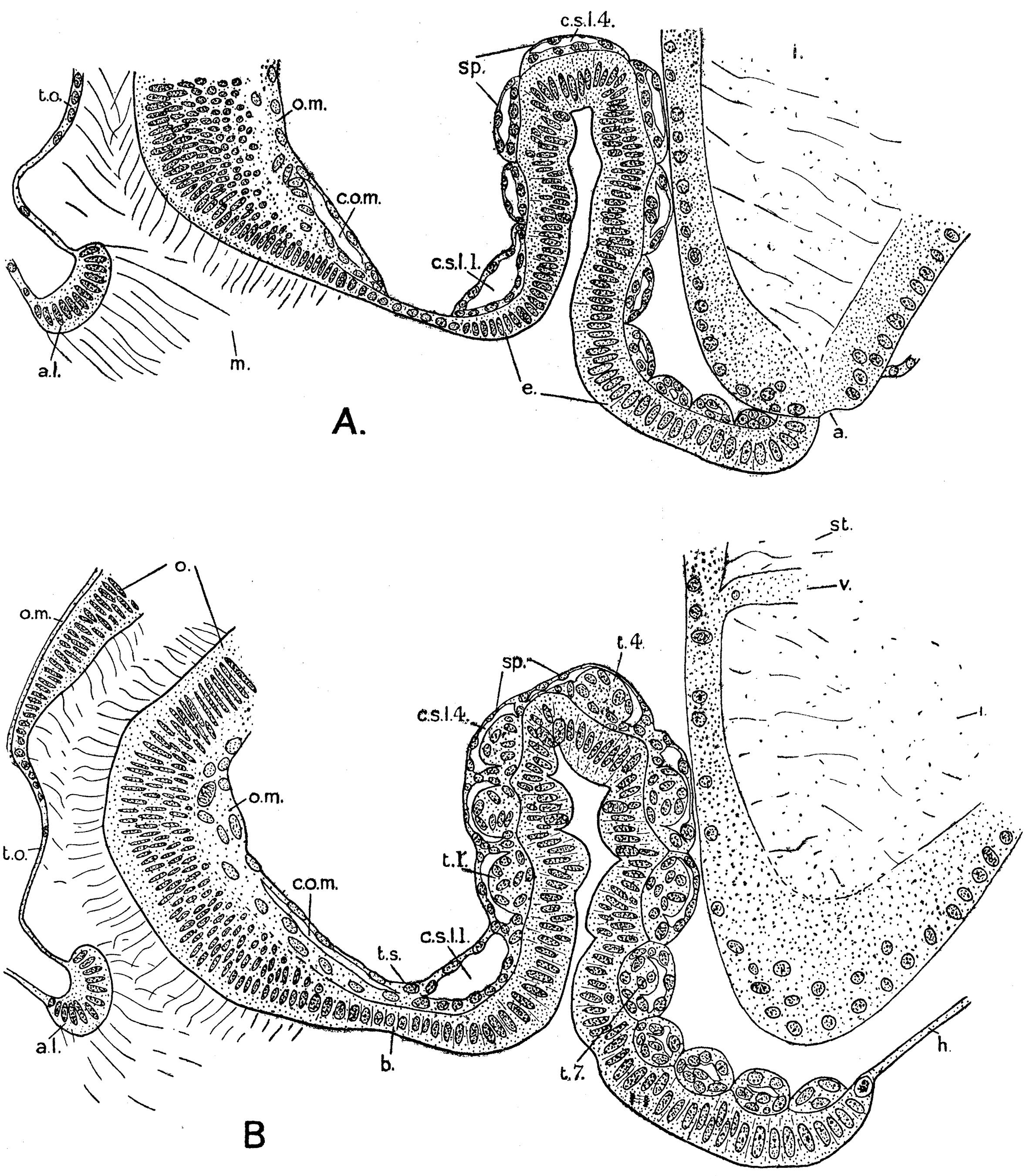
In the preceding section the development of the trunk as far as six segments has been described. It is peculiar in that it is folded upon itself to form an upright pocket-like invagination of the ventral surface between the mouth and the anus. The position of the fold at the top of the pocket gradually passes backwards as development proceeds, in such a manner that the anteriormost segments successively occupy the top of the structure and pass over on to its anterior face. An examination of the series of sections, text-figs. 8 and 10, should make this point clear; thus in text-fig. 8H, where six segments are present, the third is just approaching the summit, while in text-fig. 10, at a stage when seven segments are definitely indicated, it is passing down on to the anterior face, or, in other words, the fold which was originally between segments two and three, text-fig. 8H, has passed back until it is between three and four, text-fig. 10. This process continues regularly as further segments are produced posteriorly. In text-fig. 11A, the fourth segment occupies the summit of the ingrowth, there being eleven segments altogether, while a little later, when twelve segments are marked out, this fourth segment has passed over on to the anterior face, the fifth segment being almost at the top, text-fig. 11B. This also passes over on to the anterior face, and then the final condition is reached with the summit or fold of the pocket occupying a position between the fifth and sixth segments (the fourth and fifth adult chætigerous segments). This is seen in text-fig. 24, where the septum between chætigerous segments four and five is clearly at the top of the invagination. It, however, reached this position at a much earlier stage, actually before the trunk rudiment closed round the intestine (see below).

Soon after the stage shown in text-fig. 10 has been passed, splits appear in the paired segmental blocks of mesoderm, with the exception of the posterior pair. In this way the cœlom is formed, which rapidly hollows out the blocks. The fusion of the anterior wall of each hollow block with the posterior wall of the block in front gives rise to the septa, text-fig. 11, while in a similar way the fusion of adjacent walls near the middle line gives rise to the ventral mesentery, text-fig. 13.

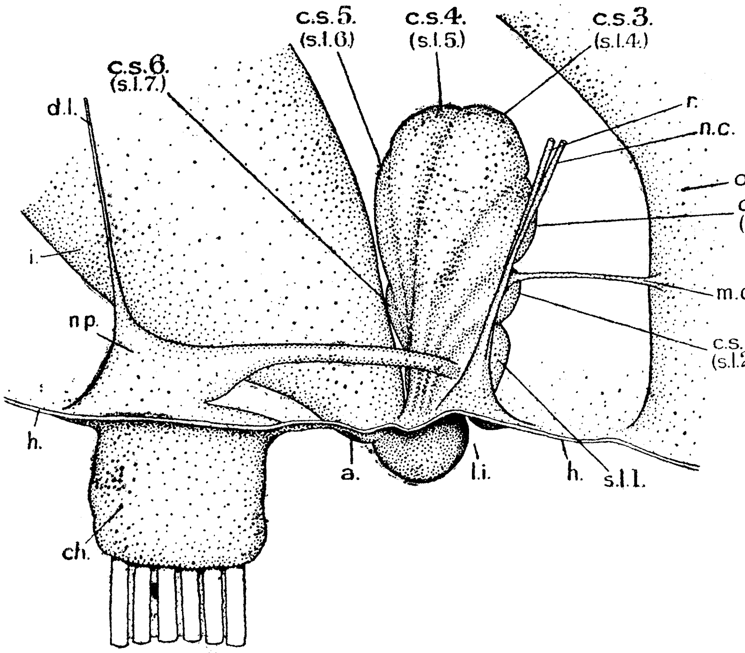
We must postpone any detailed consideration of the development of the various organs and tissues of the body segments until we have made clear the curious process by which the intestine comes to be surrounded by the invagination. We have seen how this forms an upstanding pocket immediately in front of the intestine, fig. 6, Plate 29, with the opening of the pocket crescentic in shape and tending to embrace the anus, fig. 8, Plate 30. This crescent becomes more and more curved until finally its two horns meet and fuse behind the anus, thus completely enclosing the latter within the invagination. To understand what happens to the individual segments, we must go back, however, to an earlier stage. Text-fig. 12 is a diagrammatic representation of the invagination as a solid opaque object, viewed from the right side, at a stage when nine or ten segments can be distinguished. On the anterior face are the first four segments, the fifth is nearing the top, and the remainder—of which the sixth, seventh and eighth can be seen in the figure—are on the posterior face. It is a stage a little earlier than that shown in text-fig. 11A. The important point to notice is that all the segments slope steeply downwards at the sides to reach the border of the crescentic opening at the tips of its horns. The segments on the anterior face are well in evidence, those on the posterior face are somewhat hidden behind the former. Note also that these lateral extensions pass down behind the nephridial duct, which discharges through the first segment.

This sloping off of the segments on either side of the invagination can be traced from early stages when only a few mesoderm blocks have been formed. As the main part of a block passes over the top from the posterior to the anterior face, so does its lateral extension pass around the side of the ingrowth from the posterior to the anterior face.

Transverse sections taken about the middle of the body rudiment confirm the above

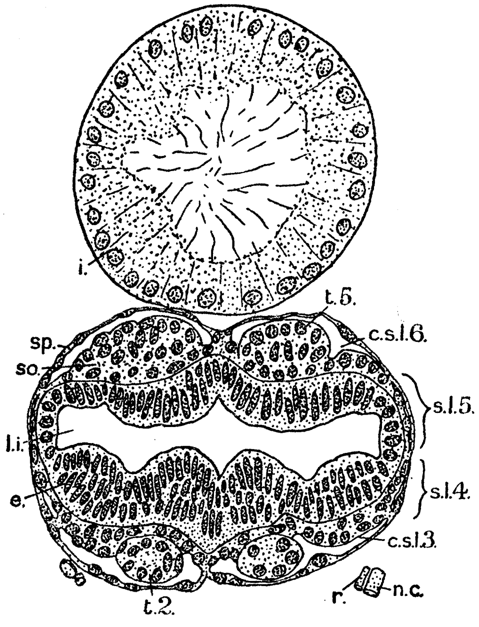


Text-fig. 11.—Sagittal sections of the worm trunk invagination. × 756. A. Section taken a little to one side of the median plane. The œsophageal mesoderm has not yet met the first larval segment. B. Section well to one side of the median plane. The œsophageal mesoderm has fused with the first larval segment. Text-fig. 20B, shows a sagittal section, close to the median plane, of the buccal organ portion of the same individual. *b.*, lateral part of buccal organ ; *c.o.m.*, cœlom in œsophageal mesoderm ; *c.s.l.*1, etc., cœlom of first larval segment, etc. ; *e.*, ectoderm of worm trunk ; *sp.*, splanchnopleure ; *t.*1. etc., first, etc., thread gland ; *t.s.*, transverse septum of buccal organ " segment " (*i.e.*, the combined segment formed by the fusion of the paired œsophageal mesoderm blocks with the first larval segment).



Text-fig. 12.—Stereogram of an early stage in the development of the worm trunk invagination. × 756. The tissues are represented as being opaque. *c.s.*1, *c.s.*2, etc., first, second, etc., chætigerous segment; *h.*, cut edge of hyposphere; *l.i.*, lumen of trunk invagination; *m.o.*, œsophageal muscle; *np.*, nephridium; *r.*, retractor; *s.l.*1, *s.l.*2, etc., first, second, etc., larval segment.

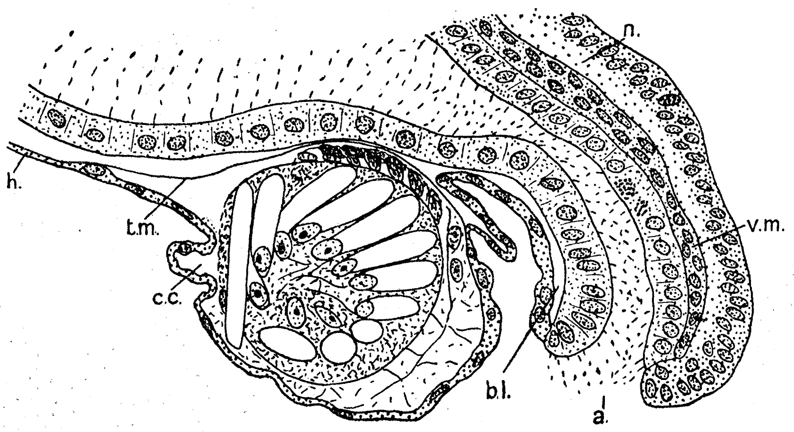
observations, which were made on sagittal sections and whole mounts. Such a section is text-fig. 13, which, while passing through the main parts of opposite segments three



Text-fig. 13.—Transverse section of trunk invagination at approximately the same stage as the sagittal section shown in text-fig. 11B. × 756. *c.s.l.*3, *c.s.l.*6, cœlom of second and sixth larval segments; *s.l.*4, *s.l.*5, dorsal portions of fourth and fifth larval segmental mesoderm blocks; *so.*, somatopleure; *t.*2, *t.*5, rudiments of second and fifth thread glands.

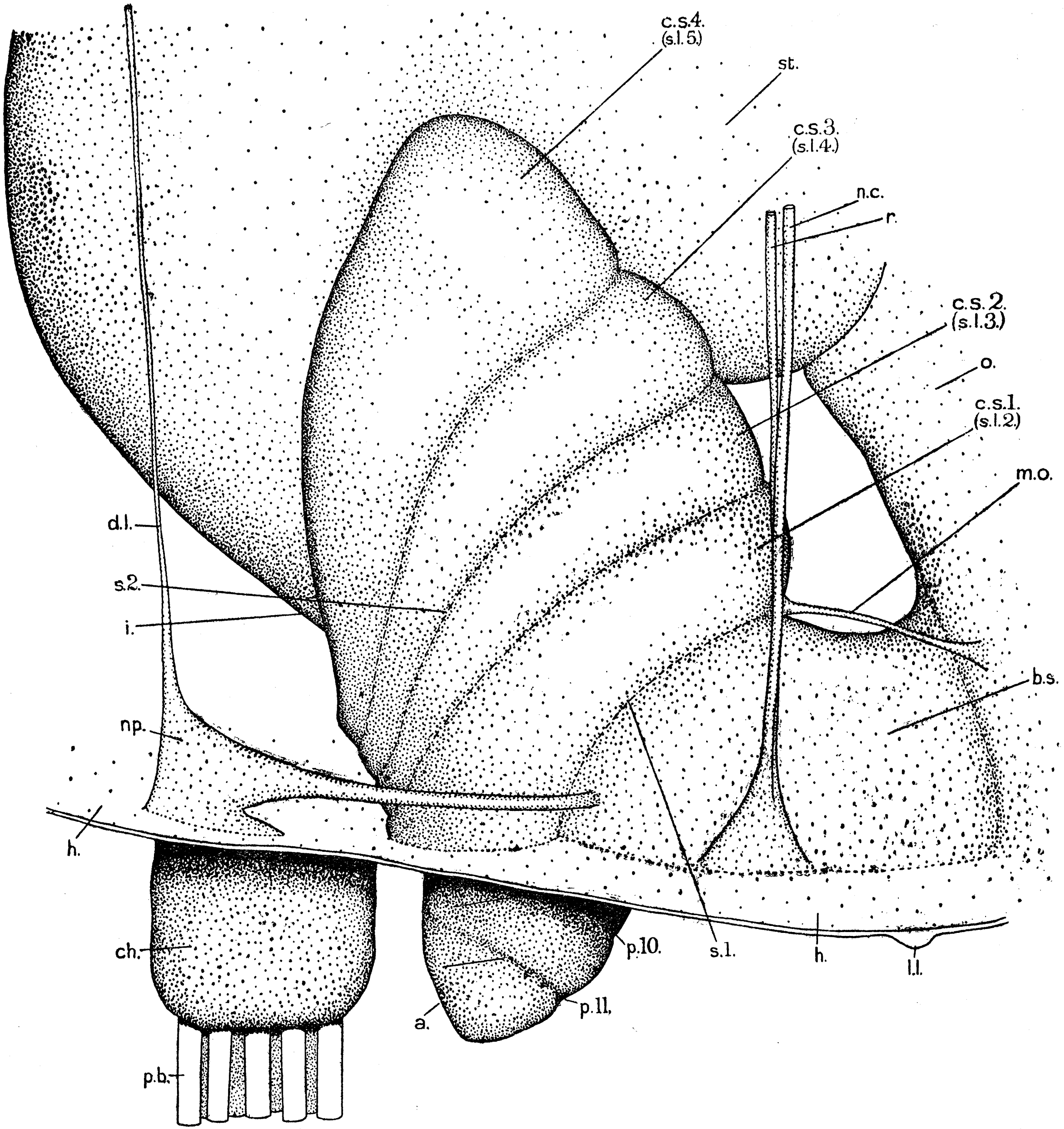
and six (chætigerous two and five) (see text-fig. 11B), also cuts the downward lateral extensions of segments four and five. These extensions are, however, often by no means easy to make out in early stages, although in favourable series they may, with care, be traced from section to section. Later on, after further growth, they are much more distinct, see text-fig. 17.

Consider now the gradual closing of the crescentic opening round the anus referred to above. The horns of this crescent are composed, as we have seen, of the ends of the downward extensions of the segments. As will shortly be apparent, these ends are dorsal, and can now be referred to as such. At the time when the horns meet, the first five segments are on the anterior face of the invagination, the remainder on the posterior face. The horns will then meet in the line of the septum, between segments five and six and the ectoderm in this line will be raised up to continue the upper edge of the invagination round the back of the intestine. For a short while this part of the ingrowth immediately behind the intestine is merely this ectodermal fold, text-fig. 14, but soon the dorsal ends of the mesoderm blocks of segment five will come into contact with one another on the outer side of the fold, and below them the dorsal ends of segments four, three and two (not one, as will be shown later). A little further growth, and we have the condition shown in text-fig. 15. In a similar manner, but on the inner side of the fold, the dorsal ends of segments six, seven, eight, etc., come into contact. The invagination thus forms a complete annular space around the intestine, high in front (ventral part of the trunk rudiment) and low behind (dorsal part of the trunk rudiment).



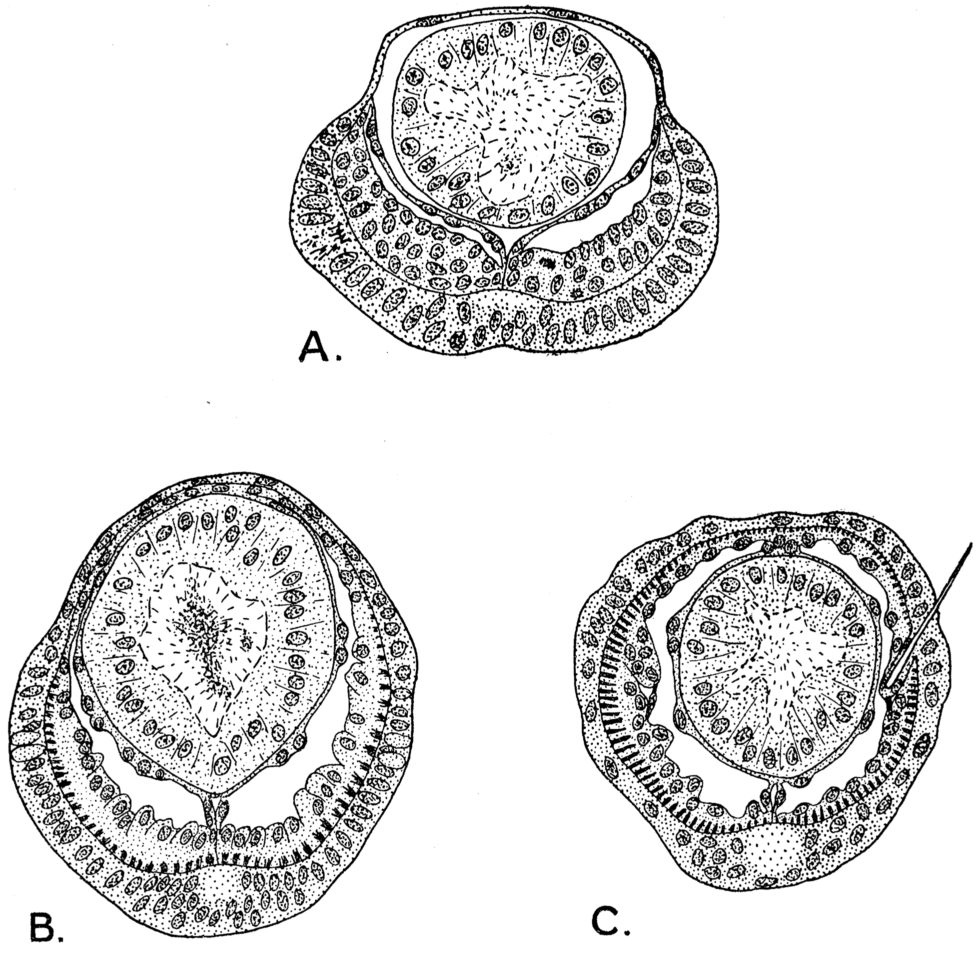
TEXT-FIG. 14.—Median sagittal section of posterior end of worm trunk shortly before the segmental mesoderm blocks meet round the intestine. × 756. *c.c.*, cœlom of larval chæta-sacs ; *n.*, ventral nerve cord ; *v.m.*, ventral mesentery.

The actual meeting of the dorsal ends of the segmental mesoderm blocks is extremely difficult to see in living specimens on account of its obscured position between the larval chæta-sacs and the gut ; moreover, it appears to take place very quickly. Sections, however, leave no doubt that the main features of what actually takes place are as described above, although they leave a little to be desired as regards the exact histological detail. There is no doubt, either, that where the dorsal ends of the



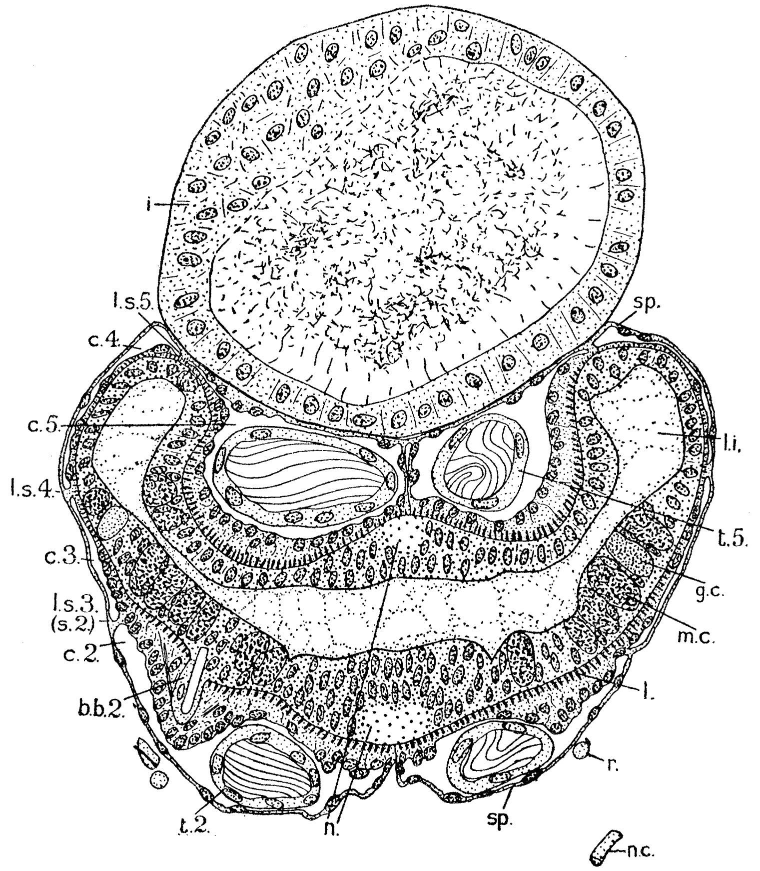
Text-fig. 15.—Stereogram of the worm trunk of a Mitraria shortly after the segments have met round the intestine. × 756. The tissues are represented as being opaque. *b.s.*, buccal organ segment formed by fusion of first larval segment with paired œsophageal mesoderm blocks; *l.l.*, posterior lateral lip of mouth; *p.*10, *p.*11, tenth and eleventh parapodia; *p.b.*, provisional bristle cut short; *s.*1, *s.*2, first and second adult septa; *s.l.*1, *s.l.*2, etc., first, second, etc., larval segment.

mesoderm blocks meet the dorsal mesentery comes into being. The process is not then essentially different from that which occurs in other and more typical Annelids. Here, as in Annelids, the segmental blocks meet ventrally to form the ventral mesentery, and extend round between ectoderm and gut to fuse dorsally, forming there the dorsal mesentery. The process here is complicated simply in that the first five segments develop inside out and that the segmental blocks of the anterior segments are unusual in tapering to very narrow dorsal extremities. This remark does not, however, apply in such a marked degree to the posterior segments (segments eight to twelve), where practically typical conditions prevail. Fig. 7, Plate 29, and fig. 9, Plate 30, show how the anus becomes carried out on the end of a projection in front of the larval chæta-sacs, and text-fig. 14 shows this projection in median sagittal section before the mesoderm blocks have finally surrounded the intestine. The projection is merely the developing tail-end of the worm, and the segmental blocks here have only to extend round between ectoderm and gut until they meet dorsally. The transverse sections of this region in text-fig. 16 will make this point clear. These segmental blocks are visible in living larvæ and whole mounts, and are shown in the drawings on Plate 29.



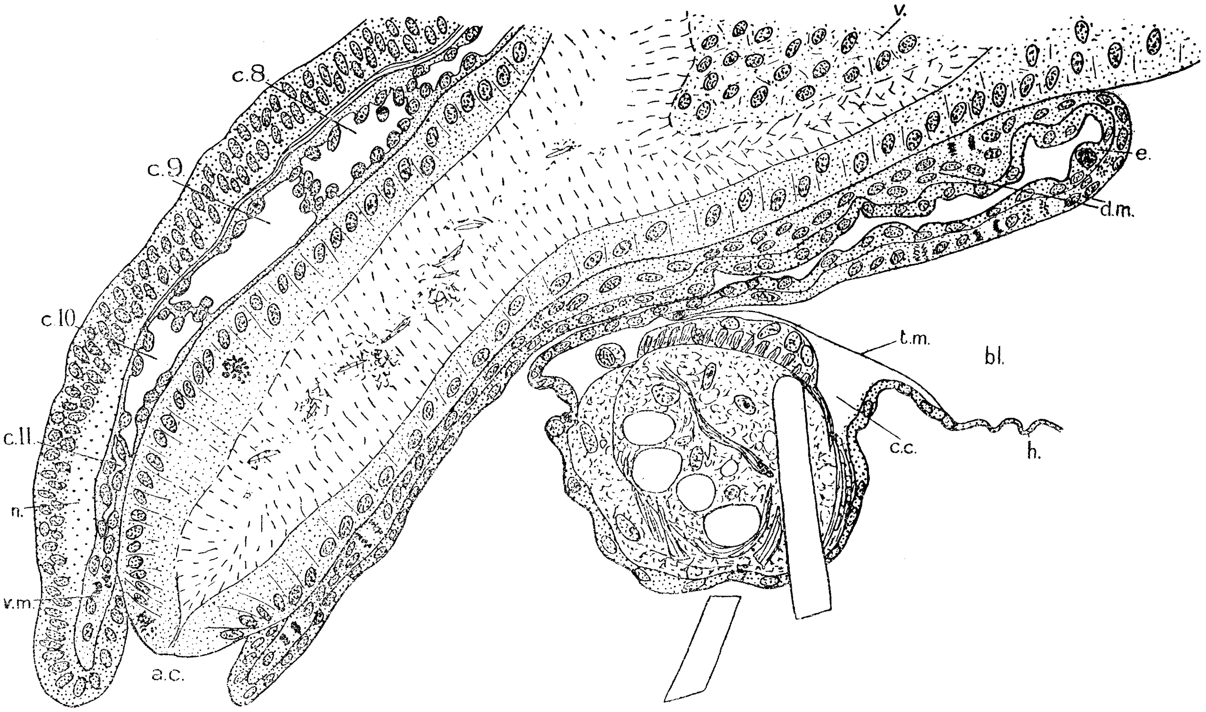
TEXT-FIG. 16.—Transverse sections of posterior trunk segments of Mitraria larvæ to show growth of segmental mesoderm blocks round to the dorsal side. × 756. A. Region of ninth chætigerous segment in a larva 20 days old. B. Region of posterior part of seventh chætigerous segment of a larva 16 days old but more advanced in development than A. This larva was from a different culture than A. The section is cut somewhat obliquely. C. Region of ninth chætigerous segment of a larva 22 days old. This larva was from the same culture as A.

So far we have neglected the first segment. This will be dealt with more fully when the development of the buccal organ is described (see p. 265), but it should be noted here that anteriorly it fuses with the mesenchyme on the œsophageal wall (see text-fig. 11 A and B), and so connects the trunk to the œsophagus. The septum between it and the second segment does not come to surround the intestine as do the remaining septa, but, running obliquely antero-dorsally, finishes its course at the anterior end of the trunk, a short distance behind the opening of the nephridial duct, text-figs. 12 and 15. Thus the position of the latter is little affected by the extension of the other segments round the gut. The development of the trunk has now been described as far as the condition represented in text-fig. 15 and fig. 9, Plate 30. The segments (excepting the first) form complete rings, broad ventrally and narrow dorsally. The anterior ones are inside out, but the sixth and succeeding ones bear a normal relation to the gut. A transverse section through the middle of the structure passes through parts of several segments, text-fig. 17, in a manner similar to a transverse section taken at a much earlier

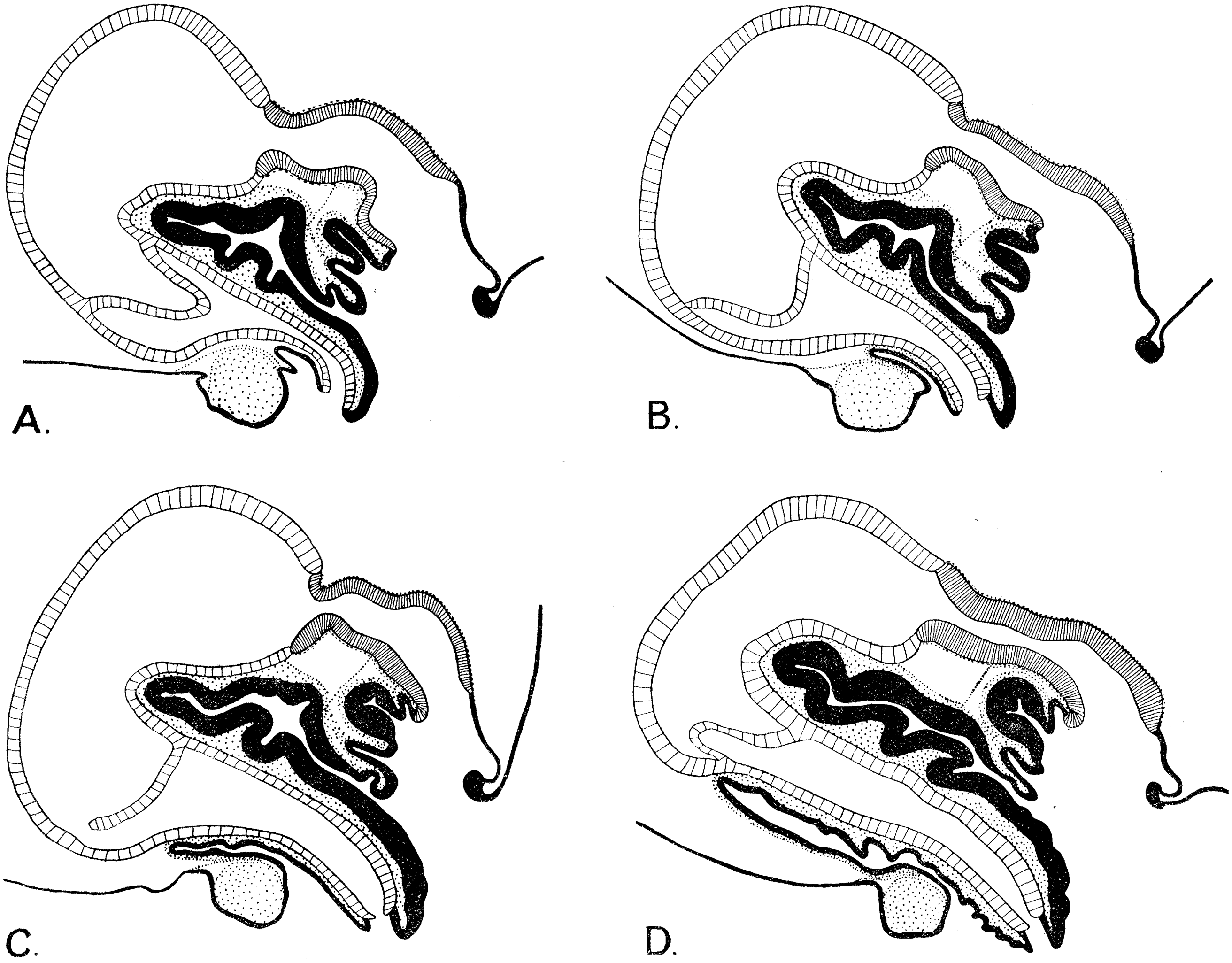


Text-fig. 17.—Transverse section through upper part of worm rudiment of a Mitraria at about the stage illustrated in the stereogram, text-fig. 15. × 756. From a preparation stained with Delafield's hæmatoxylin. *b.b.*2, second bristle bundle ; *c.*2, *c.*3, etc., cœlom of second, third, etc., chætigerous segment ; *g.c.*, gland cell ; *l.*, longitudinal muscles ; *l.s.*3, *l.s.*4, etc., third, fourth, etc., larval septum ; *m.c.*, mucus cell in ectoderm ; *s.*2, second adult septum ; *t.*2, *t.*5, second and fifth thread glands.

stage, text-fig. 13. The various tissues and organs of each segment are well developed in the more ventral parts, and, indeed, undergo comparatively little further growth until after metamorphosis. The main growth of the trunk now concerns the dorsal portions of the segments, and these grow very rapidly for about two days or less, until they are as broad as are the ventral portions. Most of this growth centres in the dorsal mesentery, which is obviously very short when first formed by the meeting of the tapered dorsal extremities of the segmental blocks. Mitotic division stages are very numerous in sections of the dorsal mesentery at this time, text-fig. 18, but it is a very puzzling fact that no division stages have been observed in the ectoderm of this dorsal region during the period of rapid growth, although a considerable number of larvæ have been studied. One can only conclude that the ectoderm is here increasing in length by changes in the shape and position of the cells on either side of the mid-dorsal line. As cell boundaries cannot be distinguished with any certainty, it has not been possible to prove this. The diagrams, text-fig. 19, illustrate the growth of this mid-dorsal region, and show clearly its increase in size relative to the mid-ventral region. They also show the way in which the latter is folded. The folds vary a little in different individuals, and, owing to contractions, in the same individual from time to time. There is generally in the region of the second segment a very characteristic projecting fold, text-fig. 19A–C, which tends to disappear in the last larval stages, text-fig. 19D. Segments seven and eight



TEXT-FIG. 18.—Median sagittal section of posterior end of worm trunk at the stage when the dorsal mesentery is rapidly forming. × 756. *a.c.*, anal chamber; *c.*8, *c.*9, etc., cœlom of eighth, ninth, etc., chætigerous segment; *d.m.*, dorsal mesentery showing many nuclear division stages; *v.m.*, ventral mesentery showing nuclear division stage.



TEXT-FIG. 19.—Diagrams of median sagittal sections of the worm rudiment to illustrate its growth in the later stages. × 240. A. From a larva 22 days old, before the mesoderm has surrounded the intestine. B. From a larva the same age as A but more advanced, the mesoderm having met round the intestine. C. From a larva the same age as A and B but still more advanced. The dorsal part of the trunk rudiment is growing rapidly. D. From a larva 27 days old and ready to metamorphose. *Ectoderm*, black ; *Mesoderm*, stippled ; *Gut*, shaded.

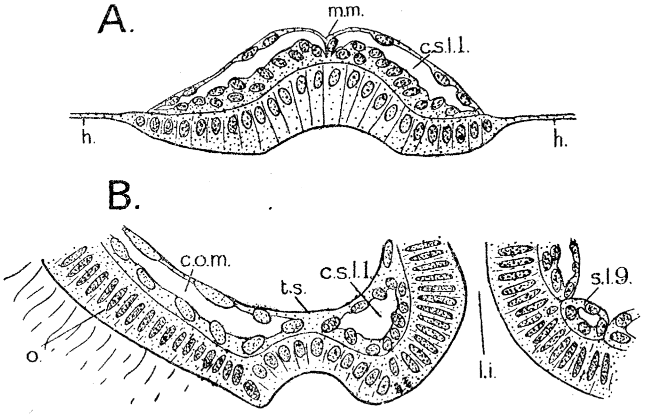
(chætigerous six and seven) develop strongly-marked folds which will be referred to later.

One point remains. It has already been shown how the larval chæta-sacs are cut off from the blastocœl by an extremely fine membrane. Some time during the dorsal meeting of the mesoderm blocks the anterior part of this membrane so fuses with the second segment that the cavity of the chæta-sacs communicates with the combined cœlom of the second and third segments, text-fig. 24 and fig. 15, Plate 32. Exactly how this takes place has not been seen on account of the tissue being so very fine and compressed between other tissues.

9. DEVELOPMENT OF THE BUCCAL ORGAN.

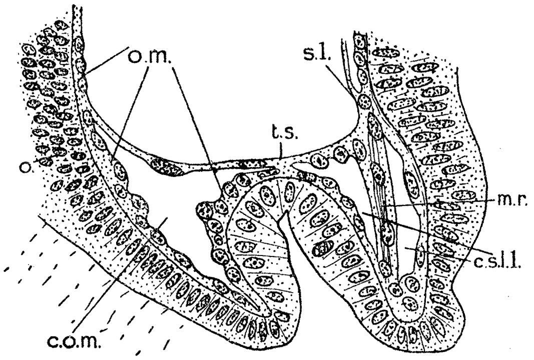
It will be remembered that in the very early stages of the development of the trunk, before it had formed any very definite invagination, there existed a thin area of tissue between the trunk ectoderm and the mouth which was devoid of nuclei, except at the sides (p. 255). This area gradually became nucleated, apparently by migration or division of the lateral nuclei. Thus arose the primitive ectoderm of the buccal organ.

The œsophagus from an early stage has a covering of what is apparently larval mesenchyme, which gives rise to the circular muscles surrounding it. This mesenchyme or œsophageal mesoderm, as it can conveniently be called to distinguish it from the mesenchyme filaments crossing the blastocœl, is thickest on the ventral side of the œsophagus, where its nuclei are fairly numerous, and are shown in text-figs. 2 and 27, *o.m.* As the trunk invagination grows, this mesoderm spreads down the œsophagus and over the primitive ectoderm of the buccal organ to meet the mesoderm of the first trunk segment, which likewise advances—but in the opposite direction—over this ectoderm, text-fig. 11A and B. They meet midway on this ectodermal floor and there fuse. Before this happens, however, the œsophageal mesoderm develops two cavities, *c.o.m.*, lying side by side with a median mesentery between them. When fusion takes place this mesentery forms a continuation of that between the hollow segmental blocks of the body segments. The posterior walls of the œsophageal cavities which fuse with the anterior walls of the first segment produce a transverse septum similar to the transverse septa of the trunk region proper. Thus this septum and the mesentery cross at right angles in the middle of the area of buccal organ ectoderm. This point marks out the summit of the ectodermal invagination which now infolds here. It is shown in median sagittal section in text-fig. 20B, and in transverse section in text-fig. 20A. In the



TEXT-FIG. 20.—Sections of early stages of buccal organ invagination. × 756. A. Transverse section immediately posterior to the transverse septum formed between the first larval segment and the œsophageal mesoderm blocks. B. Sagittal section just to one side of the median mesentery. This is from the same individual as the more lateral sagittal section shown in text-fig. 11B. *c.o.m.*, cœlom of one of the œsophageal mesoderm blocks ; *m.m.*, median mesentery ; *o.*, ventral wall of œsophagus ; *s.l.*9, ninth larval segment ; *t.s.*, transverse septum.

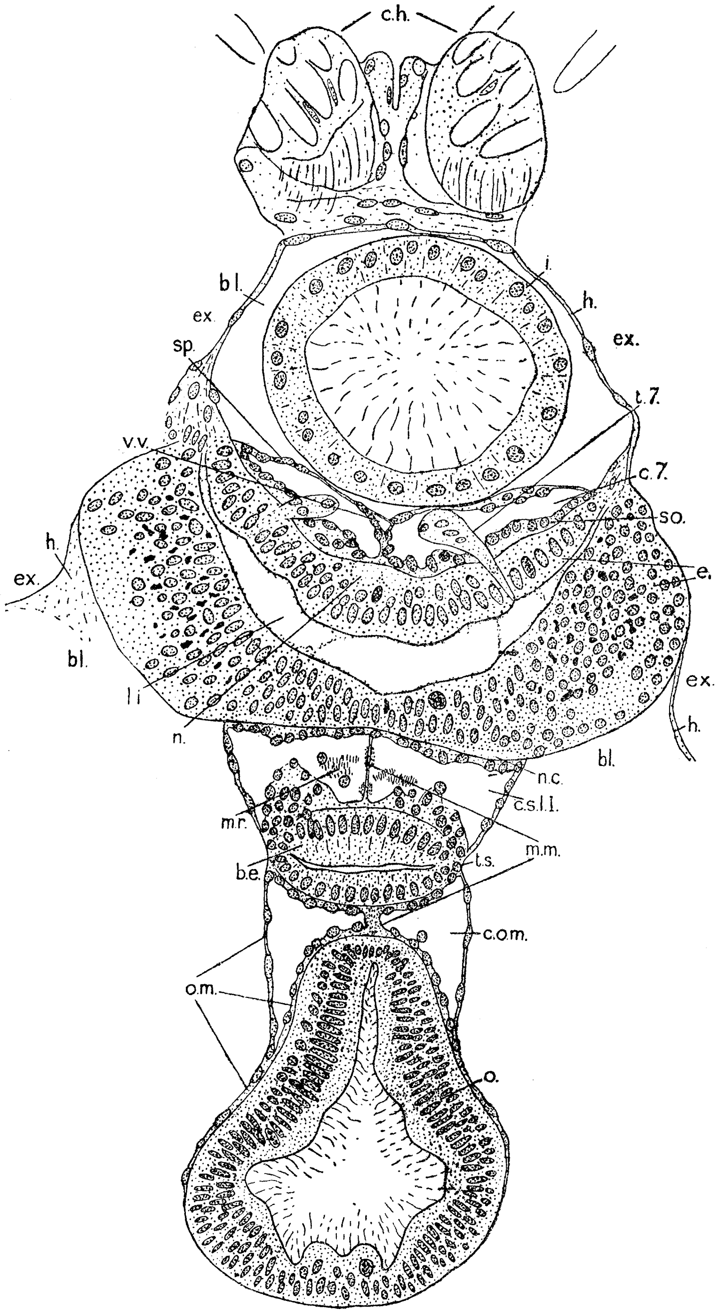
former the section passes just to one side of the median mesentery, and in the latter just posterior to the transverse septum. It will be remarked that at this stage the nuclei belonging to the œsophageal mesoderm are larger and less darkly stained than those of the first trunk segment, a difference that disappears later on.



Text-fig. 21.—Sagittal section a little to one side of median plane of buccal organ invagination of a Mitraria 20 days old. × 756. *m.r.*, median retractor of buccal organ; *s.*1, first adult septum; *t.s.*, transverse septum of buccal organ segment.

The ectoderm which invaginates to form the buccal organ slowly thickens as its component cells become taller. The nuclei have all along been fairly easily distinguishable from those of the immediately surrounding ectodermal tissue; at this stage they stain less readily, and they are a trifle more rounded in shape. Text-fig. 21 shows a stage when the invagination is fairly well advanced, and some of the muscle fibres (*m.r.*) destined to move the organ are beginning to appear. These are the paired median retractors, which ultimately run from the general longitudinal body musculature to the top of a fold that shortly appears by the posterior border of the organ. They seem to be differentiated from cells lying just on either side of the median mesentery. They are shown cut across in text-fig. 22, which is a section of the structure a little later in its development. In this figure the median mesentery (*m.m.*) is plainly visible, and so is the transverse septum (*t.s.*), although at this place it is rather thick. The mesoderm cells covering the ectoderm of the buccal organ (*b.e.*) give rise to other muscles, until finally it becomes an extremely mobile structure. The detailed development of these muscles has not been worked out, but the system, as finally developed, is described below.

In late larvæ the ectodermal cells forming the buccal organ become clear and transparent, with the cell limits fairly distinct. The rather irregular nuclei have large darkly-staining chromatin granules. At rest the organ is doubled up, text-fig. 24, and in this position is closely invested in a layer of powerful muscle fibres whose main direction is transverse. These are shown cut across (*t.p.*). Internal to these is another layer of fibres (*o.l.p.*), whose main direction is longitudinal, and which are attached at the base of the organ in front and behind. Internal to these again is a pair of muscles (*i.l.p.*), each consisting of several fibres, which run over the top of the organ, text-figs. 24 and 23, to be attached to invaginated folds of the body wall (*a.b.f.* and *p.b.f.*) anteriorly and posteriorly to the buccal organ. These folds arose since the stage shown in text-fig. 21, and a general idea of their development may be obtained from text-fig. 19. These folds are shown cut across in text-fig. 32, where it will be seen that they are merely slightly curved transverse pockets. The anterior fold is much smaller than the posterior.

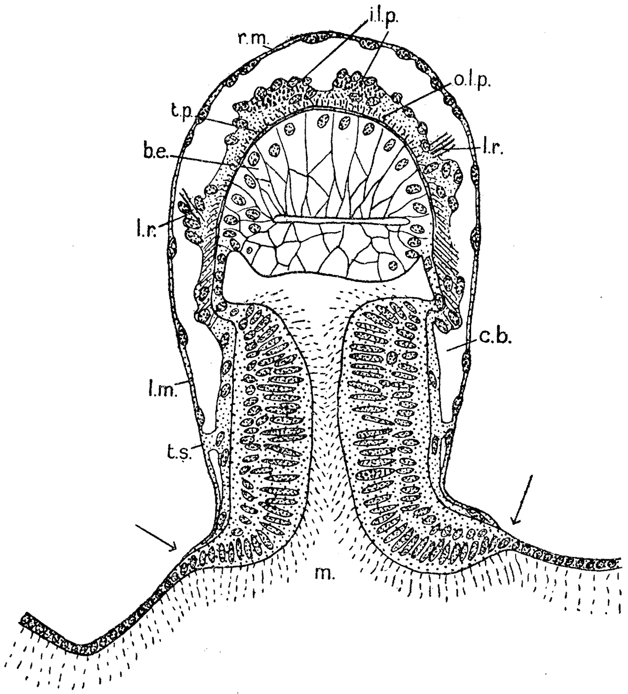


TEXT-FIG. 22.—Transverse section of worm trunk invagination, close to the external opening of its lumen, of a Mitraria 20 days old. × 630. From a preparation stained with Delafield's hæmatoxylin. *b.e.*, ectodermal cells of buccal organ which eventually become clear and transparent; *c.*7., cœlom of mesoderm block forming left side of seventh chætigerous segment (eighth larval segment); *c.o.m.*, cœlom in left block of œsophageal mesoderm; *c.s.l.*1, cœlom in left block of first larval segment. This later communicates with *c.o.m.* to form the common cœlom of a combined buccal organ segment; *e.*, ectoderm. The part near the first larval segment is turned back almost at right angles to the longitudinal axis of the invagination and is therefore cut level with its surface. The irregular black patches represent developing mucus cells; *ex.*, the external world; *l.i.*, lumen of invagination. Dotted lines represent mucus; *so.*, somatopleure or peritoneal mesoderm; *t.*7., left thread gland showing duct; *v.v.*, ventral blood vessel in ventral mesentery.

The muscles so far described are protractors, and by their contraction evert the buccal organ in the form of a tongue. It is shown partially everted in text-fig. 37A, which is a section of a young worm shortly after metamorphosis.

The pair of median retractors has already been mentioned. One is shown again in text-fig. 24 (*m.r.*), where it will be seen that it is attached to the posterior buccal fold by the side of the paired protractors. At their other ends they run into and merge with the general longitudinal musculature of the body wall. A pair of lateral retractors can be seen in transverse sections, text-fig. 32, *l.r.*, running from the sides of the organ to the body wall again to merge into the longitudinal musculature. They form incomplete horizontal partitions after metamorphosis (p. 302 and text-fig. 45).

Before this set of muscles starts to function the median mesentery (shown in text-figs. 20A, and 22) disappears, see text-fig. 23. So also does the dorsal part of the transverse septum (compare text-figs. 21 and 24) which was formed between the paired cavities of the mesoderm blocks of the first segment and those of the œsophageal mesoderm. In this way the cavities of the œsophageal mesoderm communicate with those of the



Text-fig. 23.—Transverse section of buccal organ of a Mitraria ready to metamorphose. × 756. The approximate plane of this section is indicated by the line XY in text-fig. 52. *b.e.*, clear ectodermal cells of buccal organ ; *c.b.*, cœlom of buccal organ segment ; *i.l.p.*, inner longitudinal protractors of buccal organ ; *l.m.*, lateral part of splanchnic mesodermal wall of buccal organ cœlom which after metamorphosis forms part of horizontal septum ; *l.r.*, lateral retractors of buccal organ ; *m.*, posterior part of mouth ; *o.l.p.*, outer longitudinal protractors of buccal organ ; *r.m.*, splanchnic mesodermal roof of buccal organ cœlom which after metamorphosis forms part of the first adult septum ; *t.p.*, transverse protractors of buccal organ.

first segment to form a common buccal organ cavity. The lateral portions of the transverse septum, however, remain, text-fig. 32, *t.s.* By the disappearance of the median mesentery and the dorsal part of the transverse septum resistance to the protraction of the buccal organ is removed, and in late larvæ the occasional pushing out and drawing back of this tongue-like structure in a kind of licking motion is frequently observed. In adult worms it is used in tube building (WATSON, 1901) and possibly also in feeding.

10. DEVELOPMENT OF THE ANAL CHAMBER.

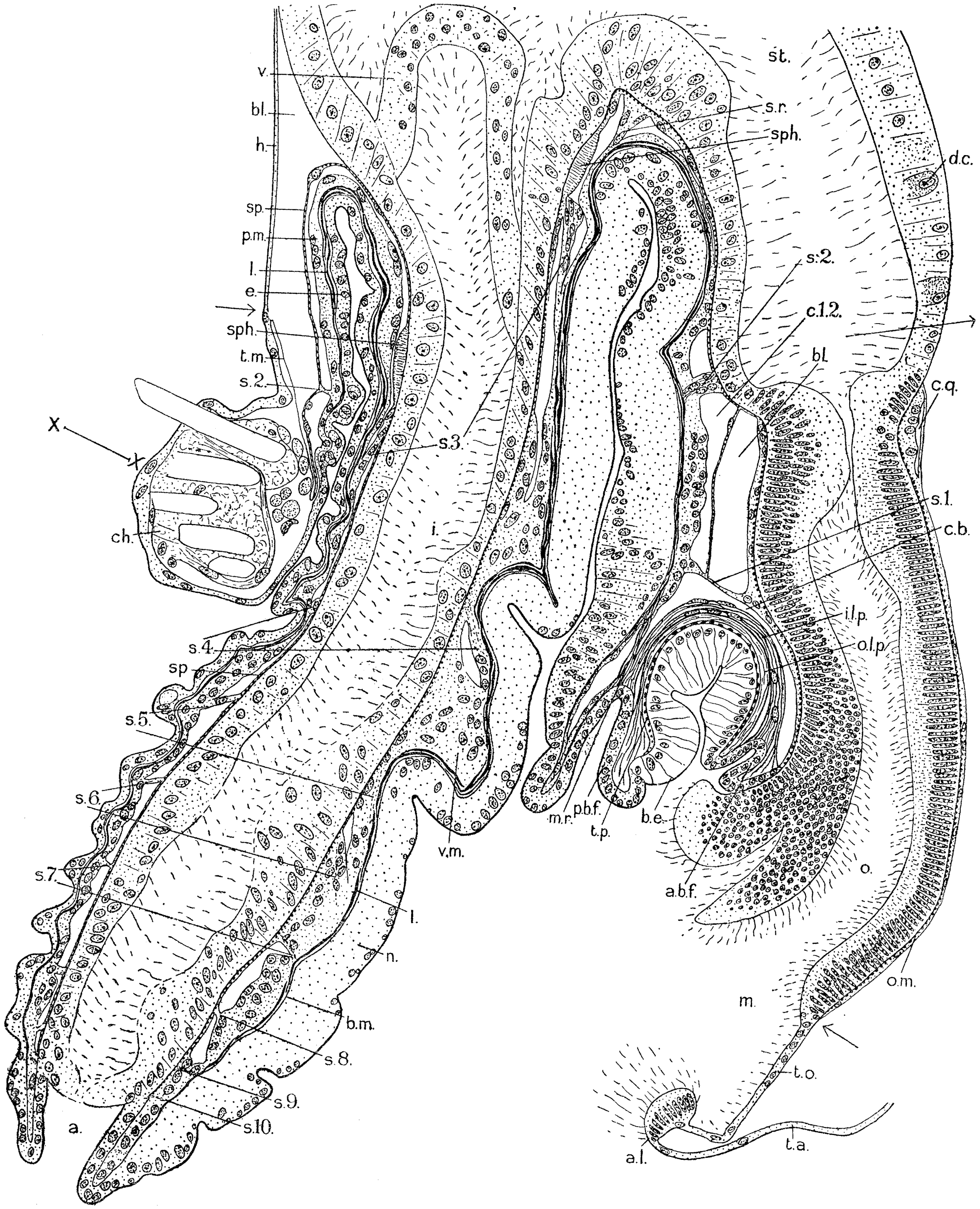
The anus during early stages discharges directly to the exterior, text-fig. 14, but later it becomes enclosed in a small ectodermal chamber, text-figs. 18 and 24. The steps by which this chamber is produced by an inturning of the ectoderm, combined with growth of this tissue—and the mesoderm which it encloses—past the anus in a posterior direction is indicated in text-fig. 19. It will be noticed that there is a marked lag in the formation of the posterior wall of the chamber, which always remains lower than the anterior wall. The latter, in turn, is lower than the lateral walls, fig. 15, Plate 32. Frontal sections show the mesoderm of the circular wall of the chamber to be hollow, and this cavity to be cut off from the body cœlom by a definite septum level with the true anus (septum 10 in text-fig. 47). The mesenteries extend into the wall and divide the cavity into right and left halves.

11. DIFFERENTIATION OF THE TISSUES OF THE WORM TRUNK.

(i) *Ectoderm.*

A.—*Epidermis.*—We have already followed the early development of the ectoderm as an invaginated pocket. The cells at this stage are all much alike, with relatively large nuclei and the cell boundaries ill-defined. The ectoderm is at first one cell thick everywhere, and remains so over the greater part of the trunk. On either side of the mid-ventral line, however, it becomes much thicker than elsewhere, and although the cell-limits are not clearly defined, the numerous rather crowded nuclei show that it must be regarded as several cells in thickness.

During the later stages of the invagination, and before the segments have surrounded the intestine, the epidermis becomes well supplied with gland cells. The content of some of these cells is coarsely granular, and stains very darkly with Delafield's hæmatoxylin, text-figs. 17, 26 and 32, *m.c.*, and Mayer's mucicarmine, remaining practically unstained with Heidenhain's iron hæmatoxylin, thus showing it to be mucus. The content of other gland cells (*g.c.*) scattered among these does not so stain, but appears dense and finely granular. Here and there empty discharged mucus cells (*d.m.c.*) can be seen. These have emptied their contents into the lumen of the invagination, which, with the two former stains, is shown to be well lubricated with mucus. Ectodermal mucus cells are very characteristic of the adult worm, where, as in later larvæ and



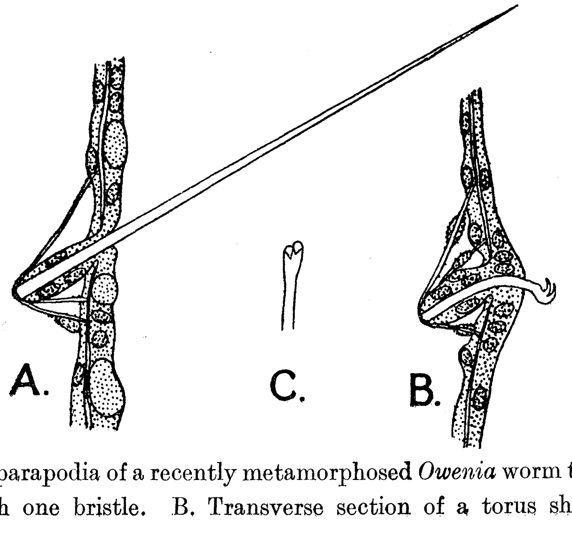
Text-fig. 24.—Slightly oblique median sagittal section of the worm trunk of a Mitraria ready to metamorphose. × 630. From a preparation stained with Heidenhain's hæmatoxylin. Portions of the ventral mesentery appear in this section, the remaining parts were in the adjacent section. The whole of the dorsal mesentery was in the adjacent section. The trunk is extended about as far as it can be at this stage. Arrows—except the one labelled *bl.*—indicate places of breakage at metamorphosis. Line XX indicates approximate plane of section shown in text-fig. 32. *a.b.f.*, anterior buccal fold; *b.m.*, basement membrane; *c.*1. 2., combined cœlom of first and second chætigerous segments; *ch.*, provisional chæta-sacs; *c.q.*, cavity in œsophageal mesoderm of unknown function. May form part of vascular system in adult; *d.c.*, dark staining amœboid cell of stomach; *o.m.*, œsophageal mesoderm and circular muscles of œsophagus; *p.b.f.*, posterior buccal fold; *p.m.*, peritoneal mesoderm; *s.*1, *s.*2, etc., first, second, etc., adult septum; *sp.*, splanchnopleure, and contained circular muscles; *sph.*, sphincter in splanchnopleure; *s.r.*, remaining ventral part of fifth larval septum; *t.a.*, thin non-ciliated hypospheral area anterior to mouth.

recently metamorphosed worms, they are specially numerous in the anterior region, text-figs. 26, 32 and 38. Similar mucus cells are present on the head.

The ectodermal cells rest on a strong basement membrane, which is always clearly distinguishable.

B.—*Ventral Nerve Cord.*—In *Owenia* the ventral nerve cord is unusual in that it is situated outside the basement membrane. It appears sometime before the mesoderm has encircled the gut, text-fig. 22, and in the early stages is clearly composed of two cords lying side by side. This distinction into two cords gradually disappears, text-fig. 17, until finally it is not visible, text-fig. 32. Anteriorly in the region of the first adult septum, text-fig. 24, it divides into right and left portions, which, after continuing laterally through the ectoderm for a short way, pass upwards through the blastocœl as the circumœsophageal commissures to the head, text-fig. 28B, *n.c.*

C.—*Parapodia.*—The first larval segment which enters into the formation of the combined buccal organ segment does not develop parapodia. The second larval segment develops bristles, and becomes the first chætigerous segment of the adult. The third and fourth larval segments likewise develop bristles. The fifth and succeeding larval segments develop bristles and uncini, the latter forming tori below the bristles. The bristle-sacs arise a little while after the stage shown in text-fig. 13 as ingrowing projections of ectoderm into the mesoderm. Text-fig. 17 shows one (*b.b.*2.) sometime after its first appearance (in the section the bristle is cut short, it passes into the lumen of the body ingrowth in the next section). The mesoderm immediately surrounding the projection gives rise to the muscles which move the bristle. Uncini appear to develop in an exactly similar manner, and arise about the same time as, or shortly after, the formation of the bristles. All these chætæ project into the lumen of the ingrowth, see text-figs. 26 and 31, and fig. 15, Plate 32, except, of course, those of the tail-end of the worm. Text-fig. 25 shows the structure of a bristle-sac and torus of a young worm immediately after metamorphosis.



TEXT-FIG. 25.—Sections of parapodia of a recently metamorphosed *Owenia* worm to show structure. × 756. A. Bristle bundle with one bristle. B. Transverse section of a torus showing uncinus. C. Front view of uncinus.

The occurrence of the chætæ is interesting. The first two pairs of parapodia soon have two bristles in each bundle, while the remaining nine pairs have only one bristle each. The number of the uncini is subject to more variation, for not only are there slight differences between larvæ of the same age and between the two sides of the same larva, but the number steadily increases with age. The following are typical counts from three larvæ of different ages :—

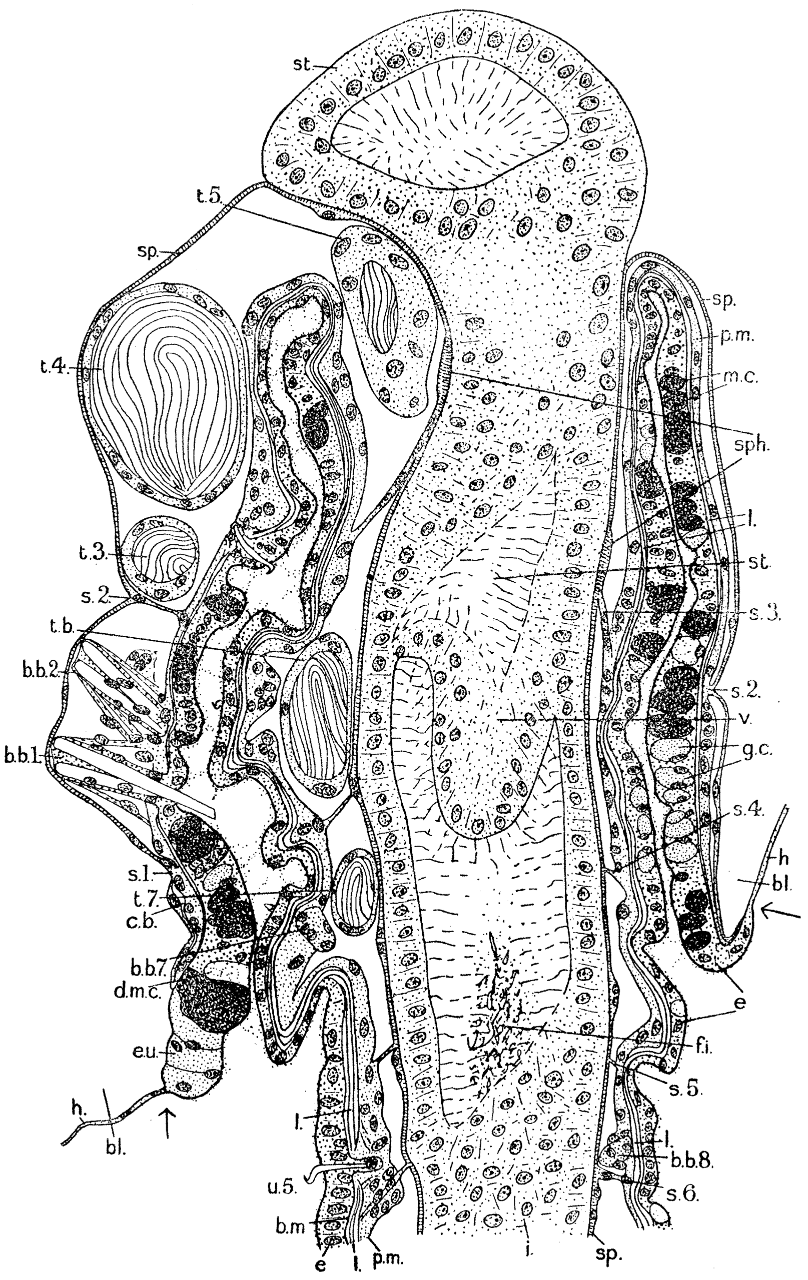
Parapodium.	Late ingrowth sometime before meeting of mesoderm round intestine.		Stage at which mesoderm is just meeting round intestine.		Last stage shortly before metamorphosis.	
	Left side.	Right side.	Left side.	Right side.	Left side.	Right side.
4	5	4	7	7	11	12
5	5	4 or 5	6	6	11	10
6	3	3	7	6 or 7	9	9
7	2	2 or 3	3	4	8	7
8	6 or 7	4 or 5	9	9	14	13
9	3	3	3 or 4	3 or 4	5	6
10	1 or 2	1	2 or 3	3	5	4
11	None	None	2	2	3	3

An interesting feature is the large number of uncini on the eighth chætigerous segment, where there are even more than there are on the fourth, the most anterior segment to bear tori. As will be shown below (p. 306), this segment marks a special transition from the type of segment in front to the type behind. Apart from this interruption of the series, the numbers on the whole progressively decrease posteriorly. The uncini, as a rule, form a single row in each torus, but on the eighth chætigerous segment they are roughly in two rows, and a tendency to form two rows is sometimes observed in the anterior tori.

(ii) *Mesoderm.*

A.—*Septa.*—As has already been pointed out, these arise by fusion of the anterior and posterior walls of neighbouring segmental cœlomic sacs (p. 256). Thus at first there is a complete series of septa, one between each segment, text-fig. 11. This condition does not persist for long, however, because shortly before the segments meet round the intestine certain septa begin to disappear. These are situated between segments two and three (adult chætigerous one and two) ; four and five (adult chætigerous three and four) ; and five and six (adult chætigerous four and five.) For a time these septa may be found between the dorsal extensions of these segments, text-fig. 17, while they have completely disappeared ventrally, except that in the last-mentioned case a narrow strip of septum remains on each side of the ventral mesentery, text-fig. 24, *s.r.* These dorsal portions disappear during the rapid growth of the dorsal part of the segments behind the intestine.

As a result of this disappearance of the septa, the first and second chætigerous segments form a common cavity, the thoracic cœlomic cavity of the adult. Likewise the third, fourth and fifth chætigerous segments form a common cavity, see text-fig. 26,



TEXT-FIG. 26.—Section of anterior part of worm rudiment of a Mitraria ready to metamorphose. × 630. From a preparation stained with Delafield's hæmatoxylin. Arrows indicate places of breaking at metamorphosis. The approximate plane of this section is shown in text-fig. 32. *b.b.*1, *b.b.*2, etc., first, second, etc., bristle bundle; *d.m.c.*, discharged mucus cell; *e.u.*, anterior lateral part of trunk ectoderm which is not covered internally by mesoderm; *f.i.*, food in intestine; *s.*1, *s.*2, etc., first, second, etc., adult septum; *sp.*, splanchnopleure, drawn thicker than it actually is; *t.*3, *t.*4, etc., third, fourth, etc., thread gland; *u.*5, uncinus of fifth torus.

and fig. 15, Plate 32. Further discussion of the relation of these various cavities and septa to one another is reserved until later (p. 305).

B.—*Mesenteries and Associated Blood Vessels.*—We have already seen how the ventral mesentery is formed by the fusion of the adjacent median walls of the segmental blocks (p. 256). What is not so clear is the formation of the ventral blood vessel situated in this mesentery. At the stage of text-fig. 13, no such vessel is present, but in text-fig. 17 it is seen on the posterior part of the invagination, but not on the anterior part. It is present, however, higher up the anterior part in the region of the third and fourth chætigerous segments. It could have been formed by the two components of the mesentery fusing above and below it, or by larval mesenchyme cells forming the dorsal wall of an essentially triangular vessel. In text-fig. 22 the dorsal wall is exceedingly thin, and suggests that the second explanation is the correct one, but it is not possible to be sure. Mesenchyme cells appear to enter into the formation of some of the blood vessels of *Polygordius* (WOLTERECK, 1905). If the same process occurs here it only does so as far forward as the septum between the third and second chætigerous segments (second adult septum). Anterior to this septum the vessel is formed at metamorphosis by another process (see below, p. 290).

The rapid formation of the dorsal mesentery has been described above. In cross section it appears triangular in form, text-fig. 32, *d.m.*, with the apex attached to the body wall, and it is solid as far forward as the septum between chætigerous segments two and three. Anterior to this, the second adult septum, it is, in the last larval stage, different in structure as it there contains a dorsal blood vessel, and below it a much larger cavity, which is probably blastocœlic in nature. It is very difficult to make out the true relations of the various parts of the mesentery at this place, as they are closely pressed together. In occasional sections, however, they can just be made out, and are clearly visible when opened out after metamorphosis. These relations are shown best in text-figs. 32 and 39. The latter figure shows the mesentery just behind the first septum a few days after metamorphosis (see p. 293), but the structure of the more posterior part immediately after metamorphosis is the same.

C.—*Splanchnopleure.*—Attention must now be directed to the thin layer of mesoderm which was split off from the thicker mass adhering to the body wall during formation of the cœlom, text-figs. 11 and 13, *sp.* This tissue, the splanchnopleure, becomes very thin, with its nuclei scattered far apart. During the later stages (stage of fig. 7, Plate 29, and onwards) of the invagination it becomes loosely attached to the stomach and intestine on either side of the middle line (compare text-figs. 13 and 17). The intestine finally becomes closely enveloped in it, text-figs. 24, 26, 32, etc., while the ventral surface of the stomach is in contact with the turned-back splanchnopleure of chætigerous segments three and four for some distance on each side of the middle line text-figs. 24 and 31, and fig. 15, Plate 32. The œsophagus, however, is quite free from it, and the splanchnopleure anterior to the first adult septum does not come into contact with the gut during larval life.

Where the splanchnopleure comes into contact with the gut it is only very slightly adherent, there is no actual fusion, and it seems likely that the gut can to some extent slide up and down within it. Even where it completely surrounds the intestine, it frequently leaves irregular spaces where it does not actually touch (text-fig. 32, just below dorsal mesentery). After metamorphosis the peri-intestinal blood sinus forms (p. 300) between it and the gut. The contact is thus of a temporary nature only.

In the later larval stages very fine circular muscles can be detected in the splanchnopleure. They are indicated rather diagrammatically in text-fig. 24, *sp.* These muscles come into action when the blood sinus has formed, and then produce the characteristic peristaltic contractions which drive the blood forwards. It is uncertain whether or not they are present anterior to the second adult septum.

Immediately in front of the third adult septum (that anterior to the sixth chætigerous segment) there forms a powerful sphincter muscle in the splanchnopleure, text-figs. 24 and 26, *sph.* This muscle was first described for the adult by ZÜRCHER (1909). During larval life it runs obliquely round the gut, its ventral part being well anterior to its dorsal. After metamorphosis this obliquity is corrected, and it then becomes functional.

D.—*Somatopleure.*—When the splanchnopleure is split off it leaves a considerable mass of mesoderm, the somatopleure, adhering to the basement membrane. In the first seven chætigerous segments a large mass in each segmental block, text-figs. 11B, and 13, is easily distinguished as the rudiment of one of the large thread-glands which are such a peculiar feature of the adult, in which they attracted the special attention of GILSON (1894), who showed that they secrete " a very thick viscous liquid containing bundles of exceedingly fine colourless threads, which are used by the animal in the formation of the membranous lining of its tube " (WATSON, 1901, p. 236). Soon after their first formation as a small, roughly spherical mass of tissue, the nuclei take up positions around the periphery and the central part becomes clearer, text-figs. 13, *t.*2, and 11B, *t.*4. This clear portion rapidly increases in extent as the gland enlarges, and it becomes apparent that it is a secretion containing filamentous structures. These structures are twisted in various ways, giving in sections a curious appearance, which is only partially conveyed in the drawings, text-figs. 17, 26, etc. The filaments are much more numerous than the lines in these drawings would suggest : they twist about in the main body of the gland, but their extremities are directed down the duct. Only occasionally staining with Heidenhain's iron hæmatoxylin, they often take up Delafield's hæmatoxylin very strongly, although in the same section the filaments of a neighbouring gland will be practically unstained. This latter remark also applies to Mayer's mucicarmine and Mayer's muchæmatin. The glands on the whole are pear-shaped in the larval stages ; most of them elongate after metamorphosis. Their ducts are fairly short, fig. 11, Plate 31, and fig. 15, Plate 32, and open just below the bristle bundles, between them and the tori, where the latter are present. The duct of an early stage is shown in text-fig. 22. The peripheral cell layer of each gland does not show any of the special characters, muscle fibres, etc., which GILSON (1894) has described for the adult. It is a layer one cell thick, and with no defined cell limits.

The thread glands vary markedly in size in the different segments ; the fourth pair is always very much larger than the others, while the third pair is smaller than the first and second pairs. The fifth pair is moderately large, the sixth smaller, and the seventh smaller still, fig. 11, Plate 31, and fig. 15, Plate 32. The glands do not function during larval life.

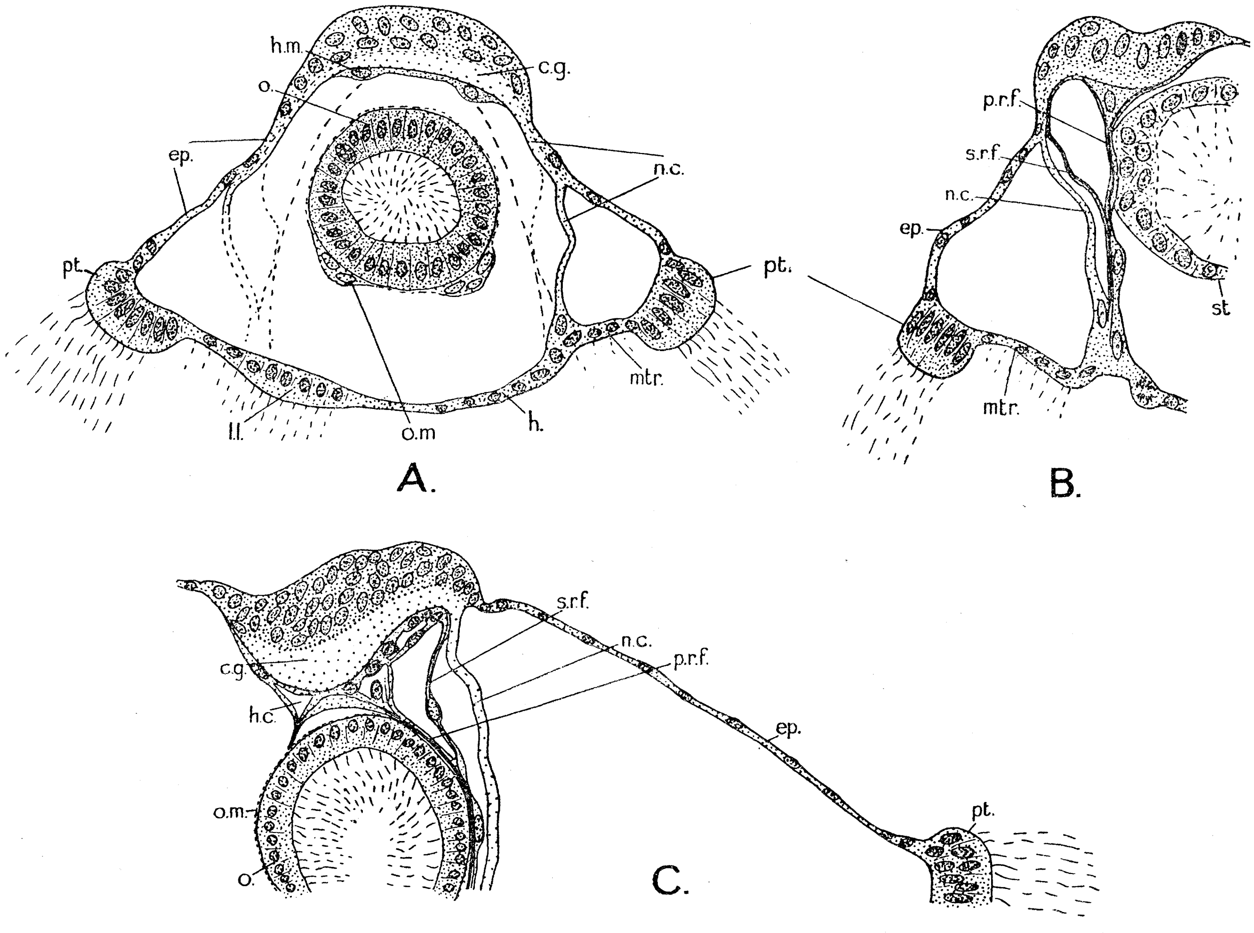
That part of the somatopleure mesoderm which does not enter into the formation of the thread glands forms a layer of cells on the basement membrane. Cell limits can sometimes be distinguished in the later stages, text-fig. 32, but at all times they are difficult to follow. Some, if not all, of these cells develop contractile filaments, and so give rise to the musculature. That moving the parapodia has already been mentioned (p. 271), and we can now turn our attention to the musculature of the trunk.

Owenia is peculiar in that only the thoracic region possesses circular muscles in the body wall, and as these do not form until after metamorphosis (see p. 302), we have only the longitudinal muscles to consider here. These arise as long filaments which run closely parallel to each other just internal to the basement membrane. They are shown cut across in text-fig. 17, shortly after their first appearance, and in text-fig. 32 at a later stage, when they are thicker and stronger. They stain darkly with Heidenhain's iron hæmatoxylin, but do not take up Delafield's hæmatoxylin. It will be noticed that the layer of these longitudinal fibres is only one fibre thick and not several fibres thick, as in the adult. In the dorsal region of the first two or three segments the fibres are very fine and spaced far apart ; further back they are almost as strongly developed dorsally as they are ventrally, text-fig. 24.

The study of the development of the muscle fibres has not produced any definite evidence in support of GILSON'S " *cellules musculo-glandulaires*," as he apparently hoped that it would (1898, p. 95). From observations on the adult, GILSON stated that inside the basement membrane the body wall consisted of a single layer of cells, glandular towards the cœlom, but containing in their bases the longitudinal contractile elements. This view was opposed by OGNEFF (1899) and ZÜRCHER (1909), who both found a definite peritoneal layer overlying the muscular layer. A summary of their views is given by McINTOSH (1917). GILSON had compared his muscular-glandular cells to the condition found in *Polygordius*, where a true peritoneal coat is absent. He reproduced figures from FRAIPONT (1887) showing the development of contractile filaments in the bases of the cells (1898, fig. 10). In *Owenia* some of the cells certainly give rise to filaments in a somewhat similar fashion, text-fig. 32, but on account of the indefiniteness of the cell boundaries, it is impossible to be sure whether all of them do so or not.

12. DEVELOPMENT OF THE HEAD.

In the early trochosphere the head from above is shaped roughly like a semi-circular plate, with the rounded border directed backwards, text-fig. 1. In its centre there is a slight pit speckled with dark brown pigment, and from this pit a few long fine cilia arise. Similar cilia are scattered over the posterior half of the plate, and there is a row of them along the anterior border. The whole upper surface of the plate is thinly speckled with brown pigment. Median sagittal sections, text-fig. 2, show that this plate consists largely of ectoderm, thickest anteriorly and thinning out behind. The nuclei are fairly densely crowded, and no cell limits are visible. Beneath it is a thin layer of mesoderm (*h.m.*), and separating the two a distinct basement membrane. In the ectoderm just above the latter the cerebral ganglion (*c.g.*) is perceptible by reason of its non-staining properties and the absence of nuclei in the actual nervous mass. Transverse sections, text-fig. 27A, show that a nerve commissure (*n.c.*) leaves each side of this mass, and after running below the ectoderm of the episphere until it is nearly half-way to the prototroch, it turns



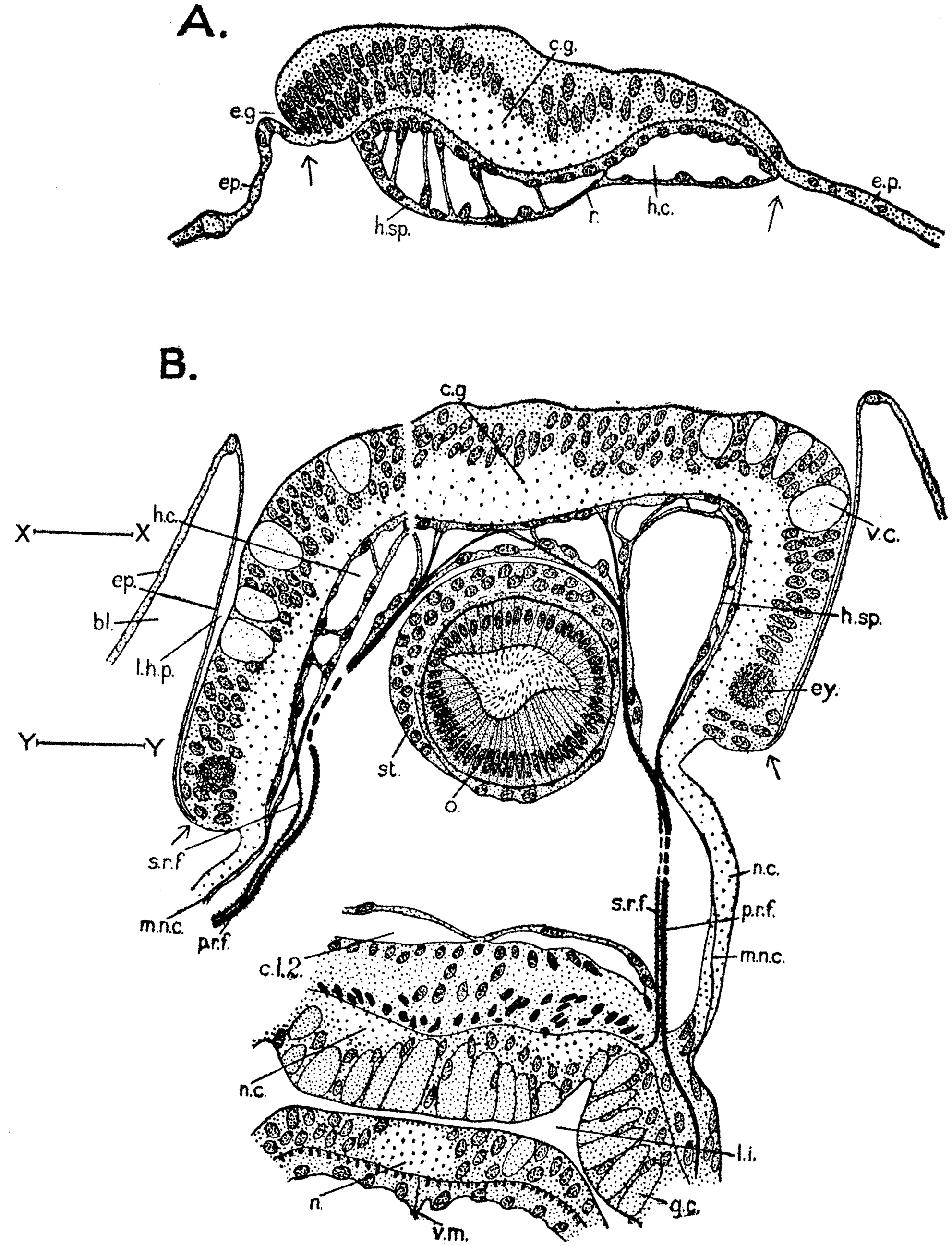
TEXT-FIG. 27.—Frontal sections of early Mitraria larvæ to show transverse sections of the head, etc. × 756. A. Larva 2 days old. Dotted lines show courses of retractor muscles, etc., in adjacent posterior section. B. Larva 2 days old but slightly more advanced in development than A. Section slightly oblique and most of right side omitted. C. Larva 16 days old. (May–June rearing. Not quite as far advanced as July larvæ of same age.) Section slightly oblique and most of left side omitted; *h.c.*, head cœlom; *p.r.f.*, primary muscle fibre of retractor; *pt.*, prototroch; *s.r.f.*, secondary muscle fibre of retractor.

directly downwards and, passing freely through the blastocœl, reaches the hyposphere wall. The point at which it leaves the episphere gradually recedes from the prototroch, owing to growth of the episphere in between, text-fig. 27B. Finally, owing to lateral growth of the head ectoderm, this point is merged into the lateral border of the head, and the nerve commissure no longer at any point comes into contact with the ectoderm of the episphere, text-fig. 27C. This is in striking contrast to *Polygordius*, where the commissure nowhere passes freely through the blastocœl, but remains in close contact with the blastocœl wall throughout its course. It is interesting that in the earliest stages of Mitraria the condition of *Polygordius* should be to some extent paralleled.

From the under surface of the head muscle fibres run to the hyposphere close by the circumœsophageal nerve commissures. The fibres were probably formed from larval mesenchyme, as in other larvæ. On either side a main or primary retractor fibre (*p.r.f.*), situated a little posterior to and internal to the commissures, arises by two roots, the internal one of which reaches the basement membrane in the median sagittal line along with its fellow from the opposite side. As this main fibre passes down through the blastocœl it is joined about the middle of its course by a secondary fibre (*s.r.f.*), which leaves the lateral border of the head close to where the nerve commissure enters the blastocœl. The two then continue in a common protoplasmic sheath as the retractor muscle. This delicate sheath is transversely wrinkled when the fibres contract. On reaching the hyposphere just median to the nerve commissure, the main fibre runs to the larval chæta-sac on its side, and is lost among the muscles on the latter's upper anterior surface. Thus contraction of this fibre, in addition to depressing the head, lifts up the chæta-sacs. The secondary fibre, on the other hand, does not appear to run to the chæta-sacs, but at a later stage passes into the developing worm trunk to act as suspensor to the folds (see p. 285).

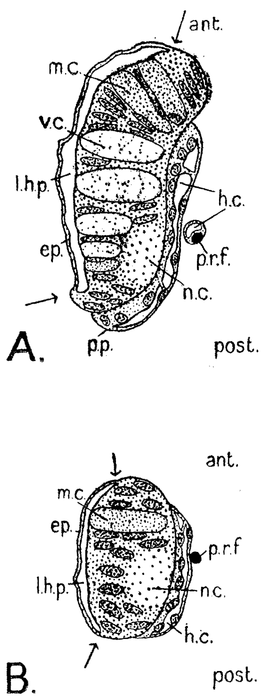
A very fine muscle fibre runs along the inner side of each nerve commissure. This can rarely be seen in the earlier stages, but is fairly distinct in older larvæ when stained with Heidenhain's iron hæmatoxylin, text-fig. 28B, *m.n.c.* At the base of the commissure it approaches the main fibre of the retractor and runs alongside it to the chæta-sacs. This single fibre can be compared to the strong lateral muscle composed of many fibres, which is situated immediately internal to the nerve commissure of *Polygordius*. In a similar manner, while the retractor of Mitraria has only two fibres, that of *Polygordius* possesses ten to fifteen.

As the head ectoderm grows laterally the mesoderm spreads below it and hollows out, text-fig. 27C, by a process of splitting. The cavity (*h.c.*) so formed is crossed anteriorly by numerous strands of tissue, text-fig. 28A, which apparently contain extremely fine contractile filaments in late stages. In this way the head acquires what can only be regarded as a cœlom, and after metamorphosis it comes to open into the anterior cœlomic cavity of the trunk (see p. 298). It must be noted that this is an entirely different cavity from the schizocœl of *Polygordius*, which is formed by a split appearing between the tentacle rudiments, ganglia, and skin of the head.



TEXT-FIG. 28.—Sections of the head of a Mitraria ready to metamorphose. × 756. Arrows indicate places of breakage at metamorphosis. A. Median sagittal section. B. Slightly oblique transverse section a little posterior to the mid-way point between the anterior and posterior borders of the head. This figure is combined from two adjacent sections as indicated by the break on the left-hand side. Lines XX and YY indicate approximate planes of sections shown in text-fig. 29A and B, respectively. *e.g.*, ciliated groove formed by epispheral fold in front of head ; *ey.*, eye ; *g.c.*, gland or mucus cell ; *h.sp.*, head splanchnopleure ; *l.h.p.*, lateral head pocket ; *m.n.c.*, muscle fibre of circumœsophageal nerve commissure ; *p.r.f.*, primary muscle fibre of retractor (dotted portion in adjacent section) ; *r.*, portion of retractor muscle fibre ; *s.r.f.*, secondary muscle fibre of retractor (dotted portion in adjacent section) ; *st.*, portion of stomach wall ; *v.c.*, peculiar clear cell.

When the ectoderm reaches the points at which the nerve commissures enter the blastocœl, the lateral borders of the head turn inwards and follow the courses of the commissures. It is as though the growing sides of the head are pulled down by the commissures and muscle fibres attached thereto. As a result the episphere ectoderm is invaginated where it is attached to the sides of the head, text-fig. 28B, to form a pocket-shaped cavity (*l.h.p.*) on either side. These can be referred to as the lateral head pockets. By the time the last larval stage has been reached, the head is a distinctly saddle-shaped structure, fig. 11, Plate 31, with its upper part exposed directly to the exterior, but with its sides forming the inner walls of the pockets, text-fig. 28B. The ectoderm has differentiated, and become well supplied, especially anteriorly, with mucus cells, text-fig. 29, *m.c.*, of the same type as those found in the trunk (p. 269). Gland cells, *g.c.*, in text-fig. 26, etc., are not present. A kind of cell peculiar to the head is present in some numbers in an area whose limits are indicated approximately in text-fig. 30B, *v.c.* These cells appear to be of the goblet type, but their contents take up none of the stains used, and in preparations they generally resemble large empty, or almost empty, vacuoles, text-fig. 28B, *v.c.* They can be distinctly seen in living larvæ, somewhat hexagonal in shape, with clear contents, and with granular tissue around and in between them. Their function is unknown.

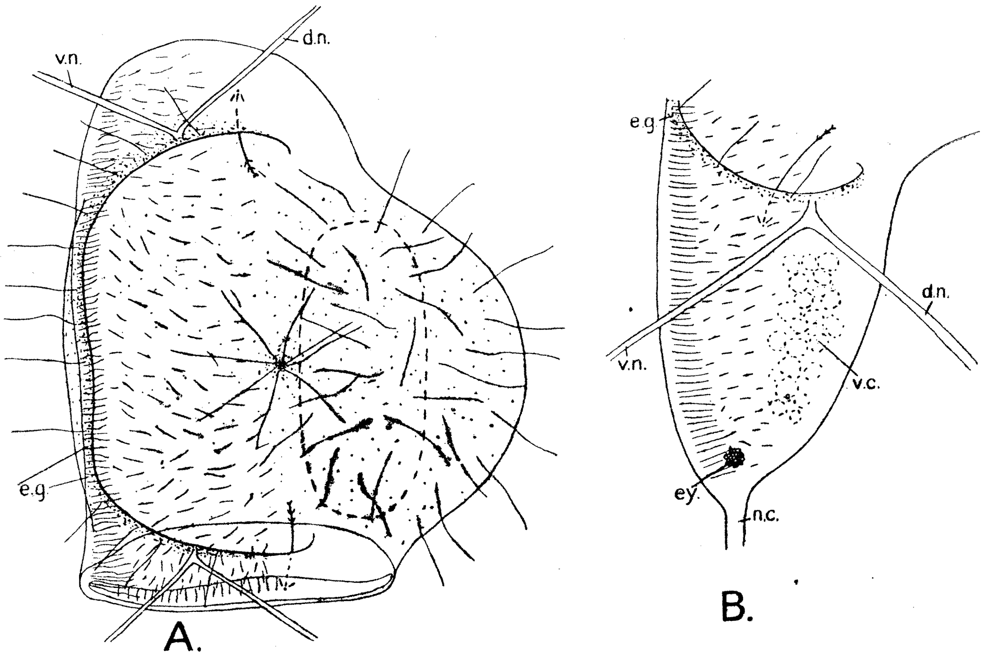


TEXT-FIG. 29.—Frontal sections of lateral parts of head of a Mitraria ready to metamorphose. × 756. Arrows indicate places of breakage at metamorphosis. A. In approximately the plane indicated by line XX in text-fig. 28B. B. In approximately the plane indicated by line YY in text-fig. 28B. The anterior and posterior ends of these sections are indicated by *ant.* and *post.* *p.p.*, posterior projection of mesoderm.

At the extreme lateral corners of the head there is an eye-spot composed of reddish-brown oily globules arranged in a cup-shaped cluster, which is practically in contact with the underlying nerve commissure and is doubtless innervated from it. The cerebral ganglion is situated immediately above the basement membrane in the central region of the head, and in sagittal sections presents a bilobed appearance, text-fig. 28A. The circumœsophageal commissures pass down close to the posterior lateral border of the head on either side, text-fig. 29. Finally, a considerable portion of the ectoderm still consists of densely crowded nuclei with no visible cell limits between them.

The mesoderm encloses the head cœlom, as has already been mentioned. It should be noticed that anteriorly a fair margin of the under surface of the ectoderm is not covered by any of the mesoderm, text-figs. 28A, 29. The posterior margin of the mesoderm is much thicker laterally, text-fig. 29A, *p.p.*, than it is medianly, text-fig. 28A. The importance of this will be seen later (p. 298). A number of very fine muscular fibres lie transversely just below the basement membrane, and some of them appear to enter the strands which run across the cœlom.

During life a dorsal view of the head shows that it is specially ciliated, text-fig. 30A. The central pit of the earlier stages is retained, and still speckled with brown pigment, and other specks of the same colour are scattered over the upper surface. From the pit some long fine cilia arise, and similar ones are distributed over the area behind. These move with a kind of trembling motion, and are supposedly sensory. A few similar cilia are to be seen along the anterior border of the head on top of a fold of the episphere which forms a groove, text-figs. 28A, 30A, *e.g.*, connecting the cavities of the lateral pockets. This fold contains a considerable amount of granular brown pigment. A row of actively-beating cilia lines the groove and passes down the antero-lateral borders of the head inside each pocket. Between this row and the pit the whole upper exposed surface of the head is covered with short scattered cilia, which are also found on the antero-lateral parts of the head inside the pockets, but not on the posterior parts in the region of the large clear cells, text-fig. 30B. These cilia are not very active and may be sensory in function.



TEXT-FIG. 30.—Sketches from life of the head of a Mitraria ready to metamorphose. Not to scale. The arrows pass into the cavities of the head pockets. The area encircled by the broken line in A shows the part of the head which is indented by a mild contraction of the retractors. A. Head from above. B. Lateral part of head from the left side. *v.c.*, peculiar clear cells of head.

13. GENERAL DESCRIPTION OF THE ANATOMY OF THE LAST LARVAL STAGE.

Before passing on to a study of the metamorphosis it will be of assistance to the reader if the main features of the anatomy of the last larval stage, the building up of which has been described in the preceding sections, be presented in a concrete form. While reading the following account, constant reference should be made to the stereogram, fig. 15, Plate 32.

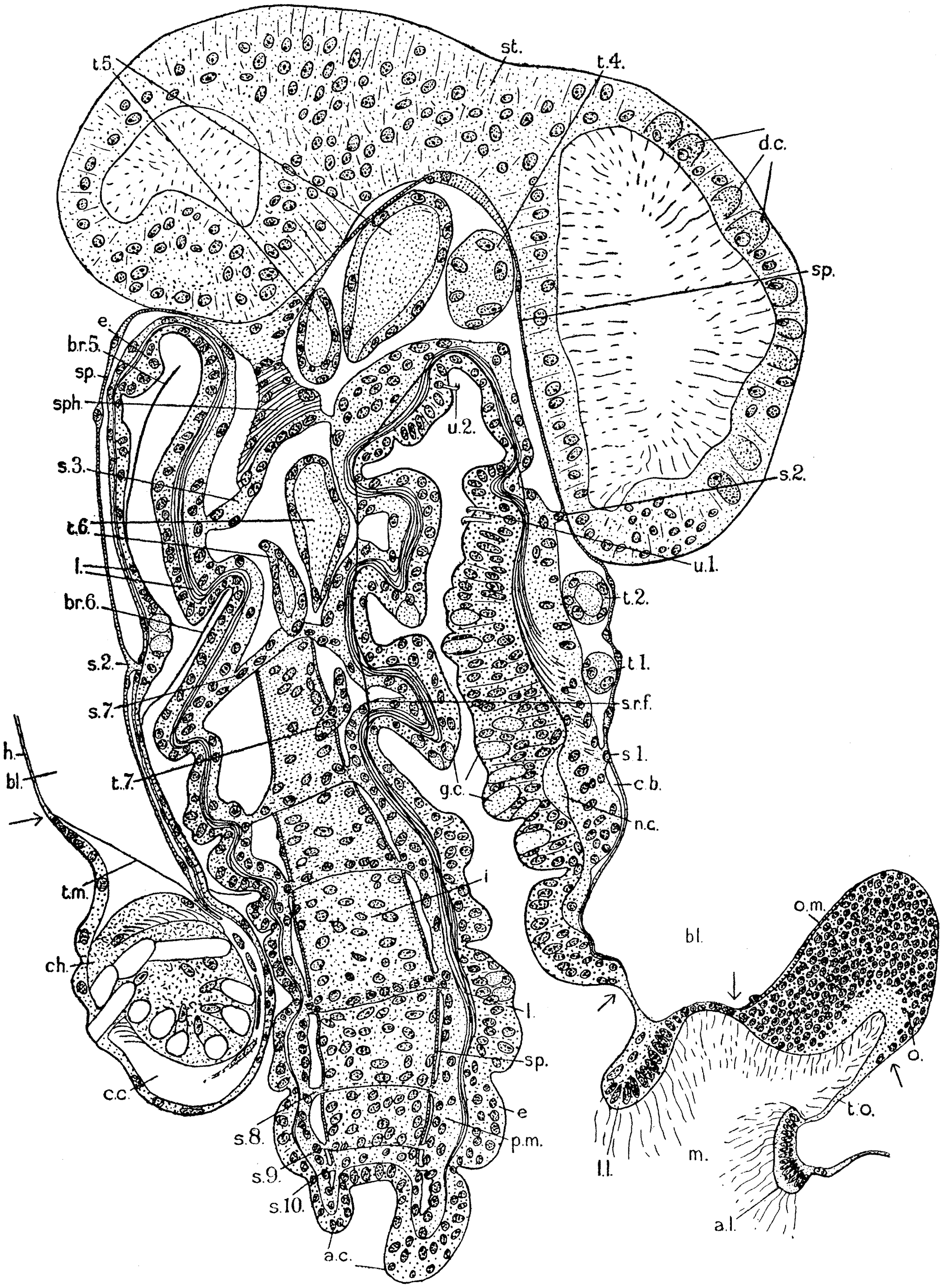
The very sinuous prototroch (*pt.* in key to Plate 32) forms a thickened rim of cells, below which is the metatroch (*mtr.*), a broad band of cilia whose function is apparently that of collecting food. These ciliated bands are carried out on the thin epi- and hyposphere tissues of the blastocœl wall. The episphere also supports the head, while to the hyposphere the remaining parts of the developing worm are attached. Fine mesenchyme strands across the spacious blastocœl (*bl.*), and serve to keep the various structures in position. The gut and trunk rudiment occupy a central position, and seem to be nicely balanced in relation to the prototroch and to the tuft of long bristles, which, in addition to defensive purposes, undoubtedly serve the larva as a counterpoise and rudder. The larval mouth receives the metatroch on each side, fig. 10, Plate 30, and is bordered by three lips, one anterior (*a.l.*) and two posterior lateral (*l.l.*). The mouth can be closed, there being one or two muscle fibres on each side for this purpose. It leads into a ciliated œsophagus, the first part (*t.o.*) of which is thin walled. The junction between the thin and thick-walled portions is shown by the dotted line in the stereogram. The œsophagus leads through a valve of cilia into the stomach. The latter is separated from the intestine by a valve only open during the passage of food. The intestine lies almost parallel to the œsophagus, bringing the anus (*a.*) close to the mouth. It is surrounded by the tissues of the worm trunk, which now claims attention.

It will be easiest to begin at the anus and work forward through the segments. The anus actually opens into a cup (*a.c.*) formed by the extension of the body wall beyond it. Just anterior to it is the parapodium of the eleventh chætigerous segment, and, as the segments are followed forward as far as the sixth chætigerous segment, nothing unusual for a Annelid is noticed. Septa occur between each segment (for the special positions of these septa, see p. 305), there are dorsal and ventral mesenteries, and the gut is surrounded by a definite splanchnopleure (*sp.*), in which there are circular contractile fibres (probably not functional at this stage). There are no nephridia, but the sixth and seventh chætigerous segments each possesses a pair of thread glands (*t.* 6. and *t.* 7.), opening between the bristle and torus. The fifth chætigerous segment also is almost normal, but the septum dividing it from the fourth has disappeared, except for a short distance on each side of the ventral mesentery, see text-fig. 24, *s.r.* It has been removed in the stereogram, fig. 15, Plate 32. It, too, possesses a pair of thread glands (*t.* 5.). At its junction with the fourth chætigerous segment a marked change occurs, for this latter segment is turned inside out and drawn back over the fifth. In like manner the third, second and first chætigerous segments are inside out, and surround approximately the sixth and seventh. All these segments possess thread glands, of which those of the fourth (*t.* 4.) are the largest and third (*t.* 3.) the smallest. The only septum is that (*s.* 2.) between the second and third. The splanchnopleure of these four segments, instead of being wrapped around the gut, is turned back away from it, and separates their cœlomic cavities from the blastocœl. In the third and fourth segments it is in contact with the lower surface of the stomach, but in the first and second it nowhere comes into contact with the gut. It is important to notice that the septum between the second and third segments comes close to the ventral part of the junction between œsophagus and stomach. It should be noticed also that the cavity (*c.c.*) enclosing the muscles of the provisional bristles communicates with the common cœlom of the first and second chætigerous segments (*c.* 1. 2.). It is cut off from the blastocœl by a very fine membrane (*t.m.*), which, during the dorsal meeting of the mesoderm blocks, became fused with the dorsal splanchnopleure of the first chætigerous segment (see p. 264).

Anterior to the first chætigerous segment is the cœlomic cavity (*c.b.*) of the buccal organ, which was formed by the fusion of the cavities of the first pair of segmental blocks and of a pair of cavities formed by the œsophageal mesoderm (p. 268). The buccal organ itself (*b.*) lies in a pocket opening into the anterior ventral part of the œsophagus, see fig. 10, Plate 30, and can be pushed out of the mouth. Its cœlomic cavity is separated from that of the first chætigerous segment by a septum (*s.* 1.), which does not, however, completely surround the trunk, but which runs obliquely forwards and upwards (in relation to the trunk) to meet the anterior extremity of the trunk at either side, where it ends. This septum is thus present only in the morphologically ventral part of the body. Its dorsal part is continued as a roof (*r.m.*) to the buccal organ cavity until it meets the lower surface of the œsophagus, and this roof is continued down on either side as the lateral walls of this same cavity.

The anterior border of the body wall ectoderm is attached to hyposphere tissue all round, except ventrally, where the buccal organ segment joins it to the œsophagus. It should be noted that the splanchnopleure of the first chætigerous segment meets anteriorly the body wall at or near this line of junction of adult and larval ectoderm. The mesoderm forming the lateral walls of the buccal organ cavity, on the other hand, mainly meets the trunk wall some little way back from the latter's edge, so leaving on either side an area of ectoderm (*e.u.*) which is not covered by mesoderm. The position of this is clearly seen in the stereogram, and is shown in section in text-fig. 26, *e.u.* In life it is usually turned back flat with the general hyposphere surface, and can be seen clearly in fig. 10, Plate 30. Although not previously mentioned, this uncovered area of ectoderm is, of course, visible in earlier stages, the mesoderm never having grown over it.

In addition to the main backward folding of the anterior segments, several subsidiary folds of the body wall occur. Of these the most striking are the overfolds of the sixth and seventh chætigerous segments, text-figs. 24, 26, 31. These were visible in much earlier stages, text-fig. 19, and they are always more prominent ventrally than dorsally. The degree of folding varies with the state of contraction and, to some extent, with different individuals. These folds are not of the same nature as those of the *Polygordius* endolarva, but they are, nevertheless, reminiscent of them, and the similarity is increased by the way in which they are suspended or retained in position. In *Polygordius* muscle



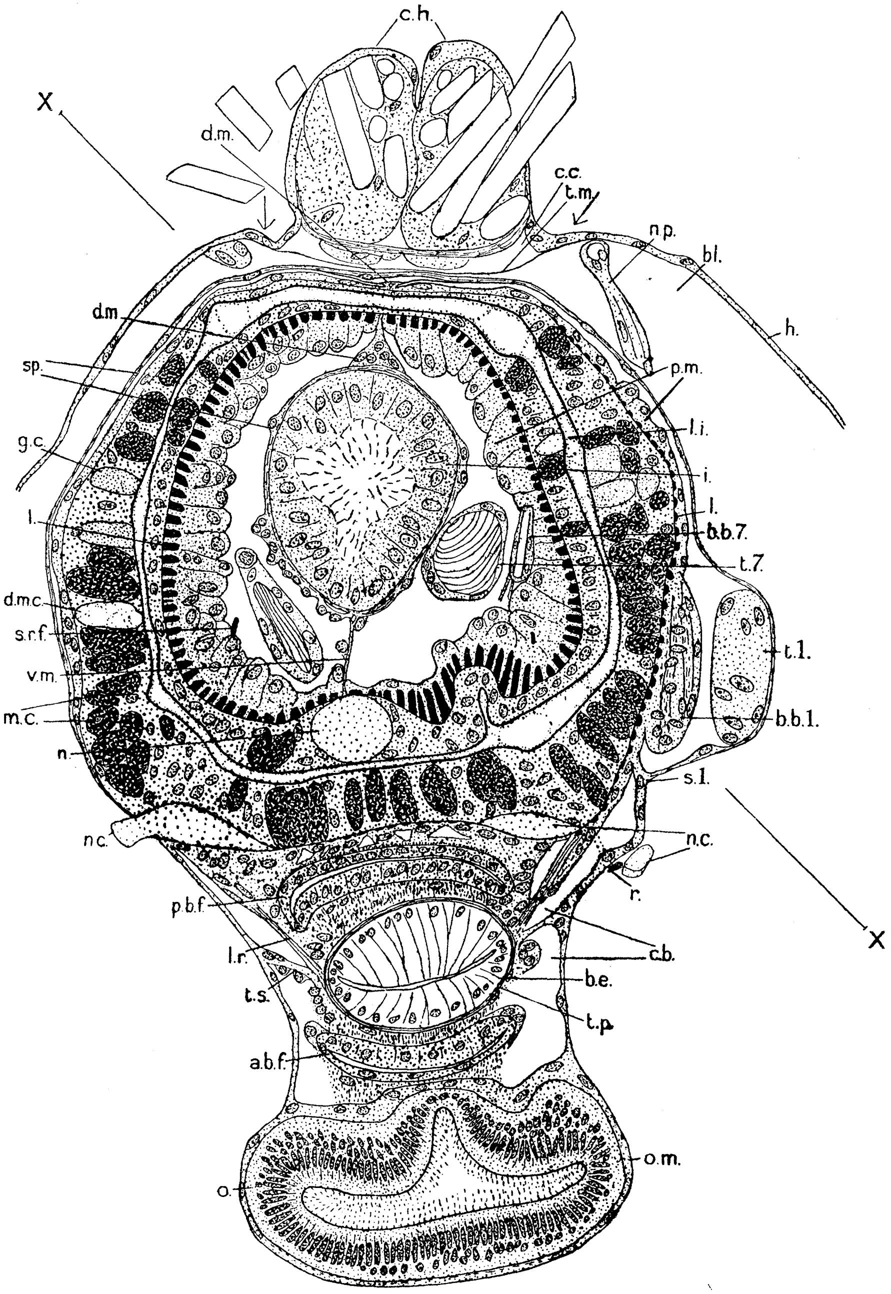
TEXT-FIG. 31.—Lateral sagittal section of worm trunk of a Mitraria ready to metamorphose. × 630. From a preparation stained with Heidenhain's hæmatoxylin. The trunk in this specimen is contracted. Arrows indicate places of breakage at metamorphosis. *a.c.*, walls of anal chamber; *br.*5, *br.*6, bristles of fifth and sixth parapodia; *g.c.*, gland cells or mucus cells; *s.*1, *s.*2, etc., first, second, etc., adult septum; *s.r.f.*, secondary retractor fibre acting as suspensor to the folds of the body wall; *t.*1, *t.*2, etc., first, second, etc., thread gland; *u.*1, *u.*2, uncini of first and second tori.

fibres from the retractor are attached to the ventral folds on either side, and here, in like manner, one of the retractor fibres (the secondary, see p. 278) enters the trunk and runs along each side as far as the anus. Here and there it is attached to the basement membrane, text-fig. 31, *s.r.f.*, among the longitudinal trunk muscles (in *Polygordius* it is attached to the longitudinal muscles themselves), but wherever there is a fold it leaves the basement membrane and pursues a straight course to the other side, where it again becomes attached to the membrane. Where the folds are large, as in those we are specially considering, this may mean that it actually passes through the cœlom. In the course of its journey across the folds it presumably passes between the cells of the mesoderm and not through them, text-fig. 32, *s.r.f.*, but the cytology is too minute to show this clearly. This pair of fibres, although extremely fine threads —much finer than the corresponding ones in *Polygordius*—stains very darkly with Heidenhain's iron hæmatoxylin, and retains the stain when that in the muscles of the body has been washed out. Thus they are made visible, and can be followed in many sets of sections, both sagittal and transverse, and have been seen in sections of larvæ a good deal younger than those in which the mesoderm blocks meet round the intestine.

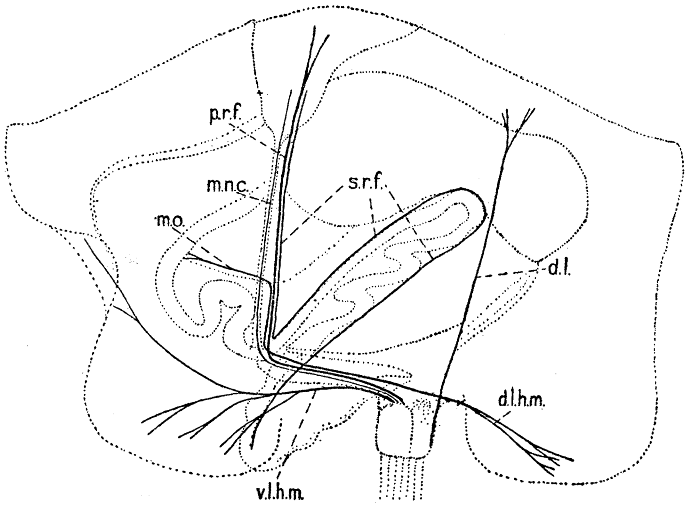
In *Polygordius* fibres from the dorsal levator assist those of the retractor in holding up the folds, but this does not occur in Mitraria, where the levators are attached to the larval chæta-sacs (p. 240).

The head has already been described in detail, and it is sufficient to mention here that it consists of a thick ectodermal layer containing the cerebral ganglion below which there is hollow mesodermatic tissue forming a head cœlom. This cavity is crossed anteriorly by fine strands of tissue. The cerebral ganglion gives rise on either side to the circumœsophageal nerve commissures, which, running down through the blastocœl, enter the trunk in the region of the buccal organ segment, and, running posteriorly towards the middle line, fuse to form the ventral nerve cord. The retractors leave the under surface of the head and run medianly to the commissures, and enter the body a little behind them. The course of the secondary fibres has just been described : the primary fibre runs in the mesoderm round the body on each side against the basement membrane, close to the anterior border of the trunk, until it reaches the larval chæta-sacs, where it is attached, see text-fig. 33, *p.r.f.* Alongside it runs the fine muscle fibre (*m.n.c.*) from the nerve commissure, and the fibre connecting the œsophageal muscle (*m.o.*) with the dorso-lateral hyposphere muscle (*d.l.h.m.*). Before it reaches the chæta-sacs it is joined by the ventro-lateral hyposphere muscle (*v.l.h.m.*).

The ducts of the larval nephridia discharge into the annular space formed by the turned-back segments of the trunk, through the buccal organ segment just anterior to the septum dividing it from the first chætigerous segment, key to Plate 32 (*np.d.*).



Text-fig. 32.—Transverse section of worm trunk of a Mitraria ready to metamorphose. × 630. From a preparation stained with Delafield's hæmatoxylin. The approximate plane of this section is indicated by the line XX in text-fig. 24. The outer everted part of the worm trunk is cut a little nearer the anterior end on the left-hand side of the figure than it is on the right-hand side. Arrows indicate places of breakage at metamorphosis. Line XX indicates approximate plane of section shown in text-fig. 26. *a.b.f.*, anterior buccal fold. The short lines above and below this fold represent the longitudinal protractor muscles near their places of insertion; *b.b.*1, *b.b.*7, first and seventh bristle bundles of left side; *l.*, longitudinal muscles, here shown black, actually not stained with Delafield's hæmatoxylin; *l.i.*, lumen of invagination (dotted strands represent mucus); *m.c.*, ectodermal mucus cell; *n.c.*, circumœsophageal nerve commissure. The one on the left of the figure is shown entering the ectoderm of the trunk; *p.b.f.*, posterior buccal fold. The short lines above and below this fold represent the median retractor and longitudinal protractor muscles near their places of insertion; *r.*, retractor. In the next section the secondary retractor fibre crosses the buccal organ cœlom to enter the peritoneal mesoderm of the trunk. Its course is shown by the dotted lines; *t.*1, *t.*7, first and seventh thread glands of left side.



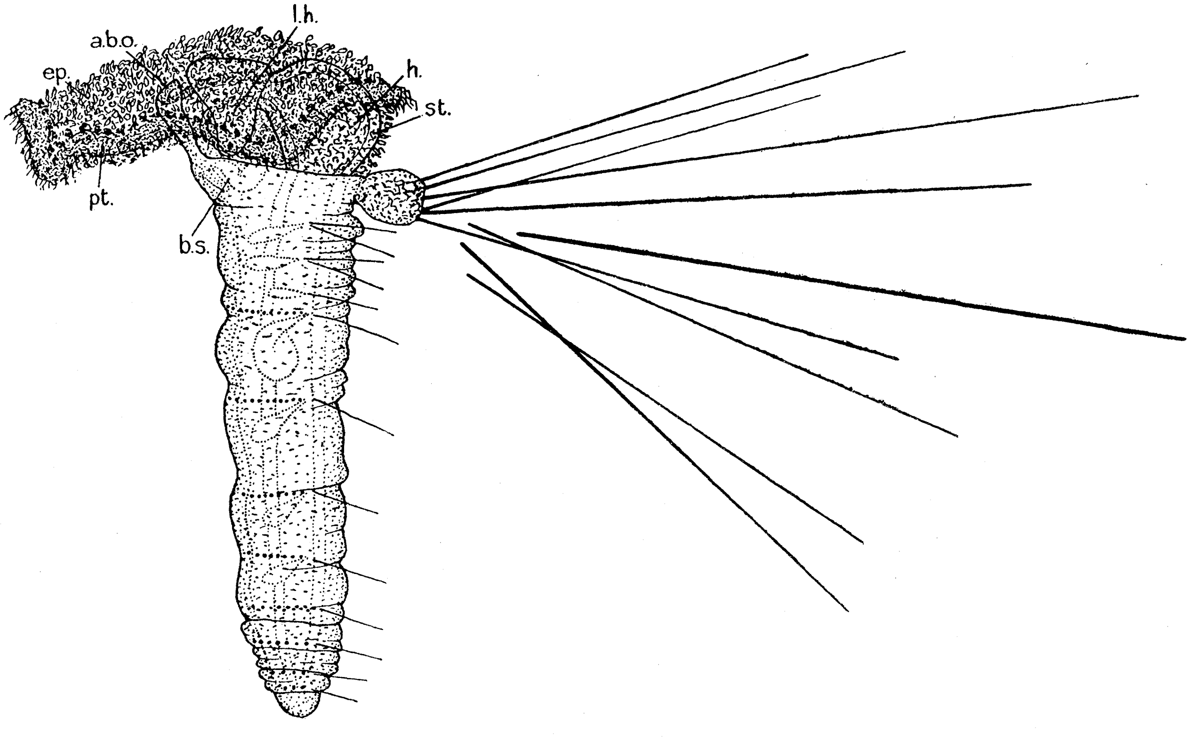
TEXT-FIG. 33.—Diagram to show distribution of larval muscle fibres in a Mitraria ready to metamorphose. Not to scale. *d.l.h.m.*, dorso-lateral hyposphere muscle; *v.l.h.m.*, ventro-lateral hyposphere muscle.

14. GENERAL ACCOUNT OF THE METAMORPHOSIS.

Metamorphosis takes place with extreme rapidity, and involves such tremendous structural changes that its investigation is attended with especial difficulty. I have watched it on many occasions, both through a hand lens, when the larvæ were in dishes submerged in a considerable depth of water, and also with the microscope, when they were confined in cavity slips, but still able to move actively about the cavity. While the speed at which the essential change takes place renders it difficult to observe fine details, and these have had to be worked out laboriously from sections and whole mounts, the general nature and order of the changes is fairly easily observed.

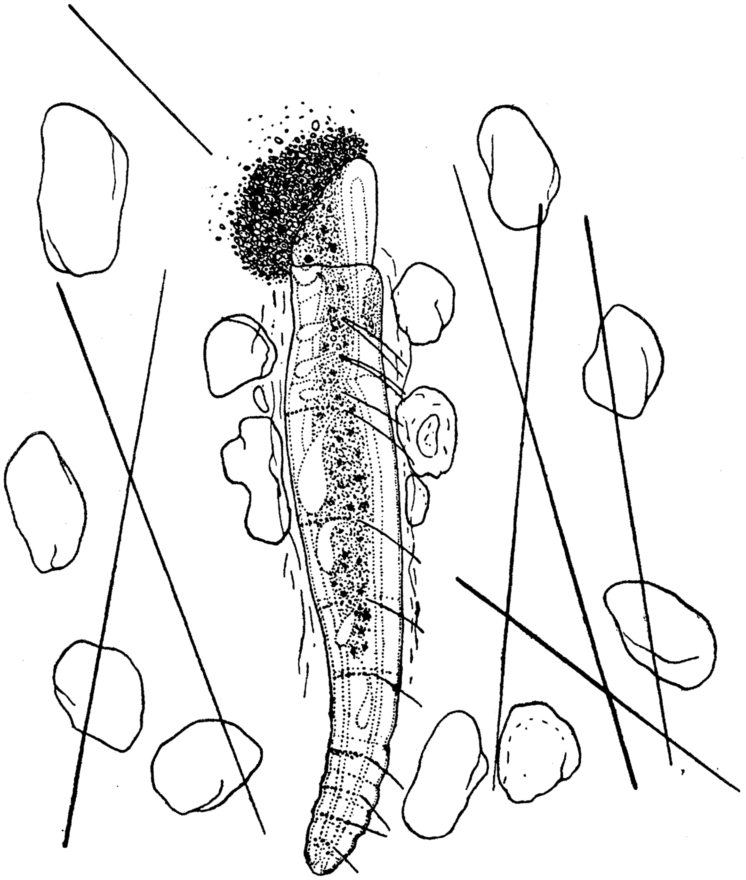
Prior to actual metamorphosis, the worm trunk frequently undergoes spasms of squirming, brought about by the longitudinal muscles, the motion being mainly lateral. Some time later, maybe half an hour or more, the larva actually metamorphoses. It may be that these movements are attempts to break the suspensors (secondary retractor fibres) holding together the body folds. The suspensors are too fine to be seen in a living Mitraria, but in the larger endo-larva of *Polygordius* WOLTERECK (1902) distinctly saw them break at metamorphosis. The suspensors have not been detected in sections of worms just metamorphosed, and it is, I think, a legitimate assumption that they are broken, possibly by these movements, and that as soon as they are broken the larva metamorphoses.

The great change is initiated by the straightening out of the trunk folds by means of a vigorous squirming motion produced by the longitudinal muscles. The wriggling is mainly in a lateral plane. This extension of the trunk takes place in 20–30 seconds, the head at the same time being pulled down by the retractors and probably also by the muscles running along the nerve commissures. As the trunk is extended the gut is dragged into it. The condition some 15–20 seconds after the process began is shown in fig. 12, Plate 31. A few seconds later the long bristles fall off, and the chæta-sac protuberance so left invaginates and subsides. The prototroch, metatroch, episphere and hyposphere tissues begin to crumple, although the cilia go on beating for a minute or so longer. The head meanwhile approaches the trunk, with which it is to unite, the whole of the gut by this time having been dragged into the latter. About this time a new mouth is formed by the breaking across of the œsophagus behind the larval mouth along the dotted line in fig. 15, Plate 32. The edges of this new mouth come into contact with the anterior border of the head, and later fuse with it completely. The crumpling prototroch, metatroch, episphere and hyposphere tissues, text-fig. 34 (but see legend), rapidly break down into a mass of irregularly-sized and shaped granules, caused apparently by the entire disintegration of the cells. Three or four minutes from the onset of metamorphosis the tiny worm so produced is busily engaged in swallowing its old larval tissues, wriggling violently the while. They are not completely histolysed when it begins to swallow them two or three minutes from the start of the whole process,



Text-fig. 34.—Pen and ink copy of pencil sketch from life of a metamorphosing Mitraria. × 156. This metamorphosis was not quite normal in that the body folds were not properly straightened out and the chæta-sacs did not finally invaginate. It took place more slowly than usual for which reason it was possible to make a rapid sketch. In its broad features, however, the metamorphosis was normal and the crumpled histolysing larval tissues pass through the characteristic stage here shown, although not as a rule before the head is in its final position at the anterior end of the trunk. *a.b.o.*, turned up anterior border of œsophagus which will fuse with anterior border of head ; *e.p.*, episphere crumpling and breaking up ; *h.*, hyposphere crumpling and breaking up ; *l.h.*, lateral part of head ; *pt.*, prototroch crumpling and breaking up.

and for a few seconds swallowing is difficult, but as they rapidly disintegrate it becomes easier, so that in a minute or two the mush pours down the œsophagus in a slow, steady, unbroken stream. The ingestion is aided by movements of the buccal organ and by the œsophageal cilia. Text-fig. 35 shows a young worm four or five minutes after metamorphosis began, and the larval tissues are already half swallowed. A thin film of mucus has appeared around the anterior half of the body ; it is secreted apparently by some of the numerous ectodermal mucus cells. This film increases in size and thickness, so that soon the whole body is enclosed in a delicate transparent tube. Grains of sand and other debris stick to the outside of the tube, and serve to keep its wriggling occupant on the bottom. Ten, fifteen or more minutes from the time that it ceased to be a larva the last of the larval tissues enters the stomach. The worm, fig. 13, Plate 31, can use its mouth as a sucker to attach itself to the substratum, but its powers of suction are feeble, and it is easily sucked up in a pipette.



TEXT-FIG. 35.—Pen-and-ink copy of pencil sketch from life of an *Owenia* worm about 5 minutes after the beginning of metamorphosis. × 110. The worm is swallowing the broken down larval tissues, which can be seen pouring into the stomach through the œsophagus. A mucus tube is being secreted and sand grains adhere to it. The cast-off provisional bristles are strewn around among the sand grains.

During the next few hours the tube is thickened by further secretions of mucus, and the head becomes firmly united with the body and with the anterior end of the œsophagus.

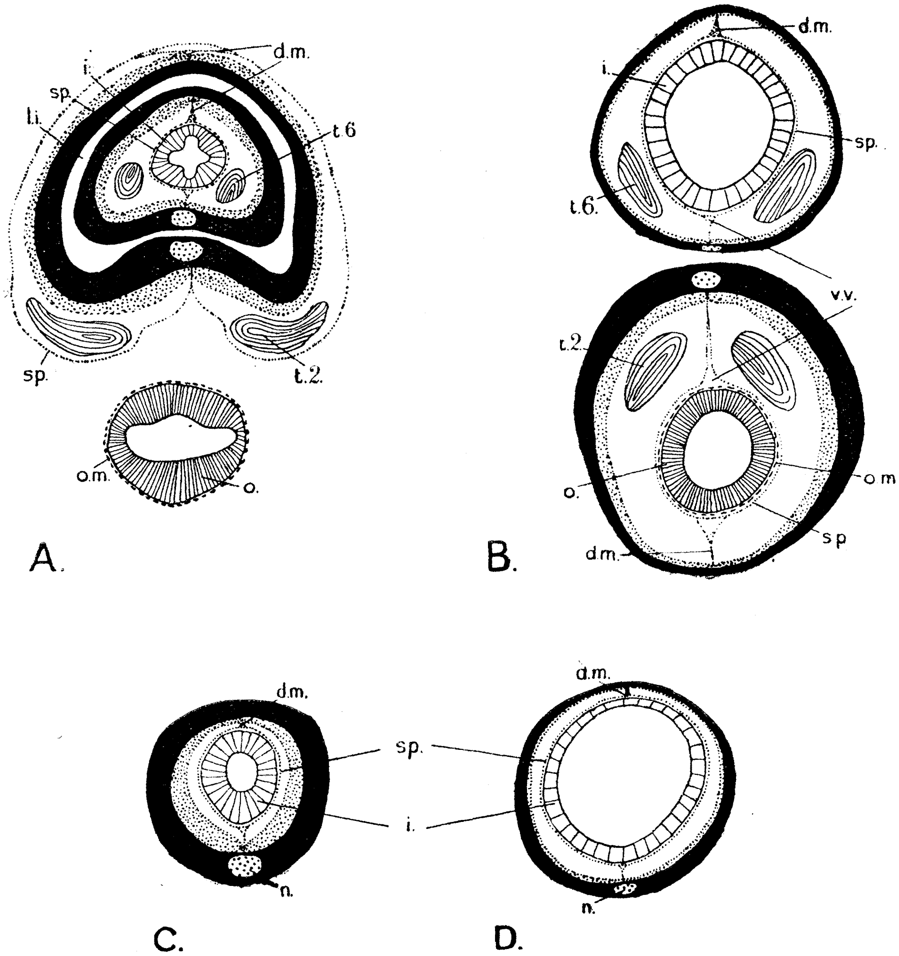
15. DETAILED STUDY OF THE METAMORPHOSIS.

(i) *The Straightening of the Trunk.*

The anatomy of the trunk has already been made clear, when it was seen that the first four chætigerous segments and the buccal organ segment are turned inside out and drawn back over the succeeding three or four segments. The body wall is much folded, and kept so by a pair of fine suspensor fibres (p. 285). The latter appear to be broken at metamorphosis (p. 287), allowing the trunk to stretch and flatten out the folds. At the same time, by a wriggling motion, the everted segments are inverted into the normal position from behind forwards. As this is done the anterior part of the gut is dragged in, and becomes completely surrounded by the splanchnic layer of mesoderm of the anterior segments. The posterior part of the gut probably slides through the investing splanchnic sheath for a short way as the body folds straighten out. The second adult septum (*s.*2.) which, in the late larva, encircled the worm rudiment, text-fig. 24 and fig. 15, Plate 32, now comes to encircle the gut, text-fig. 37A, and fig. 16, Plate 32, and takes up its final position surrounding the junction between œsophagus and stomach. It will be seen that ventrally it almost occupies this position in the larva, but that dorsally a long length of gut must be passed through it during the change to adult conditions. After metamorphosis it is clearly seen that this septum is strongly muscular, and forms a powerful sphincter at the stomach entrance, text-fig. 37A.

Anterior to the second septum the way in which the splanchnic mesoderm folds around the œsophagus is a little complicated. In the larva between the second and first septa it is nowhere in contact with the gut, and is, indeed, situated some distance away. The condition before and after metamorphosis is shown in the diagrammatic sections, text-fig. 36A and B, and it will be seen how, below the œsophagus (above it in the figure), two lateral layers come together to form the greater part of the adult ventral mesentery. These layers subsequently fuse, except for a space which forms the ventral blood vessel, see text-fig. 38, *v.v.* Other less well-marked spaces appear to be left between the splanchnic mesoderm and the larval mesoderm on the œsophagus, and these no doubt are the origins of the adult blood vessels of this region described by ZÜRCHER (1909). A further result of this folding together of the mesodermal layers will be referred to below (p. 296).

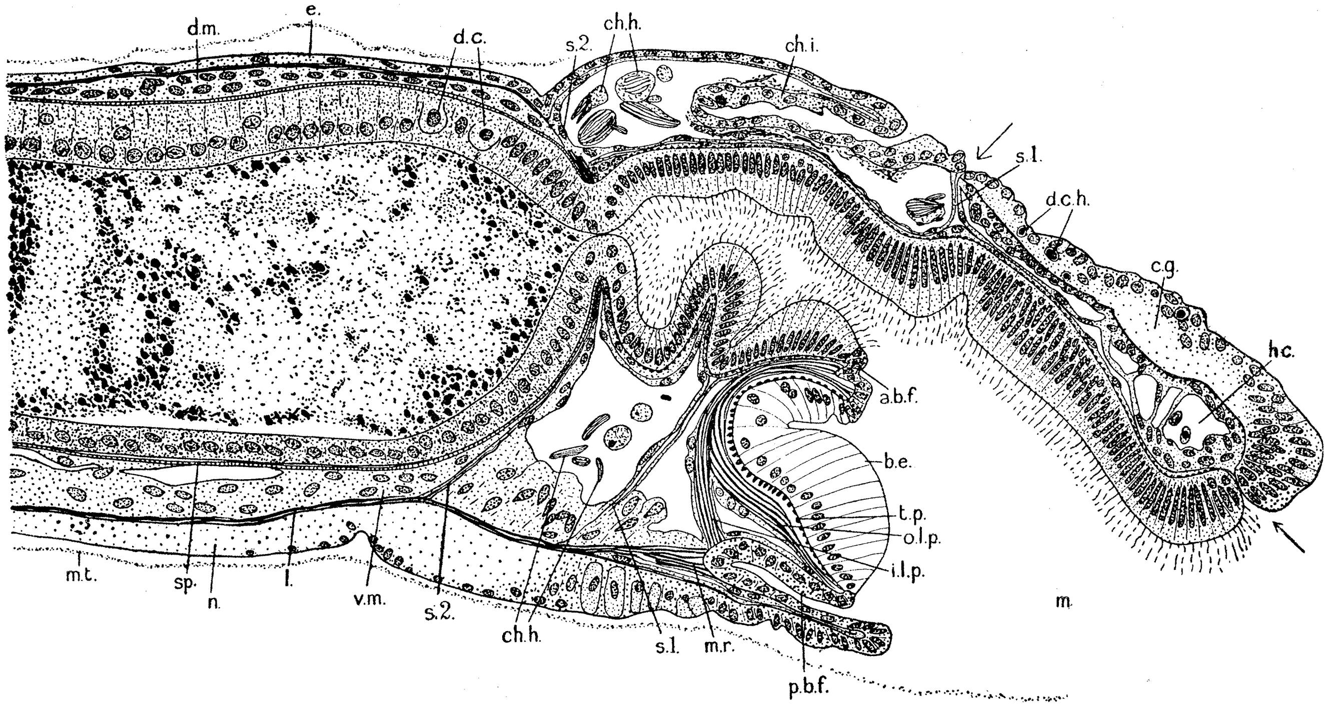
As the trunk extends, the part which, in the larva, is confined within the everted anterior segments and the tail region not only elongates but increases in diameter, the body wall at the same time becoming much thinner. This is graphically illustrated in text-fig. 36A and B, which are from sections of the same regions of the body before and after metamorphosis. It is doubtful whether the gut shares in this general increase in diameter by a process of thinning of its walls, or whether the apparent increase is due merely to an opening out of its longitudinal folds, text-figs. 32 and 36A, or through a more anterior and wider portion of the gut being dragged into the region of the particular section during the elongation of the trunk. Possibly both processes are



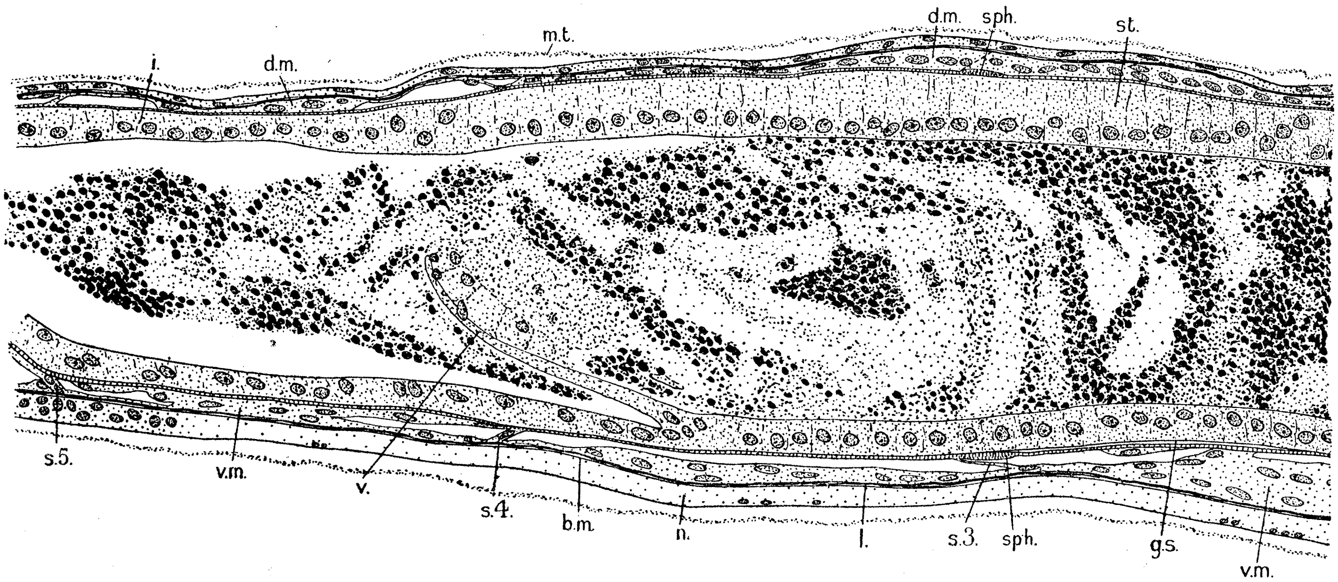
TEXT-FIG. 36.—Diagrams to scale of transverse sections of worm trunk before and after metamorphosis. × 315. A. Transverse section in region of the second and sixth chætigerous segments before metamorphosis. B. The same regions after metamorphosis. C. Transverse section in the region of the tenth chætigerous segment before metamorphosis. D. The same region after metamorphosis. *Trunk ectoderm*, black ; *mesoderm*, stippled ; *gut and other structures* as indicated by lettering. *d.m.*, dorsal mesentery ; *t.*2, *t.*6, second and sixth thread glands.

involved. The anterior everted segments do not, however, undergo this increase in diameter, and the body wall there retains its thickness, text-fig. 36A and B, and compare text-figs. 24 and 37A and B, and text-figs. 32 and 38. We thus see how the posterior part of the trunk is in the larva compressed by the everted segments, and how its increase in diameter at metamorphosis ensures that the young worm shall taper gradually.

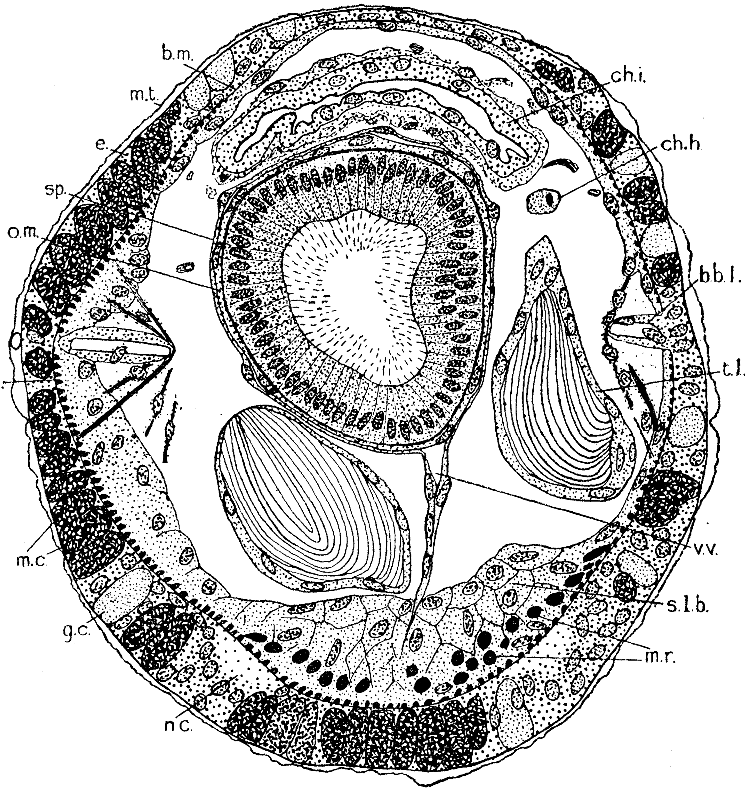
When the trunk has been elongated the long provisional bristles of the larva fall off, and then the tissues of the chæta-sacs, which would otherwise remain as a protuberance, invaginate into the body cavity at the extreme anterior end of the trunk, text-fig. 37A, *ch.i.*, and fig. 16, Plate 32. Here the ectoderm which originally covered them forms at first a sac-like invagination opening to the exterior by a dorsal pore, but this pore closes after a time and the tissues, mesodermal and ectodermal, of the sacs break into pieces and float away in the cœlomic fluid, text-figs. 37A, and 38, *ch.h.*, and fig. 16,



TEXT-FIG. 37A.—Median sagittal section of anterior end of young *Owenia* worm immediately after completion of metamorphosis. × 630. From a preparation stained with Heidenhain's hæmatoxylin. The head has not yet fused with the trunk and the arrows show where fusion will take place. Letters as in text-fig. 37B.



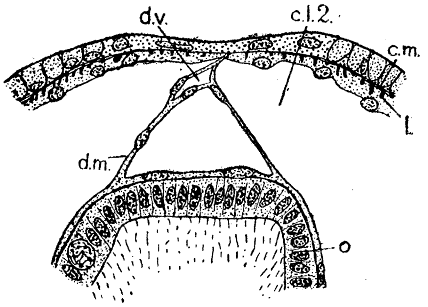
TEXT-FIG. 37B.—Posterior continuation of the section shown in text-fig. 37A. The dark masses in the gut represent the histolysed prototroch and other larval tissues which the worm had just finished swallowing when fixed. The valve between stomach and intestine is breaking down. Parts of the dorsal and ventral mesenteries were on the adjacent section. *ch.h.*, portions of larval chæta-sacs histolysing; *ch.i.*, invaginated ectoderm of larval chæta-sacs; *d.c.*, dark staining amœboid cells of stomach preparing to be extruded into gut; *d.c.h.*, degenerating cells in head ectoderm; *g.s.*, gut sinus; *h.c.*, head cœlom, containing free histolysing cells; *m.t.*, mucus tube just secreted; *s.*1, *s.*2, etc., first, second, etc., adult septum; *v.*, valve between stomach and intestine in process of breaking down.



Text-fig. 38.—Transverse section of first chætigerous segment of *Owenia* worm not more, and possibly considerably less, than 8 hours after metamorphosis. × 756. The splanchnopleure has not yet fused with the œsophageal mesoderm. From a preparation stained with Delafield's hæmatoxylin. Muscles here shown black, actually unstained. *s.*1.*b.*, basal part of first adult septum; *t.*1, first thread gland.

Plate 32. The fate of these loose pieces has not been made out: they seem to disappear entirely. At any rate, after a few days, all trace of the chæta-sacs is lost.

The invagination of the chæta-sacs during metamorphosis raises a minor problem which has not been entirely solved. While they are still breaking up the dorsal mesentery is present, posterior to them in front of the second septum. Its structure has already been referred to (p. 274). After they have disappeared it stretches the whole way between septa 1 and 2, text-fig. 39. How does the mesentery arise in the position previously occupied by the chæta-sac invagination? I have in some sections seen signs of it between the chæta-sacs and the œsophagus, and possibly as the sacs break up and disappear it grows until it reaches the body wall. It might also be regenerated from behind forwards.



Text-fig. 39.—Slightly oblique transverse section of the dorsal mesentery of an *Owenia* worm several days after metamorphosis. × 756. The section is situated immediately posterior to the first adult septum in the position formerly occupied by the larval chæta-sac invagination. *c.m.*, circular muscles; *d.m.*, one side of dorsal mesentery; *d.v.*, dorsal vessel.

After metamorphosis a few degenerating cells can be seen in the tissues, usually mesodermal, of the trunk, and floating in the cœlom. They are always few in number and they soon disappear.

(ii) *The Fusion of the Head with the Trunk.*

By far the most complex process of the metamorphosis is the fitting of the head on to the trunk and the details of this have only been worked out with great difficulty. The minute size is chiefly responsible for those points that still remain doubtful.

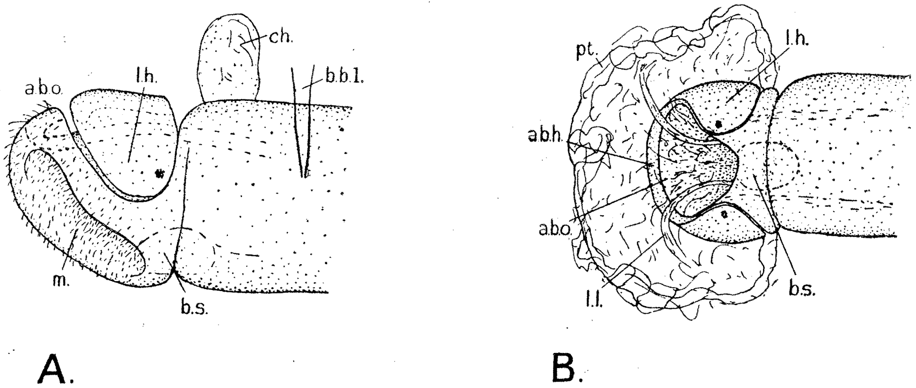
In the early stages of the great change, while the trunk is being extended, the head is pulled down towards its final position by contraction of the retractors, and most probably also by the contraction of the muscle fibres running along the nerve commissures. The latter must in some way be shortened, but the manner in which the nervous tissue undergoes this change is a problem which also puzzled WOLTERECK in the case of *Polygordius* (1902). When the gut has entered the trunk the posterior border of the head ectoderm—which has broken away from the episphere tissue in the positions shown by the arrows in text-fig. 28 and key to Plate 32—approaches and comes into contact with the anterior ectoderm of the trunk, by now broken away from the hyposphere tissues in the positions shown by the arrows in text-figs. 24 and 26 and key to Plate 32. Meanwhile, as previously mentioned, a new adult mouth has formed by the breaking across of the larval œsophagus in the line of junction of the thick œsophageal walls and the thin larval tissue (*t.o.*), see text-fig. 24 and dotted line in fig. 15, Plate 32. The dorsal and lateral edges of this new mouth turn back a little to meet the ectoderm of the anterior border of the head, text-figs. 34, 37A, and fig. 16, Plate 32. A few hours later complete fusion of the ectoderm of the head with the œsophagus anteriorly, and with the ectoderm of the trunk posteriorly, has taken place, text-fig. 42, of a worm less than eight hours after metamorphosis ; also text-fig. 44 of a worm a few days later. The rough sketches reproduced in text-fig. 40 will give some idea as to conditions ventrally. There the ventral surface of the trunk is joined to the mouth by the buccal organ segment, and is not separated from it by the head, as is the case dorsally and laterally.

The history of the changes affecting the mesoderm is much less simple, and the two stereograms should constantly be consulted throughout the following description.

It will be best to begin by considering the formation of septum one at the anterior end of the trunk during metamorphosis. It has already been described (p. 283) as present in the larva as a fine membranous partition posterior to the buccal organ on the ventral side, from whence it runs obliquely forwards and upwards (in relation to the trunk) to meet the anterior border of the trunk on each side. It there ends, so far as it is composed of the fused posterior wall of the buccal organ cavity with the anterior wall of the first segment. The latter component, however, continues along and is attached to the anterior edge of the trunk ectoderm dorsalwards, ending finally as the very thin membrane (*t.m.*) separating the larval chæta-sac cavity from the blastocœl. It will be seen that, considered as a septum, it is very much distorted, and that it merges imperceptibly into the general splanchnic mesoderm of the everted segments, from which, indeed, it can only be distinguished theoretically at this stage.

The posterior wall of the buccal organ cœlom which enters into the formation of the ventral part of the first septum is, of course, continuous with the mesodermic membrane forming the roof and sides of that cœlom. This latter membrane is attached to the ventral surface of the œsophagus and to the trunk, where its line of attachment is peculiar in that it leaves on either side the area of thick trunk ectoderm already mentioned (p. 283), which is devoid of any mesodermal covering (*e.u.*).

It is necessary now to visualise the relative changes in position of these mesodermal membranes when the everted part of the trunk is inverted. It is easiest to imagine this if the larval chæta-sac protuberance is neglected for the time being, and the splanchnic mesoderm regarded as being attached in a simple line to the anterior edge of the trunk right round the dorsal side. This protuberance, and its invagination when the bristles fall off, can easily be put into the mental picture afterwards. Considering now this simplified dorsal part, it is easy to see how, as inversion takes place and the splanchnic mesoderm wraps round the gut, the posterior wall of the future first adult septum, as outlined above, can be formed into a vertical sheet stretching between the anterior end of the trunk wall and the œsophagus, see fig. 16, Plate 32, and text-fig. 37A. The presence of chæta-sacs and their invagination merely complicates the process slightly, without affecting this end result.



TEXT-FIG. 40.—Pen-and-ink copies of rapid pencil sketches from life of metamorphosing larvæ. Not to scale. A. Lateral view. Metamorphosis is nearly complete but abnormal in that the larval chæta-sacs have failed to invaginate. Head not yet fusing with trunk and œsophagus. B. Ventral view. Earlier stage than A. Probably normal. Larval tissues still crumpling prior to swallowing. The anterior border of the œsophagus should be a little further forward to meet the anterior border of the head. *a.b.h.*, anterior border of head; *a.b.o.*, anterior border of œsophagus; turned upwards and backwards in A.

Ventrally, the conditions are more complicated, but if a rubber model made to resemble fig. 15, Plate 32, is imagined to be cut along the mid-dorsal line, and the everted part of the trunk to be folded right round, bringing the cut edges together above the œsophagus, the reader will gain some idea of the spatial relations of the parts which could be, and are, brought about by inversion at metamorphosis. He must consider at the same time the mode in which the mesentery ventral to the œsophagus is formed, as already described on p. 290, and he will then realise that this will result in a certain amount of folding of the splanchnic mesoderm of the first chætigerous segment against the mesodermal membrane which, in the larva, forms the roof of the buccal organ cavity. This is indicated in fig. 16, Plate 32,* and text-fig. 37A, and where it occurs a gap is left to form a blood vessel (*l.v.* in key to Plate 32).

There is one more added complication, for it appears that the area of ectoderm uncovered by mesoderm is increased a little by the mesoderm in the region of the nephridiopore (which has not been detected after metamorphosis) peeling off the ectoderm for a short way in a posterior direction. It is impossible to see this happening, but the increase after metamorphosis of the distance from the anterior edge of the trunk of the place at which the dorsal border of that part of the first septum which is formed by fusion of the posterior wall of the buccal organ cavity and the anterior wall of the first chætigerous segment meets the body wall, see line BA, figs. 15 and 16, Plate 32, seems to indicate that something of this nature happens. This I believe to be the true interpretation, but I must again emphasise the great difficulty introduced into all these observations by the minute scale of the original worm.

It will be well at this stage to give a description of the anterior end of the trunk when it has, in the above manner, been made ready to receive the head. Reference to fig. 16, Plate 32, will make the description clear if the head tissues there illustrated are neglected.

Anterior to the second adult septum (*s.* 2.) is the common cœlomic cavity of the first two chætigerous segments. It is traversed by the œsophagus, and divided into two lateral chambers by the ventral and dorsal mesenteries; the latter is incomplete anteriorly in the position occupied by the sac-like invagination produced by the breaking down of the larval chæta-sacs. Each lateral chamber is occupied by the thread glands associated with the parapodia. The anterior extremity of this common cœlomic cavity is limited by the first septum (*s.*1.), which runs in an obliquely sloping direction forwards and upwards. At its most dorsal point this septum is attached to the anterior edge of the trunk ectoderm, immediately in front of the larval chæta-sac invagination; laterally its line of attachment gradually recedes from the edge of the trunk, and the distance becomes greater as it passes around and down each side. Approximately the lower third of its area is in contact with and fuses with the mesodermal membrane enclosing the buccal organ cœlom, which lies in front. This membrane forms a roughly horizontal sheet or septum (*l.m.*), which is fused with the ventral surface of the œsophagus to spread outwards and downwards on each side until it reaches the body wall. Posteriorly

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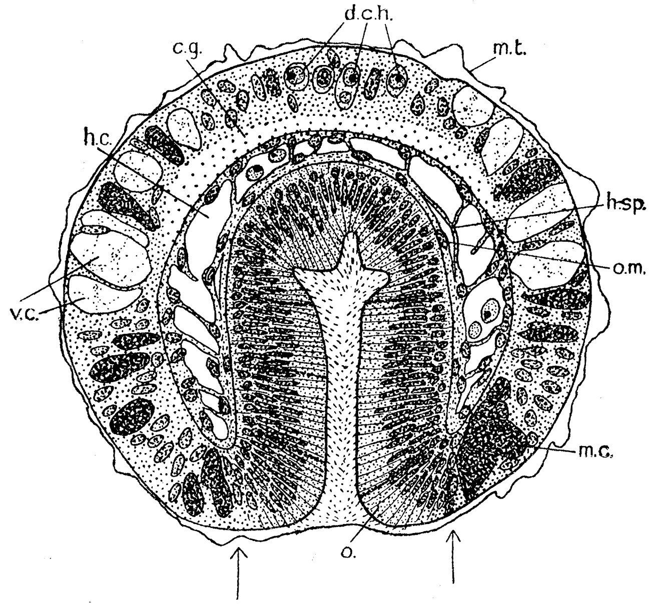
* Owing to great reduction in making the block this does not show at all clearly in the half-tone reproduction, but it is plainly indicated in the key to Plate 32, *r.m.*

it meets the first septum in a line (BA) running outwards and downwards from the ventral surface of the œsophagus, and there it turns down almost vertically to fuse, as has just been said, with the lower third of the first septum. It will be seen that as a result of this configuration of the mesodermal sheets, there is left on each side at the anterior end of the trunk a roughly triangular area of ectoderm (*e.u.*), uncovered by mesoderm internally and lined inside only by its basement membrane.

Passing through the first septum is the œsophagus. As has just been shown, its ventral surface is fused with the horizontal membrane, but elsewhere it is free and projects forwards in a great downward curve to where its turned-up edge (*o.e.*) forms the mouth.

The structure of the head has been fully described on p. 280. It will be remembered that it is essentially a saddle-shaped structure containing a definite cœlomic cavity, bounded above by a thick wall and below by a thin sheet of mesoderm. The cœlom is traversed anteriorly by a number of fine mesodermal strands. When this structure is pulled down by the muscles attaching it to the trunk it comes to rest with its under surface, the thin sheet of mesoderm (*h.sp.*), resting on the anterior end of the œsophagus (see also text-fig. 41). A slight change in actual shape of the head appears to take place, which brings the edges of its thick upper ectodermal wall in contact with the edge of the œsophagus anteriorly, and with the edge of the trunk posteriorly, fig. 16, Plate 32, and



TEXT-FIG. 41.—Transverse section of head of *Owenia* worm not more, and possibly considerably less, than 8 hours after metamorphosis. × 756. The head splanchnopleure has not yet completely fused with the œsophageal mesoderm; spaces are left between the two layers which are possibly stages in the formation of blood vessels. From a preparation stained with Delafield's hæmatoxylin. Arrows indicate where the head ectoderm has recently fused with the œsophagus. *h.sp.*, splanchnopleural mesoderm of head.

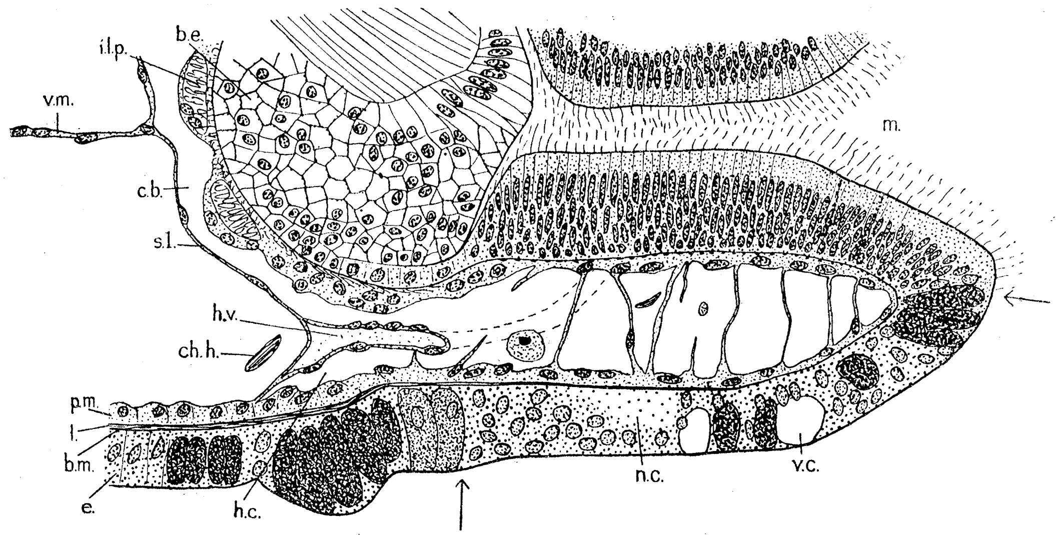
text-figs. 37A, 41, 42. Meanwhile the lower mesoderm sheet undoubtedly stretches so that not only does it cover the whole upper surface of the œsophagus exposed anteriorly to the first septum and the upper surface of the horizontal septum on each side, but it is tucked into the lateral triangular cavities of the trunk, where the ectoderm (*e.u.*) is internally exposed. It forms a lining (*h.s.e.*) to this ectoderm and also covers (*h.s.s.*) the anterior surface of the first septum, with which it is to fuse indistinguishably. It is interesting to recall at this point the fact mentioned on p. 280, and illustrated in text-fig. 29, that in the later larva the mesoderm of the head shows a definite posteriorly directed projection (*p.p.*) on each side. This is the part of the mesoderm that stretches to fill the triangular trunk cavities in the manner just described.

The formation of certain blood vessels of the head region takes place at metamorphosis, and is illustrated in fig. 16, Plate 32, where their distribution is shown. In some respects this interpretation of the method of their formation is hypothetical, for there is great difficulty in seeing them clearly at all points; they are very small. It seems certain that where the splanchnic mesoderm of the first chætigerous segment folds against the larval roof of the buccal organ cavity a gap is left on each side to form a vessel. This vessel (*l.v.*), as already referred to on p. 296, communicates with the just-formed ventral vessel and runs outwards transversely until, about half-way towards the body wall, it passes into another vessel. The latter (*h.v.*) runs forwards, and is almost certainly formed by the leaving of a gap between the horizontal septum and the lower mesodermal membrane of the head, which fuses with it. This horizontal vessel can be distinctly seen shortly after metamorphosis, text-fig. 42. The greater part of the horizontal septum and the head mesoderm with which it is fused disappear almost immediately to allow free communication between the buccal organ cœlom and the cœlom of the head. As in the adult only that part between the vessel and the still remaining lateral portions of the transverse septum of the buccal organ cavity (p. 269), which are now in an almost horizontal position, remains to support the vessel (see p. 302).

Some time after metamorphosis it is quite easy to trace each horizontal vessel into a vessel (*o.v.*) running up towards the dorsal surface of the œsophagus, where it joins its fellow from the other side to continue as a single dorsal vessel; this is seen prominently in text-figs. 44, 45, *d.v.* The latter passes backwards to join the vessel in the dorsal mesentery, which has by then been completed after disappearance of the larval chæta-sacs. I believe that this dorsal vessel and the branches joining it anteriorly to the horizontal vessel are formed by the leaving of gaps between the lower mesoderm sheet of the head and the surface of the œsophagus, which is covered with a layer of larval mesoderm, somewhat after the manner shown in fig. 16, Plate 32. It has, however, not been possible to make this out at all clearly from sections, but occasional appearances suggest that this surmise is correct, and that other finer vessels are formed in this region in a similar way, text-fig. 41, between the points indicated by *h.sp.* and *o.m.*

Wherever the tissues of the head meet those of the gut and trunk, fusion takes place in a few hours. Thus septum one of the adult becomes a composite structure formed partly from the larval trunk and partly from the larval head. A few days after metamorphosis muscle fibres can be seen passing through this septum on each side of the mid-dorsal line, from the longitudinal muscles of the body wall to the upper surface of the œsophagus over which they spread.

Mention has already been made of the shortening of the larval circumœsophageal nerve commissures which must take place to allow the head to approach the trunk (p. 294). When it is considered that these commissures enter the ectoderm of the trunk by penetrating the basement membrane some little distance behind the most anterior edge of the trunk, it will be obvious that when the head is finally in position they must, although shortened, pass, after leaving the head close to the eye region, for a little way internal to the basement membrane of the body ectoderm. This can actually be seen in sections of the young worms, and sometimes the stain picks out the retractors running alongside them. The distance is quite short, being about 8 μ.



Text-fig. 42.—Slightly oblique frontal or horizontal section of right side of head of *Owenia* worm not more, and possibly considerably less, than 8 hours after metamorphosis. × 756. From a preparation stained with Delafield's hæmatoxylin. Arrows indicate where fusion of the head ectoderm with the trunk and œsophagus has recently taken place. ***h.c.***, head cœlom. A few histolysing fragments of tissue are present in this cavity; ***h.v.***, horizontal blood vessel. The dotted lines show its course in the next section; ***s.1***, first adult septum.

A few minutes after metamorphosis starts the cœlom of the head is found to contain a number of particles, cells and apparently muscle tissue in a state of histolysis, text-figs. 37A, 41, 42. The origin of these is obscure, but they may possibly be portions of the retractor attachments which are no longer required, and also certain cells of the head ectoderm that break down, text-figs. 37A, 41, *d.c.h.*, and are probably cast out into the cœlom. These cells may perhaps have been associated in some way with the long sensory cilia of the larval head, which are also lost at this time.

(iii) *The Structural Changes of the Gut.*

As the gut is dragged into the trunk during metamorphosis, the previously uncovered portion is not only invested in a sheath of splanchnic mesoderm, but the portion which had previously been so covered is slid further down the sheath for a little way. This is evident if we compare the position of the intestinal valve in text-figs. 24 and 37B, noting its relation to the third adult septum. As previously stated (p. 275), the sheath is not actually fused with the endoderm, and a few days later a considerable space, the peri-intestinal blood sinus, *g.s.* in text-fig. 37B, is to be observed between the two. The circular muscles of the sheath then set up regular peristaltic waves which sweep forwards as far as the second septum, where the sinus ends. The sphincter situated in the sinus wall, immediately anterior to the third adult septum, after metamorphosis runs straight round the gut instead of obliquely, as in the larva.

At or immediately after metamorphosis the cilia of the stomach and intestine are lost, and at the same time the intestinal valve breaks down, text-fig. 37B, its remains probably being digested along with those of the prototroch. According to McIntosh (1917), the adult *Myriochele* possesses such a valvular process at the posterior end of the gut, in which respect it differs from *Owenia.*

In the anterior wall of the stomach there are in the larva a number of amœboid cells, which are easily distinguished from neighbouring cells by their strong staining properties. They have already been described (p. 251). On metamorphosis they round up, text-fig. 37A, *d.c.*, and within a few hours have all been extruded into the lumen of the gut, text-fig. 43, *c.*, where they mingle with the remains of the prototroch and other larval tissues, and are doubtless digested along with them. They do not appear to be replaced, and the gut of the young worm a few days old is devoid of them or any similar cells.

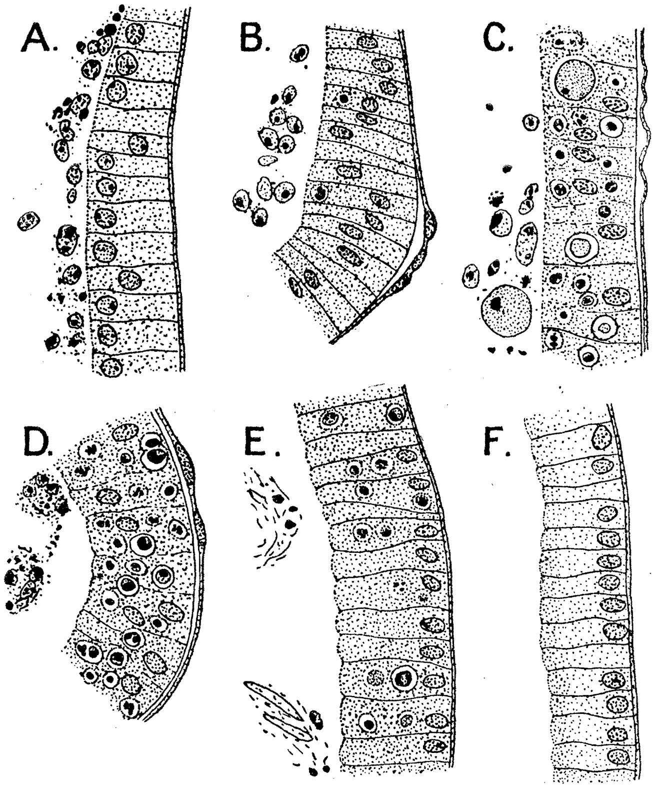
The remaining cells of the stomach and intestine undergo some sort of re-organisation. Their nuclei during larval life are at the ends of the cells nearest to the gut lumen, but immediately after metamorphosis they begin to pass to the other ends, close to the sinus, where they remain, text-fig. 43. Nuclear division stages have not been seen in the gut wall during this period.

The œsophagus does not appear to undergo any marked structural changes, and it retains its cilia. The anterior portion becomes rounder in cross-section after metamorphosis.

(iv) *The Fate of the Larval Tissues.*

We have already seen how the prototroch, metatroch, episphere and hyposphere tissues histolyse and are swallowed at metamorphosis. The nephridia and the various contractile fibrils traversing the blastocœl of the larva presumably share the same fate. The nephridia cannot be distinguished in sections of young worms just metamorphosed, and they would be quite obvious if retained. In the larva fixed during the process of inversion of the trunk, fig. 12, Plate 31, the dorsal levators, to which the nephridia are attached, are actually just breaking away from the episphere, and it is a legitimate conclusion, in the absence of contrary evidence, that they are both swallowed and digested in the gut.

The digestion of the larval tissues is a process of peculiar interest, inasmuch as it is *intracellular*. According to YONGE (1931), digestion in Annelids is "*exclusively extracellular*," and so it is in both larvæ and young worms of *Owenia*, when food consists of diatoms and other organisms swallowed in the usual way. With its own tissues, and its own tissues only, however, it digests by the more primitive intracellular method. This is illustrated in text-fig. 43. Very soon after metamorphosis, as the nuclei sink down through the cells, particles of the histolysed larval tissue contained in the gut are taken up in amœboid fashion by cells of the stomach and intestine (not the œsophagus), text-fig. 43B. In a short while each cell contains a considerable number of such



TEXT-FIG. 43.—Sections of the stomach wall of *Owenia* worms at varying times after metamorphosis to show intracellular ingestion of the histolysed larval tissues. × 756. In each case the lumen of the gut is on the left and the splanchnopleure on the right. In sections B to D the lumen of the gut sinus is seen. *A*. Immediately after metamorphosis, and before ingestion has begun. *B*. A little later. Ingestion beginning. *C*. Later still. Most of the cells now contain one or more food vacuoles. The large rounded cells have resulted from the rounding up of the dark staining amœboid cells of the larval stomach. One has been cast out into the stomach lumen, the other is either in process of being cast out or is being digested. *D*. Not more than, and probably considerably less than, 8 hours after metamorphosis. All the cells gorged with fragments of larval tissue. *E*. About 2 days after metamorphosis. Digestion of larval tissues nearly complete. Very few fragments of this tissue still remain in the lumen of the gut, which in addition now contains diatoms recently swallowed. *F*. About 2 days after metamorphosis. Digestion of the larval tissues completed.

particles, text-fig. 43C, and in a few hours becomes literally gorged with them, and the cell boundaries are rendered indistinct and difficult to follow, text-fig. 43D. The particles consist of broken-down nuclei, which stain up very darkly on account of their condensed chromatin, and of fragments of cytoplasm without nuclei. The particles are enclosed in definite food vacuoles, the outlines of which vary in degree of distinctness, and are seen in what appear to be various stages of digestion. Finally, after about two days little or no larval tissue remains in the gut, and the worm actively swallows diatoms for food. The cells gradually empty, and resume a normal appearance, text-fig. 43E and F.

It may be as well to note that there is no question of the particles being passed through the wall of the gut into the peri-intestinal blood sinus or into the cœlom. Very few broken-down cells are ever seen in these cavities, and those that are have already been mentioned (p. 294). They appear before the gut cells have begun to ingest the histolysed larval tissue.

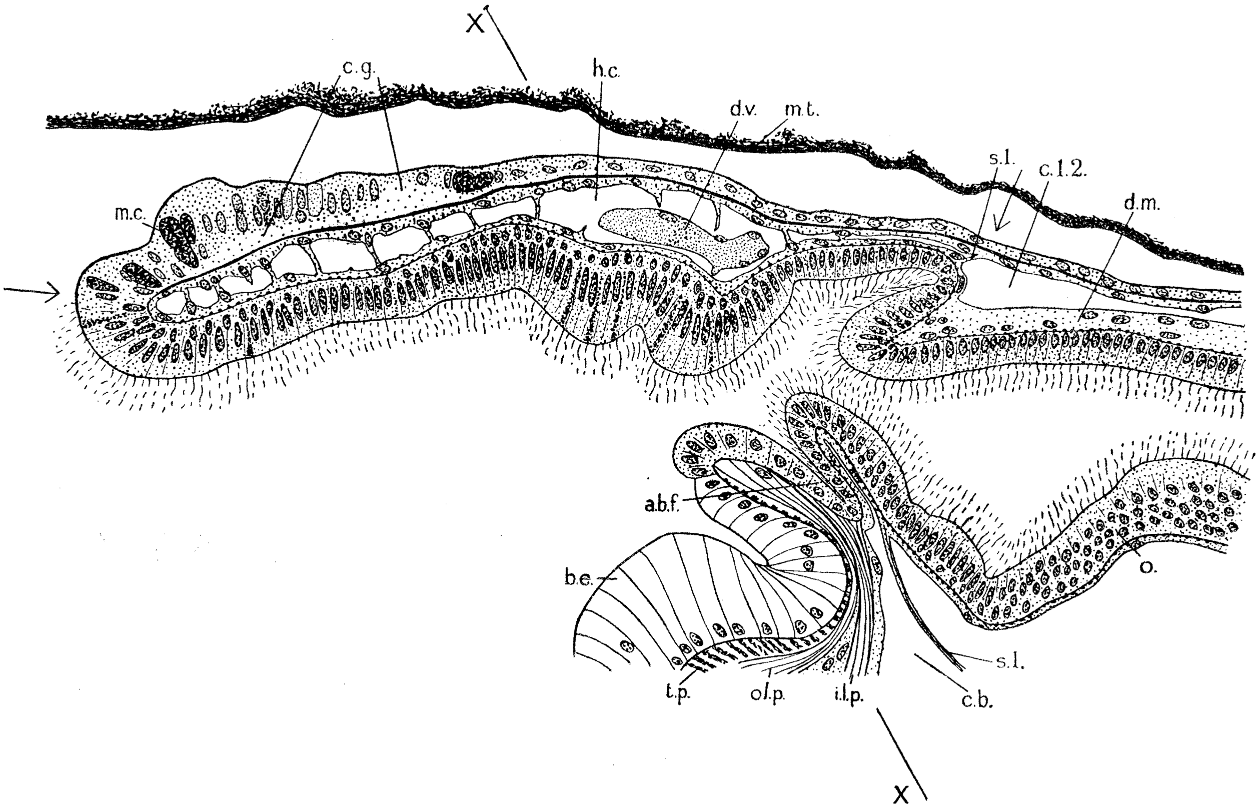
16. Later Development of the Young Worm.

Within a few hours after metamorphosis the head has completely fused with the trunk and œsophagus, text-fig. 42, and within two days the last traces of the chæta-sacs have disappeared (see text-fig. 44 of a worm a little older than this), and the prototroch is in the last stages of digestion. The buccal organ cœlom communicates with that of the head, the two forming a common cavity anterior to the first adult septum. This cavity is crossed by the strands of mesoderm tissue of the head region and by an incomplete horizontal partition formed by the lateral retractors to the buccal organ, text-fig. 45, *l.r.* This incomplete horizontal septum is described by Gilson (1897, p. 381), and is illustrated by Zürcher (1909). Compare especially the latter's figure 17, plate 17, with my text-fig. 45. Attached to the upper surface of this partition on each side is the horizontal vessel (Zürcher's "Kiemenstämme"), the attaching membrane of which appears to have been formed partly from the old true horizontal septum and partly from the transverse septum which is present on each side of the lower part of the buccal organ cœlom in the late larva. The position of attachment has, however, shifted away from the buccal organ, and down the lateral retractors.

Circular muscles are not present in the newly-metamorphosed worm: they appear two or three days later, text-fig. 45 *c.m.*, between the longitudinal muscles and the basement membrane, but only anterior to the second adult septum. They are much weaker than the longitudinal muscles.

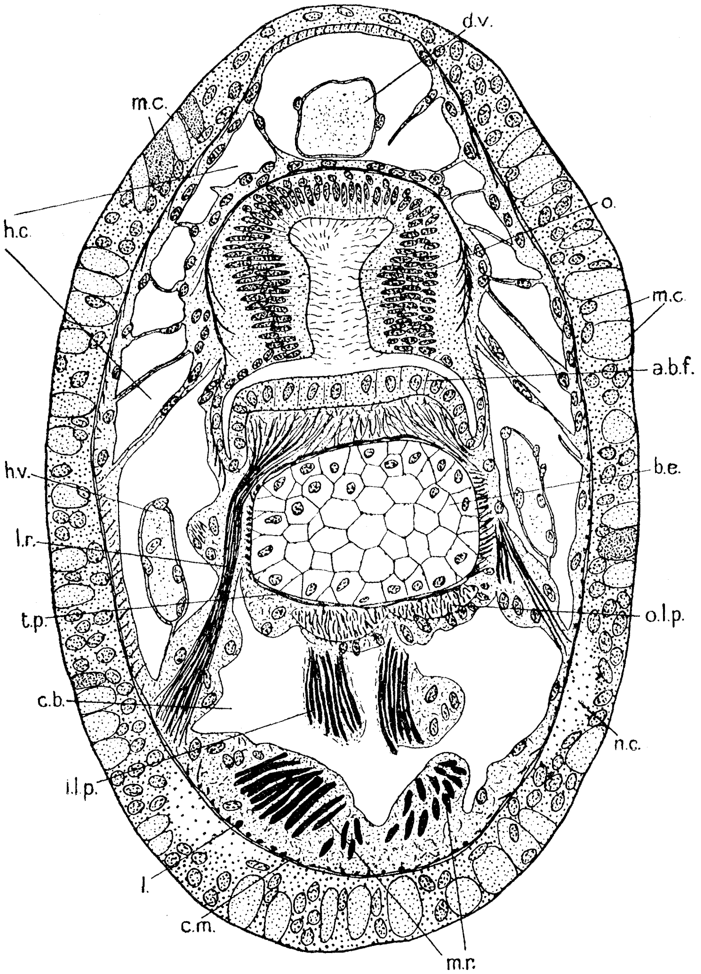
The worm grows larger fairly rapidly, not so much by a multiplication of segments as by a general increase in body size. Thus a young worm about a month after metamorphosis, fig. 14, Plate 31, has only acquired two more segments, but has trebled its length. The tube is greatly thickened, and probably in this secretions of the thread glands are used, although it is difficult to be sure. The tube is well coated externally with sand grains, arranged irregularly, and not in the characteristic imbricating manner of the adult. The conical ends of the tube are elastic, and close the openings when the worm retracts. The worm can reverse its position in the tube as well as rotate in it at a fairly rapid rate.

A few days from the time of settling down the gut sinus becomes very distinct. It runs from the end of the tail to the second adult septum, where it ends. The blood at this stage is colourless, and is driven forwards by peristaltic waves sweeping along the sinus in an anterior direction. According to ZÜRCHER (1909), the sinus communicates at the second adult septum with the dorsal blood vessel—which is present only in the thorax and in the head—and with a network of small vessels covering the œsophagus. The ventral vessel, on the other hand, is continuous from the first adult septum to the tip of the tail. In the adult it bears peculiar ampullæ, and what may be the rudiments of these have been seen in late larval stages (see text-fig. 32, on either side of where the ventral mesentery (*v.m.*) joins the intestine) and in transverse sections of young worms.



TEXT-FIG. 44.—Median sagittal section of head of young *Owenia* worm several days after metamorphosis. × 756. From a preparation stained with Delafield's hæmatoxylin. Arrows indicate where fusion of head with trunk and œsophagus has taken place. Line XX indicates approximate plane of section shown in text-fig. 45. *d.m.*, ventral part of dorsal mesentery. The dorsal part is in the adjacent section; *d.v.*, dorsal blood vessel.

I have, unfortunately, not been successful in rearing worms to a much later stage than that shown in fig. 14, Plate 31. This is about a month old after metamorphosis, and has thirteen chætigerous segments and fairly numerous bristles and uncini. The blood is now red. The head or prostomium is especially interesting in that it shows the



Text-fig. 45.—Oblique transverse section of head of a young *Owenia* worm several days after metamorphosis. × 756. From a preparation stained with Heidenhain's hæmatoxylin. The approximate plane of this section is indicated in text-fig. 44. *h.v.*, horizontal blood vessel.

rudiments of the three pairs of branched branchial processes characteristic of the adult. It shows the adult collar posterior to the eyespot and it should be noticed that this collar does not follow the line of fusion of the head with the trunk ; it is much anterior to it. On the collar there is a band of brown stippled pigment, and another occurs just in front. This is an adult feature. As first pointed out by Watson (1901), there is in the adult a pore at the anterior extremity of the prostomium occurring actually between the head ectoderm and the œsophageal tissue. It might be thought that this pore would be left when the anterior border of the head fuses with the œsophagus, but it has not been detected in the oldest worms examined, and it seems evident that it opens up at a later date.

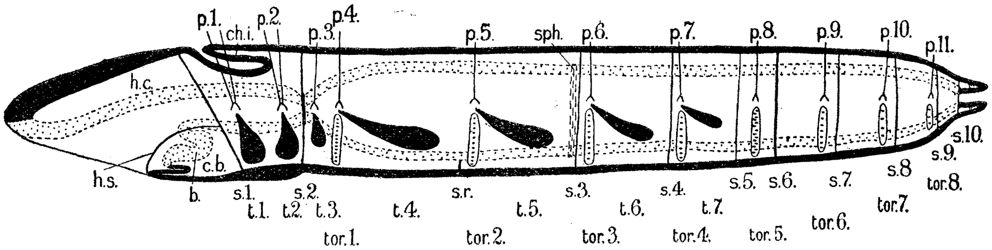
A noticeable feature of these young worms is the black colouration of the middle section of the gut. It is divided into two portions, a short part anterior to the third adult septum, then a gap in the region of the sphincter, followed posteriorly to the septum by a long portion, which gradually becomes lighter in colour as it extends backwards. The same regions can be seen in adults. Sections of worms fixed in osmic or Flemming without acetic show the cells of this region to be crowded with small, greenish-brown refringent spherules, which in the mass give a black appearance reminiscent of a fat absorption picture. Other worms fixed in Bouin do not give this picture; in such preparations the cells merely present a vacuolar appearance. It seems clear, however, that these spherules are not fat droplets, as they are not dissolved out of the sections after many days in xylol. Their nature remains unknown.

17. The Septa of the Young Worm.

Considerable confusion exists in the literature as to the positions of the various septa in the adult. Confining our attention at first to the second adult septum, we have seen how it is situated between the second and third chætigerous segments, text-fig. 27, and figs. 15 and 16, Plate 32, so closing posteriorly a thoracic region of two fused segments, the first and second chætigerous. Von Drasche (1885) supposed this region to consist of the first three bristle-bearing segments fused together, and Gilson (1894) also fell into this error for a time, but corrected it in a later paper (1897), where he demonstrated the true position of this septum. In spite of this correction, Watson, a few years later, still writes that "internally there is a septum between the buccal segment and those which follow it, *but the next three segments are without septa and enclose a single chamber*" (1901, pp. 232–233) (italics mine). Zürcher (1909) correctly describes the thoracic chamber as composed of two fused segments only, but McIntosh (1915) unfortunately follows Watson. My own work on the embryology makes it quite clear that the second adult septum is situated between the second and third parapodia, and this, and the remarks below concerning the more posterior septa, have been confirmed by examining adults cleared in cedar wood oil, when the positions of the septa and their relations to the parapodia can be seen with ease.

No less confusion exists with respect to the succeeding septa. Thus Watson's statement (accepted by McIntosh (1915, p. 359)) that "each of the 'abdominal' segments is internally cut off from its neighbour by a septum" and that "the anterior extremity of each is indicated by a bundle of dorsal or dorso-lateral setæ" (Watson, 1901, p. 233) is not entirely correct. Neither is Gilson's figure (1897, fig. 25, Plate III) free from error.

Referring to my diagram of the septa of a young worm just after metamorphosis, text-fig. 46, it will be seen that the second septum is followed by a region of three fused segments, although a trace of the septum immediately anterior to the fifth pair of parapodia is present on each side of the ventral mesentery (see p. 272). Whether this remains in the fully-grown adult or not is uncertain. This region of three segments is bounded behind by the third adult septum, which is characterised by the presence immediately in front of it of a sphincter in the walls of the gut sinus (see p. 275). All the succeeding segments are limited by septa. It will be seen from the diagram that whereas in bristle segments three, five, six and seven the parapodia are placed at the anterior extremities of the segments, just behind a septum, in bristle segment nine, and all succeeding segments the parapodia occur at the posterior extremities just in front of a septum. Segment eight is a transition segment; it is relatively short, and carries its parapodia roughly midway between its two limiting septa. It will be remembered that this transitional pair of parapodia is further distinguished by the proportionally large number of uncini which it carries (p. 272). The septa which limit this transitional segment are of two kinds. That in front, the fifth adult septum, is thin and weak, and has an open gap between it and the body wall on either side; the one behind is strong and muscular, and has no such gaps. Now according to Gilson, conditions are reversed, septum five is strong and muscular, and septum six weak with lateral gaps, and he shows a horizontal section in detail of this part of the adult (1897, fig. 19, Plate III). To make sure, I cut sagittal and horizontal sections of this region of four adults, taking great care to be quite certain of the anterior end of each piece cut out from the worm. These confirmed my previous observations on the very young worm, and, while the histology seemed to agree with Gilson's drawing, it did so in the reverse order: his septum five should thus be labelled six and his six five.

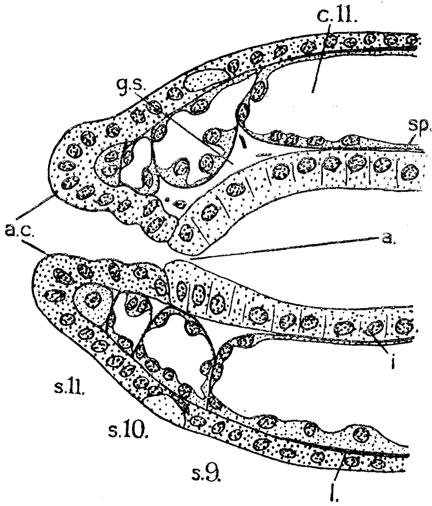


Text-fig. 46.—Diagram to show relative positions of parapodia and septa in a recently metamorphosed *Owenia* worm. Not to scale. *b.*, buccal organ; *h.s.*, horizontal septum; *p.*1, *p.*2, etc., first, second, etc., parapodium; *sph.*, sphincter in splanchnopleure anterior to third adult septum; *s.r.*, remaining ventral part of fifth larval septum; *t.*1, *t.*2, etc., first, second, etc., thread gland; *tor.*1, *tor.*2, etc., first, second, etc., torus.

Gilson's next error, in his fig. 25 (1897), is to put the ninth parapodium (sixth torus) behind the seventh septum. We have seen that it occurs in front. As a result he came to regard his sixth septum as a kind of extra one contained within the eighth chætigerous segment. It is now quite obvious that nothing of the kind exists.

The last definite septum in the young worm is the tenth adult, situated at the base of the circular wall enclosing the anal chamber, text-fig. 47. The eleventh can be seen developing further back, well within this wall, but there is as yet no definite cœlomic cavity behind it. It thus appears that the growing point is situated at the top of the anal wall, and that the segments as they are formed slide forwards until they encircle the endoderm of the gut. This surmise, however, has not yet been proved on account of the material of these growing young worms being rather scanty.

Little can be made out of the structure of the septa at this stage. Muscle fibres are clearly visible in some preparations, especially in septa two, six, seven and eight. The septal valves appear to be developing—to judge by slight thickenings caused by clusters of nuclei in the positions they will occupy in future—but it is really too much to expect to be able to see whether they are definitely perforated or not. Septa three and four may possibly have lateral gaps of a less extent than those of septum five, but it is difficult to make certain. Septum three possesses such gaps in the adult, and apparently also septum four (Gilson, 1897, p. 394—his "Fentes dorsales").



Text-fig. 47.—Horizontal longitudinal section of tail end—a little contracted—of an *Owenia* worm shortly after metamorphosis. × 756. *a.c.*, lateral walls of anal chamber; *c.*11, cœlom of eleventh chætigerous segment; *g.s.*, gut sinus containing a few fragments of histolysing tissue.

18. Conditions under which Metamorphosis takes Place.

About four weeks after fertilisation the fully developed larvæ were ready to metamorphose (July rearings). During their pelagic development they swam strongly, with an upward tendency for most of the time. On occasions when the plunger plate stopped accidentally for a few hours the majority would crowd up against the surface film, some being caught and destroyed (see p. 321). Once they were ready to metamorphose, however, a change took place, for they then tended to sink, and, when the plunger plate was stopped so that it no longer stirred up the water and with it the larvæ, the majority swam slowly along in contact with the bottom, or rested thereon without much movement. Every now and then larvæ would leave the bottom and swim up towards the surface, but sooner or later would sink to the bottom again. Larvæ kept in finger-bowls behaved in exactly the same way, and thus it is likely that in the sea they seek the bottom when ready to metamorphose.

Metamorphosis is conditioned by the character of the bottom, particularly by the presence or absence of fine sand or grit. The following experiments performed in the summer of 1930 illustrate this statement in a striking manner.

Experiment 1.—A number (80–100) of fully developed Mitrarias were removed from the plunger jar in which they had been reared and placed in a clean finger-bowl. About 12 selected at random were removed from the latter and placed in a small glass dish containing water from the same plunger jar. Sand from Torquay had previously been sprinkled thickly over the bottom. Half-an-hour later several had metamorphosed into little worms and these already had mucus tubes, to the outsides of which sand grains were attached. Two hours later all but one larva had metamorphosed. In the clean finger-bowl none of the much more numerous larvæ metamosphosed during the same period.

Experiment 2.—Late larvæ were transferred from a plunger jar to a clean finger-bowl. Two small petri-dishes of the same size were taken and filled with equal parts of sea-water. To one dish fine sand from Torquay was added. Some of the water from this dish was then pipetted into the other dish (care being taken to avoid sand grains), mixed round and then water was pipetted back to equalise the levels again. This ensured that any substances which might be dissolved from the sand were present in both dishes. Twelve larvæ were then placed in each dish. They were taken at random from the finger-bowl but only from among those resting on the bottom. Three were pipetted up at a time and the lots of three put alternatively in the dishes. Within a few minutes some of the larvæ metamorphosed in the dish containing sand, the majority had done so an hour and a half later and all had undergone the change within four and a quarter hours. In the dish without sand none metamorphosed during this period, but one began to do so a few minutes after the dish had been disturbed while examining it. Sand was now added to this dish and within 15 minutes all the 12 larvæ had metamorphosed or were undergoing metamorphosis.

Experiment 3.—Fully developed larvæ were removed from a plunger jar and put into a clean dish. Two perfectly clean finger-bowls were taken and filled with filtered outside sea-water. With a pipette five larvæ were selected at random from among those resting on the bottom of the dish and placed in one of the finger-bowls. Another five similarly selected were put into the other bowl. This was repeated alternately until there were 35 larvæ in each finger-bowl. Torquay sand, previously washed in sea-water, was spread in a thin layer over the bottom of one of these bowls, and soon some of the larvæ metamorphosed and the majority had done so 4½ hours later. The bowls were left overnight and by the next morning all the larvæ in the one with sand had metamorphosed but in the other none had done so. In the latter bowl, however, eight larvæ had been caught in the surface film and destroyed. From among those still unmetamorphosed five, selected at random and placed in a clean dish with well-washed sand, metamorphosed about 4 hours later. Those remaining in the bowl without sand were still Mitrarias, but sand was now added and soon some of them metamorphosed and all but one had done so 7 hours later.

Experiment 4.—This was a repetition of experiment 2, but was performed on the next day. In 2½ hours all the larvæ in the dish with sand had metamorphosed except one individual which did so an hour later. In the clean dish after 3½ hours no larva had undergone the change. Sand was then added to this dish and very soon some larvæ began to metamorphose, and in less than an hour all were little worms.

Experiment 5.—This was of a different nature from the preceding and was started with the idea of finding out what would happen if the larvæ were kept continually in perfectly clean surroundings. A clean finger-bowl was filled with filtered outside sea-water and *Nitzschia* was added for food. Fourteen fully developed larvæ were put into the bowl, which was covered with a glass plate. Two days later none had metamorphosed. They were not examined again until a week later—owing to my absence caused by influenza—and it was then found that eleven had metamorphosed. Of these eight were definitely abnormal in various ways. Three were normal and had evidently grown a little since metamorphosis and it is significant that they had an accumulation of very fine grit and little masses of *Nitzschia* on their tubes and one had a single fine sand grain attached. Of the remaining larvæ, two were very abnormal with reduced prototrochs and half-everted trunks and were past producing normal worms. The third larva also looked abnormal about the prototroch but the trunk and apical plate seemed normal. It was removed to a small dish with sand

and within half-an-hour it metamorphosed. The worm produced was slightly abnormal in that the gut was not dragged through the second septum quite far enough dorsally (see p. 312).

Further experiments of the nature of the last one were projected but were unfortunately unavoidably prevented. On my return after a week's absence the larvæ in the plunger jars had all metamorphosed and the worms so produced had nearly all of them got strong mucus tubes covered with fine grit and *Nitzschia*. The grit was probably dust from the atmosphere. It was too late in the season to rear again and the younger cultures I had were unhealthy.

The above experiments clearly indicate that fine sand, such as the adults live in, stimulates in some way the fully developed Mitraria and causes it to metamorphose. It is evident that the larvæ can exist in a condition ready to metamorphose over a period of at least some days, and when we consider that they are at this period seeking the bottom, we begin to realise the significance of the rapid metamorphosis. Until the larvæ reach the bottom, and maybe until they encounter a substratum suited to their adult life, they retain their full pelagic structure and the protection of their long bristles. Having reached a suitable bottom, they can change with the least possible delay into typical members of the benthos.

In my opinion it is contact stimuli with grit which occasions metamorphosis, although it is possible that there may be some less simple mechanism involved. It is important to note that a larva does not metamorphose immediately it first comes into contact with the sand; the period between first contact and metamorphosis appears to vary widely with different individuals. They may rest for a time on the sand grains, swim over them more or less in contact, or may swim up to the surface (in bowls) and down again several times before the great change takes place. Always, however, they finally become little worms, some a very few minutes after first contact and probably the majority between 10 minutes and half-an-hour, although some may take several hours before they change.

These considerations suggested further experiments, some prevented by unavoidable circumstances, others carried out with varying success. The results being indefinite, they may be summarised briefly and in a general manner. Thus it was found that sand sprinkled so sparsely that the individual grains were far apart caused larvæ to metamorphose after many hours, while those in the clean control dish did not do so, with one exception which is of special interest. This larva had been caught in the surface film, probably just before it was found, but unlike all other such larvæ I have seen, it was not destroyed but was in a half-metamorphosed condition. The trunk was extended with the gut almost completely dragged in and the head was pulled down to near its final position. The epi- and hyposphere tissues were crumpled but not disintegrated and the cilia were still beating strongly. On being released from the surface film it swam about without any further change for 7 hours. It was then transferred to a small glass dish with sand grains, and 20 minutes later as it had not changed it was left for the night. The next morning it was a perfectly normal worm in a well made mucus tube to the outside of which sand grains and grit were attached.

An attempt was made to determine the influence of various substrata on the metamorphosis. Pieces of rough surfaced rock, sandstone and concrete were thoroughly cleaned but it seemed impossible to eliminate all loose particles of grit and eventually the larvæ metamorphosed and covered their tubes with these particles. The same remark applies to very coarse shell gravel. A sheet of ground glass caused metamorphosis after several hours but apparently not so much on its ground surface as between its edges and the side of the dish, which it nearly fitted. Some of the worms so produced were abnormal. Fine sands and grits appeared to be the most efficient substances in inducing metamorphosis. Muds, particularly sticky muds such as china clay, powdered magnesium carbonate, powder from shells ground up in a mortar, stuck to and smothered the larvæ so that few metamorphosed and then usually abnormally. In the perfectly clean control pots none of the larvæ metamorphosed until sand was added.

As it was likely to be difficult to section worms inside their gritty tubes a number of larvæ were put into a dish strewn with pyroxylin fibres, well washed in sea-water. After many hours half of them metamorphosed, usually after getting entangled among the fibres. The much less readiness with which they would metamorphose among these fibres, even when entangled, than they would in a dish with sand, was very noticeable. Subsequent examination of the young worms by sectioning and whole mounts showed that most if not all were slightly abnormal in that the gut had not been dragged through the second septum to the normal position, a small portion of the stomach remaining anterior to it dorsally (see p. 312). Larvæ metamorphosed among sand grains showed only a small proportion of abnormalities.

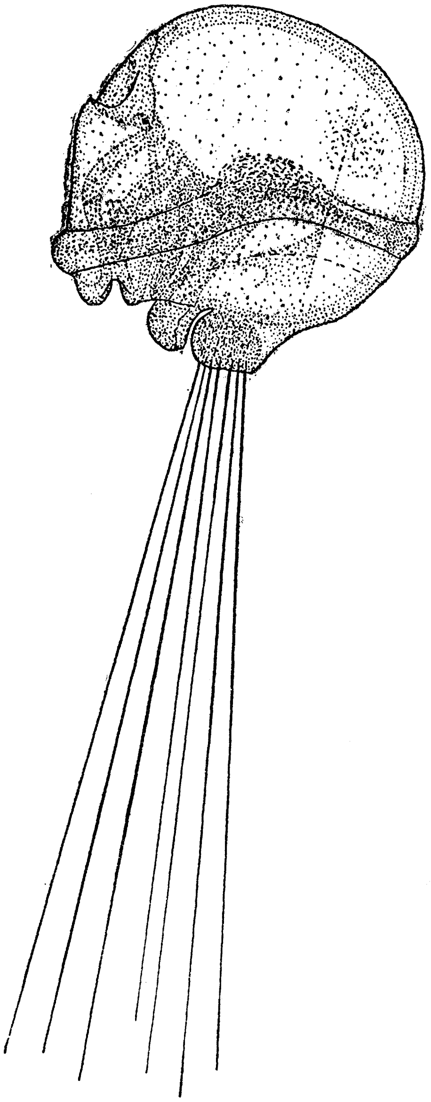
19. ABNORMALITIES.

(i) *Abnormal Larvæ.*

In some cultures a very interesting abnormality occurred, namely, the under development of the prototroch. The larvæ at first would appear to be normal and would increase in size in the usual way, but after about a fortnight it would be noticed that the prototroch was not as large and sinuous as is usually the case by the time that the trunk rudiment is well in evidence. Nor throughout the further development would the prototroch approach the normal condition. In mild cases the dorso-ventral and lateral diameters of the larva would be about normal but the four upward arches would be lower than usual. Yet the trunk and head rudiments would develop normally and even metamorphosis take place, although in the latter case there would be a high proportion of abnormal worms. In one extreme culture the prototroch remained perfectly circular or almost so, text-fig. 48, with a diameter of about 300 μ (normal dorso-ventral diameter 560 μ, lateral diameter 410 μ). In some individuals, as in that shown in text-fig. 48, the lateral portions of the prototroch became somewhat disorganised but in others this did not occur. The hyposphere was hemispherical instead of flattened, while the elliptical non-ciliated area between the anterior lip and prototroch was absent. Thus, apart from their bristles, the larvæ simulated the *Polygordius* type. The trunk and head developed normally in most instances except that they were somewhat reduced in size. In some individuals metamorphosis took place and a few, but by no means all, produced perfectly normal but dwarfed worms, averaging 600–700 μ in length (normal 800–900 μ). In July the time of development was much longer than usual, taking about 6 weeks instead of 4, and even then many individuals showed only early stages in the development of the worm rudiments.

In larvæ in which the prototroch is underdeveloped its tissues contain an unusually large amount of yellow-brown speckled pigment. Also the dark irregular globules, which are normally present along the upper border of the prototroch rim (see above, p. 246) frequently form abnormally large masses. Thus the whole organ has a much darker appearance than usual.

The cause of this curious abnormality is obscure. So far as known the conditions under which the abnormal larvæ formed were identical with those under which normal ones developed. They were given the same food and were treated in the same way. Some were in plunger jars, others in finger-bowls. It may be that the eggs or the sperm were not perfectly mature and yet normal, although undersized, worms sometimes resulted from these stunted creatures. In one case a plunger jar contained larvæ all fertilised together but the eggs and the sperm had been obtained from a number of adults. Some of these larvæ had well developed prototrochs, while in others this organ was reduced. In both types the worm rudiments appeared to be identical. The water was thick with *Nitzschia.* Here the only variable factor was the source of the eggs and sperm and it may be that these differed in potentiality. It is perhaps significant that in the case of the extremely stunted larvæ mentioned above the development time was greatly lengthened and the worm rudiments smaller than usual, for if the metatroch is a food collecting area it is likely, other things being equal, that these larvæ would not get as much food as do normal ones.



TEXT-FIG. 48.—Mitraria of *Owenia fusiformis* with abnormally underdeveloped prototroch but normal worm trunk invagination. × 156. Sketch from a mounted specimen. Actual length from top of episphere to tips of bristles approximately 925 μ. Diameter of prototroch approximately 310 μ.

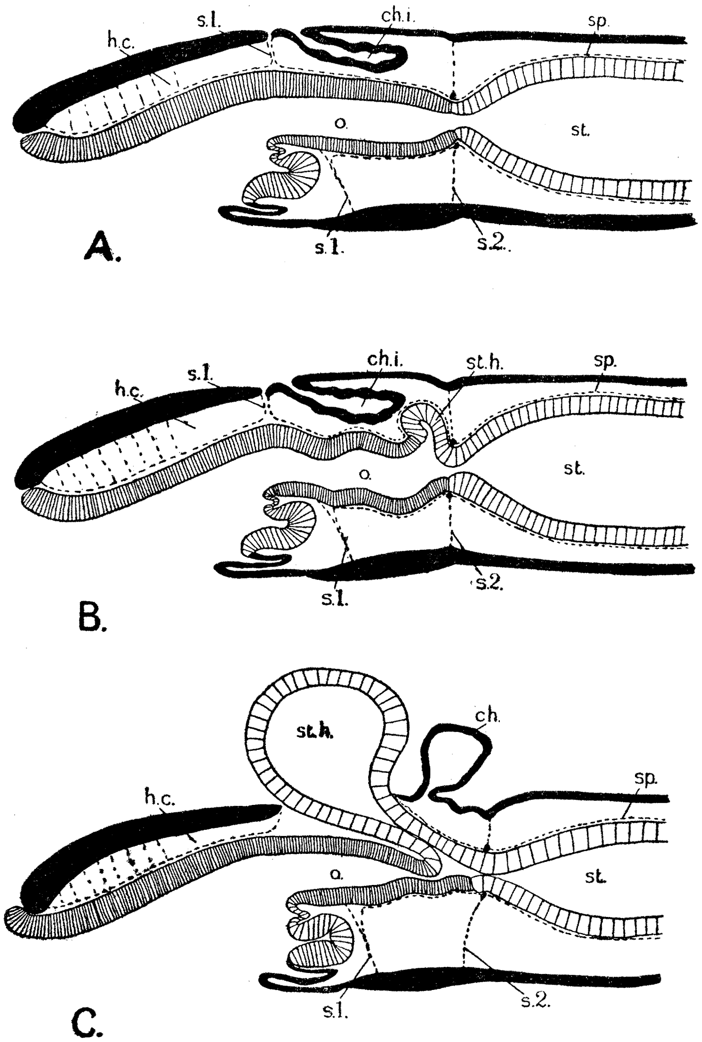
Occasional larvæ were abnormal in other ways. In one case a whole culture lost its provisional bristles at an early stage and after developing a little longer died off. Sometimes larvæ were seen in which the head was entirely missing, or only properly formed on one side. Sometimes the prototroch was asymmetrical and in one case an extra loop was seen. In another specimen four larval chæta-sacs were present, all with long chætæ. But cultures which showed many abnormalities rarely contained healthy larvæ and conversely the best cultures with fine healthy Mitrarias—and only these were used in the study of the development—rarely contained striking abnormalities.

(ii) *Abnormal Metamorphosis.*

Metamorphosis does not always take place normally. It has already been seen that unnatural conditions such as those produced by entangling among pyroxylin fibres (p. 310) or by keeping the larvæ indefinitely in clean surroundings (p. 308) result in metamorphosis which is not normal. Also larvæ which are not perfectly healthy will metamorphose in an abnormal manner and weak cultures do so without being supplied with sand.

It has been seen (p. 290) that during the change from larval to adult life the dorsal part of the stomach has to pass through the second septum and that the latter is provided with a powerful sphincter which comes to surround the junction between œsophagus and stomach. This normal condition is illustrated diagrammatically in text-fig. 49A. The abnormal metamorphoses referred to above are abnormal in that the gut is not dragged through quite far enough and the sphincter constricts a greater or less portion of the stomach anterior to the septum. In slightly abnormal cases only a small portion of the stomach is so constricted, text-fig. 49B, and should it be very slight indeed the worm can sometimes put the matter right within a few hours. When, however, the amount of stomach in front of the septum is at all great it is never able to do this and ultimately perishes. An extreme case of frequent occurrence in weak cultures is shown in text-fig. 49C, where the bulge of stomach forms a hernia at the back of the head, while at the same time the chæta-sacs have failed to invaginate. The latter is also a common abnormality and occurs sometimes when the gut is nearly in the right place. A great protrusion of stomach in the neck region usually means that the trunk has failed to straighten out its lesser folds, probably through the suspensors not having broken. In a few cases the larval tissues have broken down and been swallowed without the larva even turning its anterior segments right side in.

How many larvæ show these abnormalities under natural conditions in the sea it is impossible to conjecture, but it is probably very few indeed, except maybe when they are carried into unfavourable surroundings or fail to reach the bottom within the period during which they are able to metamorphose normally. This period appears to extend over several days even in the laboratory (p. 309) and is probably longer in the sea where the larvæ are likely to be healthier. Sooner or later, however, it must come to an end, and then it is possible that efforts are made to metamorphose which do not succeed in producing normal worms.



TEXT-FIG. 49.—Diagrams of head ends of just metamorphosed *Owenia* worms to illustrate degrees of abnormal metamorphosis. A. Normal metamorphosis. B. Slightly abnormal metamorphosis. C. Very abnormal metamorphosis. *ch.*, larval chæta-sacs which have failed to invaginate ; *ch. i.*, larval chæta-sacs invaginated ; *s.*1, *s.*2, first and second adult septa. The second septum has a powerful sphincter where it surrounds the gut ; *st. h.*, stomachial hernia.

20. COMPARISON WITH LARVÆ FROM THE PLANKTON.

It had been hoped that it would be possible completely to check the reared larvæ against Mitrarias taken from the plankton. Unfortunately they are very rare in tow-nettings in the Plymouth district and special tow-nettings taken in Tor Bay a little while after the adults at Torquay were known to have spawned yielded none. On one occasion only was a living larva obtained, and that was got off Penlee Point in May,

1930. It was presumably of the same species, and careful notes were made of it while alive. At first sight it differed strikingly from reared Mitrarias, for it was almost entirely colourless, the prototroch rim instead of being yellow had only the faintest shade of cream. It has already been shown that in definitely abnormal larvæ the prototroch is always much darker in colour than in the most healthy of reared larvæ. The gut also was colourless apart from a faint cream tinge and the same applies to the body rudiment, head and chæta-sacs. The bristles were beautifully iridescent, about three times the length of those of reared larvæ and proportionally thicker. The stomach contained a dark mass of food. The prototroch was somewhat larger and more sinuous than in plunger jar specimens, the anterior part being particularly well developed and the indentations on the ventral arch very pronounced. But although in appearance this larva differed so markedly from reared ones, in its essential structure and in the size and structure of the body rudiment—which was just about to close round the intestine—it corresponded exactly to the larvæ on which this work is based.

Reared larvæ also agree perfectly with mounted specimens (kindly lent by Miss S. M. Marshall) of the Mitraria larvæ obtained in the Firth of Clyde during the spring, except that the prototrochs of the latter are somewhat bigger and the bristles about three times as long. There is also close agreement with two larvæ obtained at Port Erin in July, 1925, except that again the prototroch is a little more developed and the bristles are treble the length of those of reared specimens. There is, of course, no proof that these plankton specimens were actually *Owenia fusiformis*, but as there does not appear to be another species of *Owenia* anywhere near the British Isles it is very unlikely that they were not. There is no reason therefore to suspect that the development described in this paper is in any way abnormal, with the possible exception that the prototroch should be larger and more sinuous and the bristles longer. We have already seen how the former may vary greatly in size and shape without affecting the essential structure of the rudiments of the head and trunk. It seems evident that the highly specialised larval tissues are capable of much greater variation than are the tissues of the developing worm body.

21. Unsolved Problems concerning the Mesoderm.

The development of Mitraria presents some very interesting problems which, although incapable of solution with the knowledge at present available, should nevertheless be clearly pointed out. The first concerns the origin of the paired teloblastic cells which give rise to the segmental blocks of mesoderm (cœlomesoblast or endomesoblast). In the absence of the evidence which would have been afforded had the division stages giving rise to these cells been seen we cannot decide whether they are derived from cells in the intestinal wall or whether they are derived from ectodermal cells immediately in front of the anus. They may even simply have been extruded from either of these two places. We know that in *Eupomatus* the paired cœlomesoblast cells are for a time situated in the intestinal wall (SHEARER, 1911), they later give rise to the mesoderm bands. According to IWANOFF (1928) these cells, the derivatives of the second somatoblast (4 *d*), give rise only to the mesoderm of the first three segments and the mesoderm of the succeeding segments is derived from cells which pass into the body cavity from the ectoderm of the growth zone. He found a similar condition in Spionid larvæ, and in regeneration of both Spionid and Serpulid worms he traced, as had previous workers, the formation of the new mesoderm from cells passing in from the ectoderm. In view, however, of the different findings and criticism of succeeding workers on regeneration, admittedly on different species (OKADA (1929); PFLUGFELDER (1929); FAULKNER (1930); PROBST (1931)), we must consider this derivation of mesoderm as doubtful, and this doubt must be extended to cover the statements concerning Serpulid and Spionid larvæ, which now require re-investigation. At any rate in other Annelids (*Polygordius, Aricia, Arenicola,* etc.) the cœlomesoblast is all derived from 4 *d* and there is no reason to suspect that this does not also hold good for *Owenia.*

Assuming then that the teloblast cells of *Owenia* are descendants of the second somatoblast it is difficult to homologise them with the paired cœlomesoblast cells of *Eupomatus* or the teloblast cells of the *Polygordius* exo-larva. In *Eupomatus* and *Polygordius* there are no parapodia when these cells appear, but in *Owenia* there is a well developed pair long before the teloblast cells are seen. Unless we believe that the muscles moving the larval chæta-sacs and the apparently cœlomic cavity in which they are placed are all products of larval mesenchyme (ectomesoblast derived from the third quartet) we are tempted to conclude that they represent a segment budded off precociously from the original teloblast cells, cells which were probably homologous with the above-mentioned cells of *Eupomatus* and *Polygordius.* Yet here a difficulty arises for the later development shows that these larval parapodia do not represent a first segment of the worm trunk, for they come to be connected, not with, or in front of, the first pair of segmental blocks, but with or in front of the second pair, being definitely situated behind the first adult septum. But surely the teloblasts cannot bud off the second or part of the second segment before the first ?

Intimately connected with this problem is another. It will be remembered that the buccal organ muscles and the cœlomic cavity of that organ are formed partly from the first pair of segmental mesoderm blocks, derived from the teloblasts, and partly from blocks derived from mesoderm on the œsophageal wall (see p. 265). What is the nature of this œsophageal mesoderm ? In other larvæ we know that it is larval mesenchyme from the third quartet and that its function is to form the circular muscles of the œsophagus. It does this in *Owenia* but in addition it fuses in this peculiar way with the cœlomesoblast of the first pair of segmental blocks and at first, even after fusion, it is seen to be a distinct tissue (p. 266). In no other larva so far described does any such thing take place. In the *Polygordius* exo-larva this mesenchyme becomes two-layered and forms a cavity (schizocœle) on either side of the pharynx (WOLTERECK, 1905, *b*) but it does not come to open into a cœlomic cavity. It may be that the œsophageal mesoderm is not purely larval mesenchyme but that mixed with it there is a pair of true cœlomesoblast blocks precociously budded off from the teloblast cells. The tendency in the earliest stages of the nuclei of the œsophageal mesoderm to congregate in two places on either side of the mid-ventral line of the œsophagus, close to the insertions of the œsophageal muscles, may perhaps be a hint of this. Were it so we should like to regard the larval chæta-sacs as representing the parapodia of this segment but for the difficulty of explaining how they come to be related to the segment next but one behind.

There are in other Polychætes, such as Nereids and Terebellids, larval chætæ which are not present in the adult but these are always situated on definite segments, which remain distinct after the loss of the bristles. In the adult *Owenia*, however, no such definite bristleless segment can be pointed to as that which bore the larval chætæ, and even when the degenerating rudiments of the sacs are still present in the recently metamorphosed worm, it seems impossible to assign a segmental position to them with any degree of certainty. Occurring as they do at the anterior end of the thoracic region they might be regarded as representing a segment immediately anterior to the first chætigerous segment (between it and the buccal organ segment) and the two divisions of each chæta-sacs taken to represent noto- and neuropodia. It should be noted that owing to the absence, so far as is known, of definitely marked ganglia or other organs of a segmental type there is at present no means of proving such an assumption. On the other hand they might possibly be considered as being precociously developed notopodia of the first chætigerous segment, but this seems unlikely as the bristle bundles of this segment, in comparison with succeeding segments, are evidently notopodia themselves. If we accept either of these alternatives we have to face the difficulty, already pointed out, of explaining how these larval parapodia can be formed before any trace of the preceding segment (first larval segment, forming posterior half of the buccal organ segment) is to be seen. There remains the possibility of assuming that they are special larval structures whose mesoderm is derived from the third quartet. If Eisig (1899) is right that in *Capitella* the mother-cells of the cœlomesoblast arise from the third quartet, this assumption might not be quite so strange, but in no other form are parapodial muscles described as being derived from this quartet. But in that case one would hardly have expected them to become incorporated in the adult trunk during the later stages.

The head mesoderm is possibly larval mesenchyme. It may correspond to the single layer of thin cells found under the head of *Polygordius*, but this is not certain as Woltereck (1902) was undecided as to whether that layer was ectodermal or mesodermal in nature.

The problems raised above are not likely to be solved until the cell lineage and very early stages have been studied.

22. Hypothesis.

It does not seem possible, with the limited knowledge at present available, to attempt to explain fully the origin and evolution of cataclysmic metamorphosis. And yet some sort of tentative hypothesis seems to be called for and the following is advanced, not so much as a finished theory, as in the hope that it may lead to the advancing of something better in the near future. It deals only with the lines along which the full cataclysmic metamorphosis probably evolved and does not attempt to explain the underlying cause which was responsible for the larva evolving along those lines.

It seems legitimate to assume that in the remote past the larva of *Owenia*, or, if we like, the larva of the ancestor of *Owenia*, was a trochosphere of standard type. That is, that it possessed an ordinary ring prototroch, joined to the head by a narrow episphere and to the trunk by a narrow hyposphere and so differed little in appearance from say a trochosphere of *Eupomatus* to-day. After developing a few segments it would begin to lose its prototroch gradually, partly perhaps by absorption and partly by shedding its cells. Both these methods are usual among ordinary trochospheres. As it completed this process it would sink to the bottom and should it there find conditions favourable would grow into an adult. Let us assume that for some unknown reason the larva was induced to increase the size of its prototroch. One is tempted to suggest that a premium was put on increased swimming powers until one reflects that such an increase would be more effectively brought about by an advance in power of the cilia (those of *Owenia* are very weak) and that an enlargement of the prototroch adds to water resistance. However, for some obscure reason the prototroch was enlarged, while the head and trunk remained about the same size. This being so it is obvious that simultaneous growth of episphere and hyposphere tissues would have to take place in order to carry this enlarged organ. Thus a larger prototroch means more tissue to dispose of at metamorphosis, and while this probably still took place partly by absorption, there would be a tendency to favour rapid shedding. A condition would be reached somewhat similar to that seen in the *Polygordius* exo-larva. As the prototroch still enlarged and became more and more bent into loops—possibly by the action of the hyposphere muscles, as suggested on p. 242—the process of getting rid of larval tissues by absorption would take too long. There is little doubt that a dwindling prototroch too weak to be efficient as a swimming organ, and too large to allow its owner to burrow readily, would be a severe handicap to all directive movements by the young worm. A premium would thus be put on rapid shedding, which would gradually entirely supersede absorption. Once regular shedding of the prototroch and other tissues took place it is a small step to the habit of swallowing them. Part of the tissue is so near the mouth that it would be carried into the œsophagus by the upward beat of the latter's cilia, and provided the broken-down cells adhere together—as they do in *Owenia*—they are almost bound to be carried up and into the stomach. We may also assume that during the gradual acquisition of full cataclysmic metamorphosis the habit of only metamorphosing when the bottom was reached became slowly ingrained into the hereditary larval reflexes with obvious benefit to the individual and to the species.

It remains to attempt some explanation of the curious configuration of the worm trunk and its equally curious mode of development. Again, while we can visualise to some extent the steps by which it was brought about, we are unable to discern the underlying cause. We start with an early condition in which the worm trunk was extended in a normal fashion, probably about the time when the prototroch was beginning to enlarge. We assume that the segmental blocks of mesoderm were situated ventrally rather than laterally and that they slowly grew around to the dorsal side. It may here be pointed out that most of the organs of the anterior segments of the adult are situated in roughly the lower half of the trunk, they are the nerve cord, the parapodia and the thread glands. Dorsally there are no such large and specialised organs. It is probable that this has always been the case and that in the past, as now, the large ventral organs would develop and reach some size before the mesoderm blocks met to form the dorsal mesentery. Thus the most actively growing and differentiating region would be the lower half of the body, as it still is. So long, however, as the dorsal ectoderm kept pace relatively with the ventral ectoderm nothing unusual in configuration would result, the larva would be an exo-larva. If, however, the dorsal ectoderm were to lag behind the ventral ectoderm to a marked degree the latter would at its anterior end be folded or invaginated into the blastocœl. Perhaps contact with the mesoderm was a stimulus which induced the ventral ectoderm to grow and differentiate at a more rapid rate then the dorsal or perhaps the evolving larval chæta-sacs had some influence in slowing down and postponing growth of the dorsal ectoderm. Be that as it may a disproportionate growth in the way suggested would produce a ventral invagination, and when the mesoderm blocks did finally meet around the gut the anterior segments of the worm would be inside out.

It is interesting to note in connection with the above that in *Polygordius* the ventral part of the body wall is always bigger than the dorsal part and tends to be ahead of it in development. WOLTERECK himself suggests that the ventral nerve cord conditions a strong overbalancing of the ventral part.

23. COMPARISON OF MITRARIA WITH THE *Polygordius* ENDO-LARVA.

The *Polygordius* endo-larva is the only cataclysmically metamorphosing Annelid which has been investigated in detail (WOLTERECK, 1902), apart from the exo-larva, in which the sudden change is nothing like as fundamentally far-reaching in its effects. A comparison therefore of this endo-larva with Mitraria should be of special interest. Various points of detail have already been compared but there remain certain broad features claiming attention.

Both larvæ resemble one another in that the head is separated from the prototroch by a broad field of episphere and the trunk by a smaller area of hyposphere, both of which are destroyed along with the prototroch at metamorphosis. In *Polygordius* a series of eight radial nerves leave the apical plate and run across the episphere to the prototroch. Of these the first, third and fourth pairs proceed no further but the second pair crosses the line of the prototroch and continues to the anal plate, it forms indeed the circumœsophageal commissures of the adult. These eight nerves have frequently been compared with the eight radial nerves of Ctenophores and it is therefore striking to find that six at least of them are represented in Mitraria. It is true that the second pair, the circumœsophageal commissures, leave the episphere and pass straight down through the blastocœl long before they reach the prototroch, but it seems highly probable that this is a secondary condition, especially when we see that in the earliest stages their course along the episphere is proportionately much longer than it is later on (see p. 278). The second and third pairs of radial nerves are present in Mitraria as the fine nervures already described (p. 245), but the dorsal pair of *Polygordius* have not been seen. In *Polygordius* itself this pair is less distinct than the others and tends to run asymmetrically.

The retractors and dorsal levators are probably the same muscles in both species and the fine muscle fibril running inside the nerve commissure of Mitraria is evidently the representative of the powerful lateral muscle of *Polygordius.* The unpaired dorsal muscle of the latter is absent in Mitraria as likewise are the paired muscles which in *Polygordius* run from the apical plate to the episphere on either side of the mouth. Most of these muscles are, of course, commonly found in other Annelid larvæ.

Whether the nephridium of Mitraria is homologous with the lateral nephridium of *Polygordius* or not is uncertain. They resemble one another only in a general similarity of position and this is probably little more than coincidence. The primary nephridia of *Polygordius* are absent from Mitraria.

While there is a certain similarity in the trochosphere tissues of both larvæ, apart from the presence of provisional bristles in one of them, the worm rudiments differ greatly. We have already seen that the schizocœl of the *Polygordius* head is not the same cavity as the cœlom of the *Owenia* head (p. 278). The folding of the bodies of the two worms is also quite different. The outer fold of *Polygordius*, which is the anterior extremity of the trunk turned inside out, might be compared to the first five segments of *Owenia* likewise turned inside out, but there the resemblance really ends. In *Polygordius* the succeeding and major portion of the trunk is thrown into complicated folds and the folds of the ventral part of the body are quite independent of those of the dorsal part, both being united by thin lateral and unfolded portions of the body wall. In *Owenia*, on the other hand, the succeeding segments depart little from the normal condition of an Annelid trunk. The two main folds, which occur in the regions of the sixth and seventh chætigerous segments, continue right round the body, being merely less prominent dorsally than ventrally. The striking similarity is that retractor fibres in both act as suspensors to the folds, although their points of attachment are different (see p. 285). In *Polygordius* fibres from the dorsal levator also act as suspensors; this is not the case in *Owenia.*

When we compare the metamorphosis we likewise find differences and similarities. In both the prototroch and epi- and hyposphere tissues are thrown off to be partly swallowed in *Polygordius* and wholly or almost wholly swallowed in *Owenia*. The head fuses with the trunk in both and with the new mouth, but note that whereas in *Owenia* the ventral part of the mouth has been directly connected to the trunk ever since the œsophageal mesoderm fused with the first pair of segmental blocks to form the buccal organ segment, and only its lateral and dorsal borders break as raw edges to fuse with head tissue, in *Polygordius* the whole rim of the mouth is entirely new and the ventral part fuses with the anterior ventral part of the trunk. The gut itself is differently altered in both. In *Polygordius* the œsophagus is renewed as two lateral pockets of embryonic tissue during the later stages and these lateral portions at metamorphosis fuse dorsally and ventrally, the old parts between them being thrown out. In *Owenia* there is no such renewal of the œsophagus and the remainder of the gut also undergoes comparatively little change. As regards stomach and intestine this is probably because it is approximately the right length for the young worm, but in the *Polygordius* endo-larva it is much too short and the stomach is therefore enormously lengthened by stretching until its walls become very thin, the intestine being practically unchanged. This should be contrasted with the condition of the exo-larva, where there is too much stomach, the tissue of which has to be got rid of by masses of the cells which form its wall breaking down into the lumen.

24. SÖDERSTRÖM'S THEORY OF THE *Polygordius* METAMORPHOSIS.

So far I have accepted WOLTERECK'S work on the *Polygordius* endo-larva (1902) without reference to the criticism raised against it by SÖDERSTRÖM in three papers (1924, *a* and *b*, 1925). Although this attack is directed in particular against the former's interpretation of the structure and metamorphosis of *Polygordius* it is in general an attack also on the belief that cataclysmic metamorphosis (the two authors use the term " catastrophic " in the same sense) is a natural phase in the life-history of any Annelid species. He does indeed express his doubt that it occurs in Mitraria (1924, *a*, p. 41)—he had only METSCHNIKOFF'S account to go by—and the criticism which he applied to *Polygordius* he would doubtless have accorded to this species also and it must be met here.

A good deal of SÖDERSTRÖM'S criticism is directed against WOLTERECK'S interpretation of the way in which the body is folded in *Polygordius*. As I have not studied this in detail myself I shall not deal with this section of his papers, except to state that as his criticism of cataclysmic metamorphosis is unsound, so also do I believe that his remarks on the body folds will subsequently be shown to be likewise in error, and I therefore accept WOLTERECK'S account in preference to his.

I will try to summarise his criticisms of cataclysmic metamorphosis, as to meet them at length would be to take up too much space. Nor is the latter really necessary as they easily condense into a few main points. To begin with we can ignore the use he makes of earlier writers, HATSCHEK (1878) and FRAIPONT (1887), who believed that the throwing off of the prototroch was pathological, and KLEINENBERG (1886), who was equally positive that it was normal. SÖDERSTRÖM, with the first two, saw the breakdown of the larval tissues but contends that it is an abnormal process brought about by cover glass pressure or other unnatural means. These means are : confinement in a small quantity of water in a watch-glass during observation under the microscope, the water evaporating and bringing about increased salinity and increased osmotic pressure which then causes the delicate larvæ to crumple up ; contact of the larvæ with the sides or bottom of the containing vessel ; and trapping of the larvæ in the meniscus or surface film of the water. In one or several of these ways the delicate larvæ are broken or caused to break up, in other words "to metamorphose cataclysmically," If these conditions are eliminated cataclysmic metamorphosis does not take place. He instances a stirring jar in which he kept water currents so circulating that the larvæ did not come into contact with any of these surfaces and did not metamorphose.

Applying these criticisms to Mitraria I have frequently watched cataclysmic metamorphosis take place at the bottom of deep finger-bowls, where there was no question of cover-glass pressure or increase of salinity due to evaporation. I have tried the experiment of allowing water to evaporate in watch-glasses containing larvæ ; the result was quite indefinite and gave no indication that increasing salinity had been the factor which caused some only of the larvæ to metamorphose. Larvæ suddenly placed in water of high salinity usually contracted and finally died without metamorphosing. One or two produced abnormal worms. SÖDERSTRÖM'S salinity argument is thus not supported by experiment.

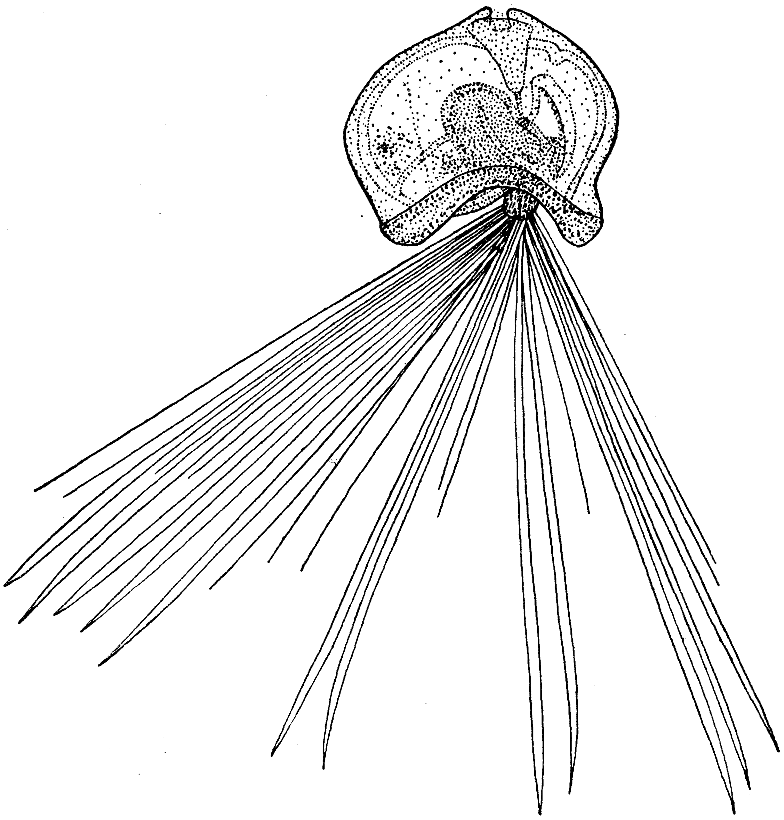
Larvæ caught in the surface film never metamorphosed (one apparent exception recorded on p. 309), they were torn up and destroyed. Many such have been seen. The tissues showed no signs of breaking down, they were torn purely mechanically and the worm trunk was never everted. Other larvæ from the same culture would metamorphose on a sandy bottom.

Contact of the *Polygordius* larvæ with the sides and bottom of the containing vessel might very well bring about metamorphosis, for we have seen that in Mitraria contact with solid matter—in its case grit—is necessary. The fact that so many metamorphosed in the tow-net, as recorded by WOLTERECK, is strong evidence that contact stimulus is a natural condition for the metamorphosis to take place. Possibly in the sea *Polygordius* larvæ do not change into worms until they touch the bottom. Therefore the fact that SÖDERSTRÖM'S larvæ in his stirring jar did not metamorphose is not surprising and is no argument against the naturalness of cataclysmic metamorphosis.

There remains SÖDERSTRÖM'S own theory of the course of the normal *Polygordius* metamorphosis to be mentioned. This he conceived as a gradual transformation process and he gives figures (1924, *a*, figs. 15–18) of what he believes to be stages in this process. He obtained these stages from larvæ brought in from the plankton and kept in large glass vessels. They showed over the course of a few days a dwindling of the prototroch and gradual elongation of the body which, however, was still very much folded at the last stage, when he was obliged to leave them. Although he never got a normal worm from these larvæ he contends that they are normal metamorphosis stages. WOLTERECK (1924, 1925) on the other hand has also seen these stages and believes that they are abnormal partially metamorphosed worms and this interpretation I, for one, accept. In Mitraria unhealthy or not quite ripe larvæ will frequently produce all degrees of abnormal or partially metamorphosed worms when stimulated but these never become normal. In strong healthy cultures producing large numbers of normal worms half-way stages with reduced and gradually dwindling prototrochs are never seen. The most conclusive criticism of SÖDERSTRÖM's theory is, however, just this : he never obtained normal young worms from his gradual transition stages, while WOLTERECK for *Polygordius* and I for *Owenia* have both obtained normal worms from larvæ which have been known to metamorphose cataclysmically. The test of normality in my case is that they have lived and grown for several weeks afterwards.

25. MITRARIA LARVÆ FROM THE FIRTH OF CLYDE.

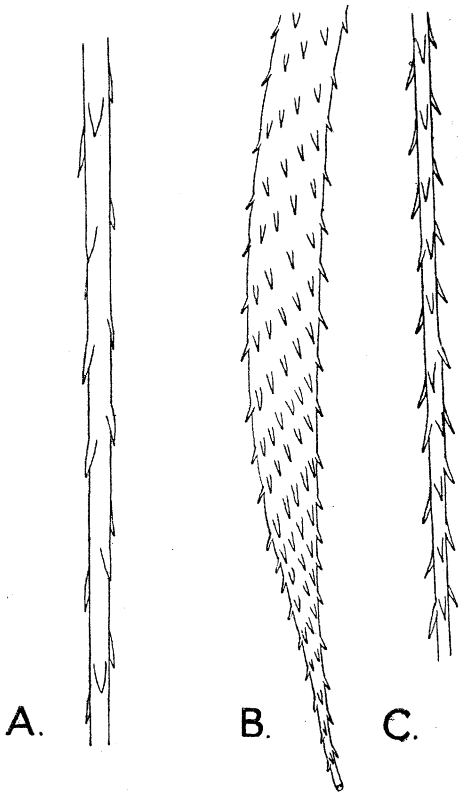
CLAPARÈDE (1860), BROWNE (1905), and MARSHALL (1925) have recorded the presence of Mitraria larvæ in the Firth of Clyde and I have taken them there myself in October, 1925. Miss MARSHALL states that they are " occasional from March till December but never common." I am indebted to her for the loan of her mounted specimens and these I find fall into two distinct groups. The first group consists of larvæ collected in the spring, May, 1923, and April, 1925, and are all certainly *Owenia fusiformis* and have already been referred to (p. 314). The second group comprises larvæ collected in the autumn, September and December, 1923, October, 1925, and October, 1926, and by myself in October, 1925. The larvæ in this second group are a perfectly distinct species which merits some attention, especially as I have been able to examine an almost complete developmental series. For convenience it can be referred to as the autumnal Clyde species. Text-fig. 50 is a sketch of a mounted specimen from my collection and is at a corresponding stage of development to that shown in fig. 7, Plate 29, of *Owenia fusiformis*. In comparing it with the latter it must be remembered that it has contracted somewhat in the fixative (absolute alcohol). The prototroch during life is probably perfectly circular, the upward bend at the side being due to contraction. Throughout its existence it never becomes sinuous, the figure shows it at its maximum development. The episphere, which is here very large in proportion to the hyposphere and is coated externally by a thick cuticular layer, is also fully developed, for in this species as in *Owenia* the larval tissues reach the end of their growth before the body rudiments make much headway. It will be noticed that this Mitraria is much smaller than that of *Owenia*, its maximum diameter being approximately 240 μ and length (chæta-sacs to head) 180 μ (*Owenia* 560 μ and 300 μ respectively). It differs strikingly in the form of its bristles, these being of two kinds, one spined capillary rods, similar to those of *Owenia* but slenderer and with more prominent spines, text-fig. 51C, the other club-shaped bristles somewhat flattened, very spinose, especially near the tip and seemingly hollow and opening by a pore at the tip, text-fig. 51B. Each larva has about twenty capillary and sixteen clavate bristles. A nephridium of the *Owenia* type appears to be present.



TEXT-FIG. 50.—Mitraria larva from the Firth of Clyde. Taken in tow-net at Millport, October 2, 1925. × 156. Sketch from the mounted specimen. Actual length, head to tip of longest bristles, approximately 670 μ. Dorso-ventral diameter (left to right across episphere) approximately 225 μ.

The development of the body rudiment follows the same general course as in *Owenia*. An ingrowth appears in front of the anus, the ectoderm is comparatively thick and segmental blocks of mesoderm can be seen. A buccal organ invagination is formed just posterior to the mouth. A late stage of the trunk ingrowth is shown in text-fig. 50. It appears to extend round the intestine in a similar complicated way to that of *Owenia*. The latest stage I have is one where it is evidently ready to metamorphose. The general relations are exactly the same as in *Owenia*, the buccal organ segment and first four chætigerous segments being turned inside out and enclosing the remainder of the trunk. The latter shows folds similar to those of *Owenia*, especially in what seem to be the sixth and seventh chætigerous segments. Thread glands are conspicuous. The head closely resembles that of *Owenia* in shape and is attached by circumœsophageal nerve commissures to the same part of the trunk. There can be no doubt that metamorphosis is cataclysmic and that it follows the same general course as in *Owenia*.

What is the identity of this larva? That it is one of the Oweniidæ is attested by the presence of thread-glands, at the same time it appears to be considerably removed from *Owenia* itself. I think it likely that it will prove to be the larva of *Myriochele*, possibly *M. Heeri*, Malm., a small worm 20–30 mm. long and 1·5 mm. in diameter (Fauvel, 1927), which is known to exist in British waters but has not yet been recorded for the Clyde. It would easily be overlooked by anyone not specially searching for it. If this Mitraria is *Myriochele* probably the Mitraria larvæ of the same general type, which have been recorded by Häcker (1898, *a*) especially, will prove to be members of the same genus. Some of Häcker's larvæ at any rate appear to belong to the Oweniidæ (see below, p. 325).



Text-fig. 51.—A. Portion of middle part of a provisional bristle of *Owenia fusiformis*. B. Tip of provisional clavate bristle of the Clyde Mitraria shown in text-fig. 50. C. Portion of middle part of capillary provisional bristle of the Clyde Mitraria. All figures × 756. In each case the distal part of the bristle is below.

26. Affinities of the Oweniidæ.

The affinities of the Oweniidæ (Ammocharidæ) are unfortunately not made any clearer by the study of the development of *Owenia*. The resemblances to the *Polygordius* type of metamorphosis are only such as would be induced by convergence, and we cannot regard the two larvæ as more closely related to one another than they are to the larvæ of other Annelids; there are as many fundamental differences as resemblances between them. The adult *Owenia* has been compared by Gilson (1898, p. 90) to the Archiannelids, on account of the almost complete absence of circular muscles in the body wall and the presence of two longitudinal mesenteries. The primitive position of the longitudinal nerve cord also recalls them. These points do not in themselves, however, prove affinity, for the first may be a secondary condition and the two latter persisting primitive ones. Moreover the Archiannelids are such an unnatural grouping of diverse forms that there is no point in claiming affinities with them as a whole, and there is no evidence that any particular genus of them should be singled out for this purpose.

The Oweniidæ show a certain degree of resemblance to the Maldanids. This was pointed out by DE SAINT-JOSEPH (1898, p. 104). The segmentation of the body and the form of the parapodia are similar, and *Myriochele* like the Maldanids lacks prostomial processes. The bristles and hooks are not so very dissimilar. In Maldanids, too, the ventral nerve cord approaches the primitive position it occupies in *Owenia* in that it is situated between the circular muscles and the epidermis. There are, of course, considerable differences: the Maldanids for instance possess a moderately developed coat of circular muscles as well as oblique transverse muscles, and they have no thread glands. In spite of these differences, however, the usual position of the Oweniidæ next to the Maldanidæ in systematic works (*e.g.*, McINTOSH, 1915; FAUVEL, 1927; HEMPELMANN, 1931) is not to be questioned at present. BENHAM (1896) placed them next to the Magelonidæ in his suborder Spioniformia, but this seems to have been based on a misconception of the nature of their branchiæ, which on p. 325 he said "appear to belong to the peristomium."

It is unfortunate that so far no undoubted Maldanid larva has been described. We have already seen (p. 235) that HÄCKER'S description of the "National" Mitrarias as Maldanidæ is based on METSCHNIKOFF'S determination of the worm he obtained from the metamorphosis of a Mitraria as a Maldanid. METSCHNIKOFF'S worm was almost certainly an *Owenia* but it is of course not proved that HÄCKER'S larvæ were not Maldanids, although there is no evidence for such an assumption. Indeed what little evidence there is is all against it.

The details of the internal organisation of his larvæ (1898, *a*) are drawn as though the author did not perfectly understand the structure, which would not be at all surprising in such unusual creatures, yet, all the same, three drawings are very suggestive. In his fig. 8, Plate I, of "*Mitraria skifera*" what are almost certainly thread-glands are clearly outlined, and the larva was in a state almost ready to metamorphose. The presence of thread-glands marks it down at once as belonging to the Oweniidæ. Another drawing fig. 6*a*, on the same plate, of a small Mitraria with capillary bristles, indicates an early stage of the trunk invagination and strongly suggests that this larva would have continued its development after the *Owenia* pattern. The same stage seems to be indicated in fig. 7 of his "*Mitraria pennata*" but in this case the larva is viewed obliquely from in front. His "*Mitraria Mülleri*" (figs. 9 and 10, Plate I) and "*Mitraria intermedia*" (fig. 13, Plate II) were evidently early stages and nothing definite can be said about them.

It is to be hoped that before long a Maldanid larva will be obtained by artificial fertilisation and described as fully as possible.

27. SUMMARY.

(1) Larvæ of *Owenia fusiformis*, Delle Chiaje, obtained by artificial fertilisation, have been reared through the metamorphosis and the young worms so produced for about a month afterwards.

(2) The trochosphere stage, figs. 3 and 4, Plate 29, attained about 2 days after fertilisation, shows a ring-like prototroch, a marked ventral flexure of the gut bringing the anus close to the mouth, and several long spiny provisional bristles which arise from a pair of chæta-sacs situated immediately dorsal to the anus. A pair of larval nephridia is present, each attached to a dorsal levator muscle, text-fig. 5A. The head contains a cerebral ganglion from which arise circumœsophageal nerve commissures which pass down through the blastocœl to be attached to the hyposphere on either side of the anus, text-fig. 27, *n.c.* Internal to them a pair of retractor muscles—each composed of two fibres—likewise connects the head to the hyposphere.

(3) Further development results in an increase in total size together with an arching up of the prototroch dorsally and ventrally and on each side, fig. 6, Plate 29. More and longer provisional bristles are acquired. At the same time the trunk and head rudiments of the future worm grow slowly until the prototroch reaches its full size about 2½ weeks after fertilisation. From then onwards they grow quickly and the larva is ready to metamorphose about a month after fertilisation, fig. 11, Plate 31.

(4) The trunk rudiment forms an invagination between anus and mouth, fig. 6, Plate 29. At first it consists of a small ectodermal pocket—often stretched out flat—but a pair of mesoderm teloblast cells soon appears and buds off paired segmental blocks, text-fig. 8. As the latter increase in number the invagination deepens and the position of its inner fold passes back in such a way that the segmental blocks pass in succession over its summit and on to its anterior face, text-figs. 8, 10, 11, until the first five pairs are so situated. The segmental blocks taper off downwards on either side to slender dorsal extremities, text-fig. 12. These extremities end close to the lateral corners of the external opening into the invagination. This opening gradually surrounds the anus until it completely encloses it and at the same time the dorsal extremity of each segmental block, except the first pair, approaches—behind the intestine—its partner of the other side and fuses with it, text-fig. 15, the dorsal mesentery forming between the two. Rapid growth now takes place in this dorsal region until the invagination is as deep there as elsewhere, text-fig. 19. In this way the intestine becomes enclosed within the trunk rudiment, the first five segments of which are turned inside out and drawn back over the succeeding segments much as the top of a stocking can be turned inside out and drawn back over the foot, fig. 15, Plate 32.

(5) During this process the segmental blocks have hollowed out to form cœlomic cavities and their walls where they touch one another fuse to form a ventral mesentery in the mid-ventral line, text-figs. 13, 17, and septa at right angles to this, text-fig. 11. Septa 2, 4 and 5 soon disappear, the first two entirely, the last all except a small portion on either side of the ventral mesentery.

(6) Meanwhile organs and tissues have differentiated. In segments two to eight a pair of thread-glands appears in each, text-figs. 11B, 13, 26, and fig. 15, Plate 32. In segment two and all posterior to it parapodia arise, notopodial bristle bundles only in the first three of them, neuropodial uncinigerous tori in addition in the fifth and succeeding.

The ventral nerve cord appears in the ectoderm and longitudinal muscle fibres in the somatopleural mesoderm, text-fig. 17. Mucus- and gland-cells become numerous in the ectoderm.

(7) The first pair of segmental blocks has a different history. They become hollow but acquire neither thread-glands nor parapodia. As they grow they advance across the floor of the blastocœl to meet and fuse with a pair of hollow blocks formed out of the mesoderm or mesenchyme which had been present on the œsophagus since early stages, text-fig. 11. The cavities of these four blocks are at first separated from one another by the median mesentery and the transverse septum formed by fusion of their walls where they are in contact. Later the median mesentery and the greater part of the transverse septum disappear, putting the four cavities into communication with one another. Before this happens, however, the place where septum and mesentery cross marks the summit of an invagination which begins immediately after the blocks have fused, text-fig. 20. This invagination forms the buccal organ. The combination of the four blocks is referred to as the buccal organ segment, and the common cœlom the buccal organ cœlom. The buccal organ acquires a complicated set of muscles to move it, text-figs. 21, 24, 32.

(8) During the growth of the dorsal extremities of the segmental blocks round the intestine the cavity containing the muscles which move the larval chæta-sacs comes to communicate with the common cœlomic cavity of the first and second chætigerous segments (second and third larval segments), fig. 15, Plate 32.

(9) While the worm trunk is developing the head also becomes larger and on each side grows downward into a projection indenting the episphere. From the extremities of these projections the circumœsophageal nerve commissures arise, text-fig. 28B, *n.c.*, which run downwards through the blastocœl to enter the trunk and subsequently fuse to form the ventral nerve cord. The cerebral ganglion, *c.g.*, is situated in the ectoderm of the head. This ectoderm develops mucus-cells and peculiar cells, *v.c.*, with clear contents. Below the head ectoderm there is a layer of mesoderm, solid in the earlier stages, text-fig. 2, *h.m.*, but later containing a cavity—head-cœlom—which is crossed anteriorly by numerous fine strands, text-fig. 28A. From the mesoderm of the head the pair of retractor muscles arises, text-fig. 28B, to run through the blastocœl to the anterior part of the trunk. A certain fibre, *s.r.f.*, in each retractor enters the trunk and serves to hold together certain folds of the body-wall, text-fig. 31, *s.r.f.*

(10) At metamorphosis the trunk folds are straightened out—the last-mentioned fibres apparently being broken—and the everted anterior segments inverted into the normal position, fig. 12, Plate 31. At the same time the stomach and œsophagus are dragged into the anterior part of the trunk as it inverts and become enveloped in the splanchnopleural membrane of the anterior segments. Meanwhile the head is pulled down by the retractors towards the anterior end of the trunk. The larval tissues, the episphere, the hyposphere, the prototroch, the metatroch and the nephridia, break away from the adult tissues (breakage positions shown by arrows in key to Plate 32) and

begin to histolyse, text-fig. 34. The posterior border of the head meets the anterior border of the trunk and the anterior border of the head meets the anterior border of the œsophagus, fig. 16, Plate 32, and text-fig. 37A. The latter is really a new mouth, formed by the breaking across of the larval œsophagus behind the old larval mouth, dotted line in fig. 15, Plate 32. Later fusion takes place at these points, shown by arrows in key to Plate 32.

(11) Metamorphosis is extremely rapid, most of the above structural changes taking about 30 seconds to complete. The long provisional bristles fall off and the larval chæta-sacs invaginate into the cœlom of the first and second chætigerous segments, there to histolyse and disappear, text-fig. 37A, and fig. 16, Plate 32.

(12) Various blood vessels are formed in the anterior region of the worm during metamorphosis by the coming together of various mesodermal membranes of head and body, fig. 16, Plate 32.

(13) The larval tissues rapidly disintegrate and are swallowed by the young worm, text-fig. 35. Swallowing is completed in about 15 minutes. These tissues are digested *intracellularly* over a period of about 2 days, text-fig. 43.

(14) The young worm begins to secrete a mucus tube for itself while still swallowing the larval tissues. Sand grains become attached to this tube.

(15) Some days after metamorphosis a definite blood sinus forms between the splanchnopleure and the gut posterior to the second adult septum. Rudiments of the adult tentacular crown appear on the head, fig. 14, Plate 31.

(16) Certain points with reference to the positions of the adult septa are cleared up. The second adult septum occurs between the second and third chætigerous segments. The eighth chætigerous segment is a transitional segment in which the parapodia are centrally placed. Segments anterior to it have the parapodia at their anterior ends, segments posterior at their posterior ends, text-fig. 46.

(17) It is shown experimentally that healthy Mitrarias require to come into contact with fine sand or grit before they will metamorphose.

(18) Various cases of abnormally developed larvæ and abnormal metamorphosis are described.

(19) Comparison of reared larvæ with larvæ from the plankton did not reveal any fundamental differences.

(20) Certain problems raised by this study of the development are pointed out. A tentative hypothesis is advanced to suggest the lines along which the Mitraria larva may have evolved from a normal trochosphere.

(21) Mitraria is compared with the endo-larva of *Polygordius* but although it resembles it in certain respects it is evidently fundamentally different and in no way related.

(22) SÖDERSTRÖM'S criticism of WOLTERECK'S account of the metamorphosis of *Polygordius* is itself criticised and shown to be unsound.

(23) A species of Mitraria larva found in the Firth of Clyde during the autumn is described and illustrated, text-fig. 50. It has spiny club-shaped provisional bristles

as well as spiny needle-shaped ones. Its prototroch never becomes sinuous but the development of the trunk rudiment is fundamentally the same as that of *Owenia fusiformis*. It is suggested that it may be the larva of *Myriochele Heeri*, MALM.

(24) The study of Mitraria does not throw any light on the affinities of the Oweniidæ but it does point to the need for an investigation of the larvæ of the Maldanids which are still undescribed.

28. LITERATURE.

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29. LIST OF ABBREVIATIONS.

The following is a list of the abbreviations used in the text-figures. It is complete except for letters used once only; these are explained in their corresponding text-figure legends. In every case letters are explained in the underlying legend the first time they occur in a figure, and whenever a supplementary note has been added referring to a particular figure only.

a., anus.
a.b.f., anterior buccal fold.
a.c., anal chamber.
a.l., anterior lip of mouth.
b., buccal organ.
b.b. 1., etc., first, etc., bristle bundle.
b.e., clear ectodermal cells of buccal organ.
bl., blastocœl.

b.m., basement membrane.
br. 1., etc., bristle of first, etc., chætigerous segment.
b.s., buccal organ segment.
c. 1, etc., cœlom of first, etc., chætigerous segment.
c.b., cœlom of buccal organ segment.
c.c., cœlom of provisional chæta-sacs.
c.g. cerebral ganglion.
ch., provisional chæta-sacs.
ch.h., histolysing fragments of provisional chæta-sacs.
c.h.i., invagination of provisional chæta-sacs.
c.m., circular muscles.
c.o.m., cœlom in œsophageal mesoderm.
c.s. 1, etc., first, etc., chætigerous segment.
c.s.l. 1., etc., cœlom of first, etc., larval segment.
d.c., dark amœboid cell of stomach.
d.c.h., degenerating cells of head ectoderm.
d.c.s., divaricator of provisional chæta-sacs.
d.m.c., discharged mucus cell.
d.n., dorsal nervure.
d.v., dorsal blood vessel.
e., ectoderm.
e.f., ectodermal fold.
e.g., ciliated groove formed by epispheral fold.
ep., episphere.
ey., eye.
g.c., gland cell.
h., hyposphere.
h.c., head cœlom.
h.m., mesoderm of head.
h.sp., head splanchnopleure.
i., intestine.
i.l.p., inner longitudinal protractor of buccal organ.
l., longitudinal muscles.
l.h., lateral part of head.
l.h.p., lateral head pocket.
l.i., lumen of invagination.
l.l., posterior lateral lip of mouth.
l.m., lateral part of mesoderm enclosing buccal organ cœlom.
l.r., lateral retractors of buccal organ.
l.s. 1, etc., first, etc., larval septum.
m., mouth.
m.c., mucus cell.
m.m., median mesentery of buccal organ segment.
m.n.c., muscle fibre of circumœsophageal commissure.
m.o., œsophageal muscle.
m.r., median retractor of buccal organ.
m.t., mucus tube.
mtr., metatroch.
n., ventral nerve cord.
n.c., circumœsophageal nerve commissure.
np., nephridium.
o., œsophagus.
o.l.p., outer longitudinal protractor of buccal organ.
o.m., œsophageal mesoderm.
o.t., external opening of thread gland.
p. 1., etc., first, etc., parapodium.
p.b., provisional bristle.
p.b.f., posterior buccal fold.
p.m., peritoneal mesoderm.
p.r.f., primary retractor fibre.
pt., prototroch.
r., retractor.
r.m., mesoderm forming roof of buccal organ cœlom, and later part of first adult septum.
s. 1., etc., first, etc., adult septum.
s.l. 1., etc., first, etc., larval segment.
sp., splanchnopleure.
sph., sphincter in splanchnopleure.
s.r.f., secondary retractor fibre.
st., stomach.
t. 1, etc., first, etc., thread gland.
t.a., thin non-ciliated hypospheral area anterior to mouth.
t.m., thin membrane separating cœlom of larval chæta-sacs from blastocœl.
t.o., thin larval part of œsophagus.
t.p., transverse protractor of buccal organ.
t.s., transverse septum of buccal organ segment.
v., valve between stomach and intestine.
v.c., clear cells.
v.m., ventral mesentery.
v.n., ventral nervure.
v.v., ventral blood vessel.

30. DESCRIPTION OF PLATES.

Plate 29.

Mitraria larvæ of *Owenia fusiformis* Delle Chiaje. Figures largely based on scale drawings from life slightly corrected after study of whole mounts and sections. The ages given are for larvæ reared in July.

Fig. 1.—Larva 24 hours after fertilisation, and before beginning to swim. × 195. View of right side. Actual length approximately 110 μ. (For description see p. 239.)

Fig. 2.—View from above of the same larva as shown in fig. 1. × 195. Diameter of prototroch approximately 110 μ.

Fig. 3.—Larva 2 to 3 days old. × 195. View of left side. Actual length, head to tips of longest bristles, approximately 450 μ. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 166 μ. (For description see p. 239.)

Fig. 4.—View from below of a larva at the same stage as that shown in fig. 3. × 195. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 166 μ, lateral diameter approximately 135 μ.

Fig. 5.—Larva about 12 days old. × 180. View of left side. Actual length, head to tips of longest bristles, approximately 640 μ. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 300 μ. (For description see p. 242.)

Fig. 6.—Larva about 17 days old. × 200. View of left side. Actual length, head to tips of longest bristles, approximately 900 μ Dorso-ventral diameter (*i.e.*, left to right across figure) approximately 500 μ. (For description see p. 243.)

Fig. 7.—Larva about 21 days old. × 185. View of left side. Actual length, head to tips of longest bristles, approximately 950 μ. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 560 μ. (For description see p. 243.)

Plate 30.

Mitraria larvæ of *Owenia fusiformis* Delle Chiaje. Figures largely based on scale drawings from life slightly corrected after study of whole mounts and sections. The ages given are for larvæ reared in July.

Fig. 8.—Larva a little earlier than that shown in Plate 29, fig. 7, viewed from below. × 132. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 560 μ. Lateral diameter of prototroch approximately 410 μ. (For description see p. 243.)

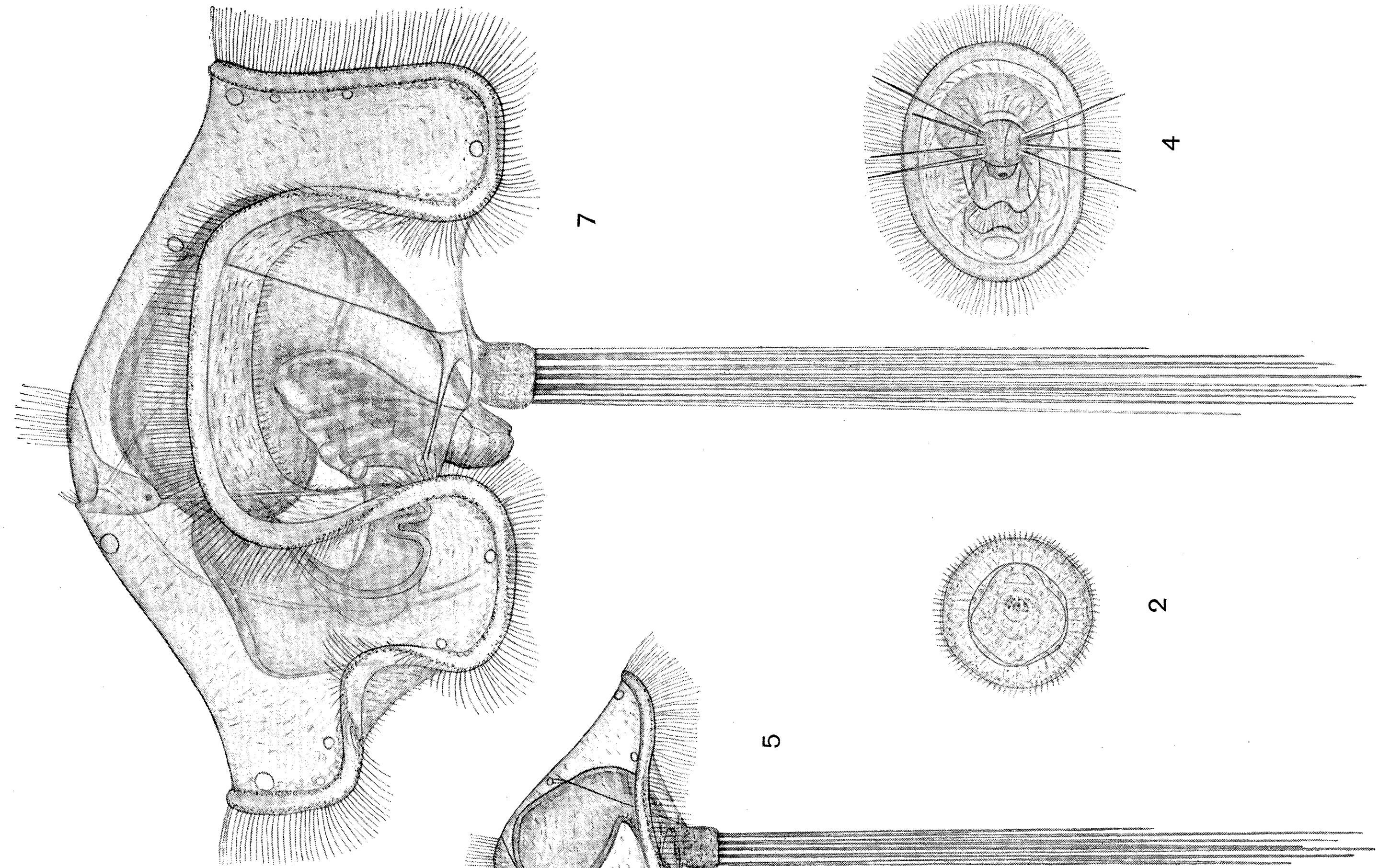
Fig. 9.—Larva about 23 days old. × 180. View of left side. Actual length, head to tips of longest bristles, approximately 950 μ. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 560 μ. (For description see p. 243.)

Fig. 10.—Larva the same age as that shown in fig. 11, Plate 32, viewed from below. × 132. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 560 μ. Lateral diameter of prototroch approximately 410 μ. (For description see p. 243.)

Plate 31.

Mitraria larvæ and young worms of *Owenia fusiformis* Delle Chiaje. All figures except fig. 2 are largely based on scale drawings from life slightly corrected after study of whole mounts and sections. The ages given are for larvæ reared in July.

Fig. 11.—Larva about 27 days old and ready to metamorphose. × 175. View of left side. Actual length, from head to tips of longest bristles, approximately 950 μ. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 560 μ. (For description see p. 243.)



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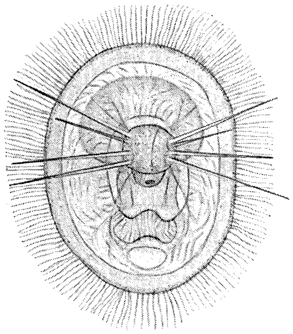

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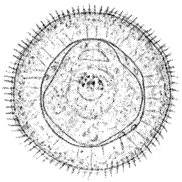

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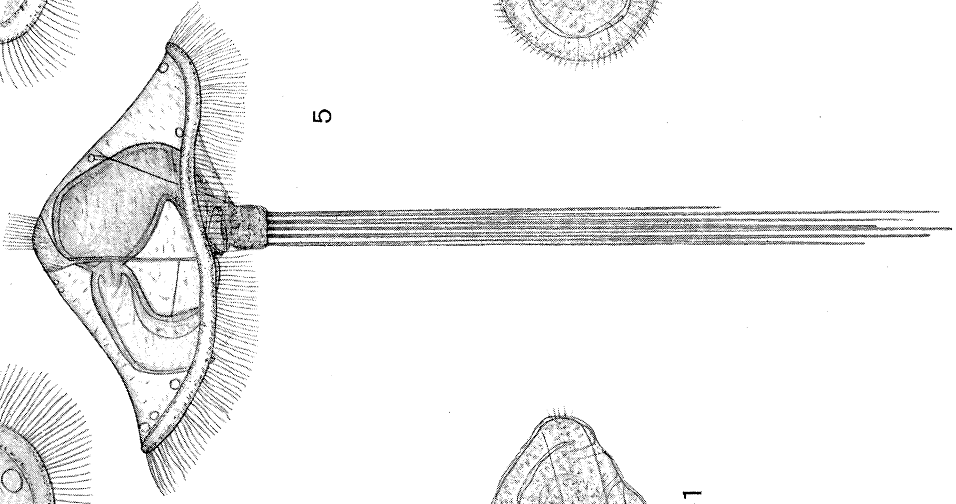

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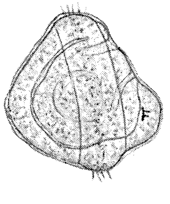

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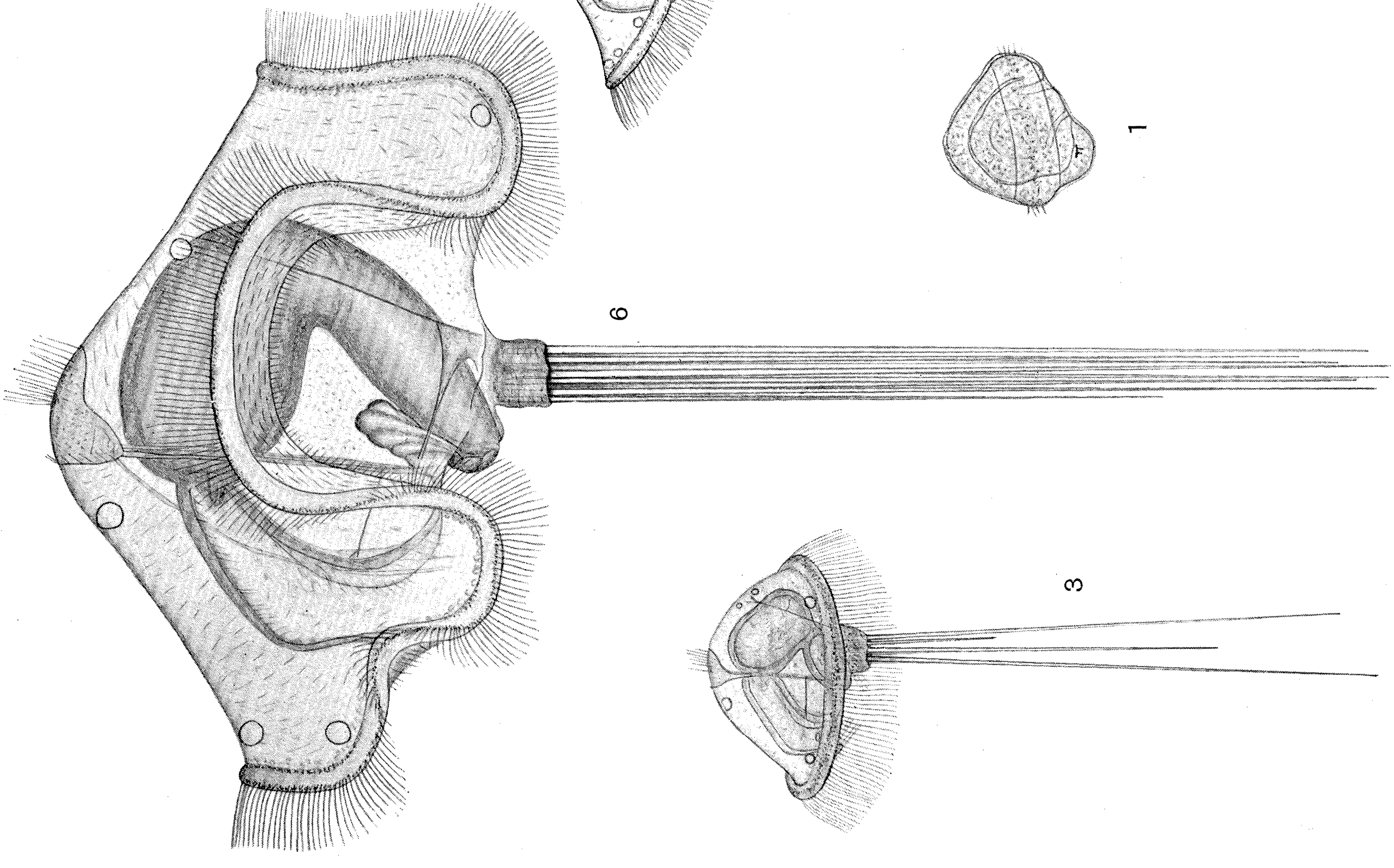

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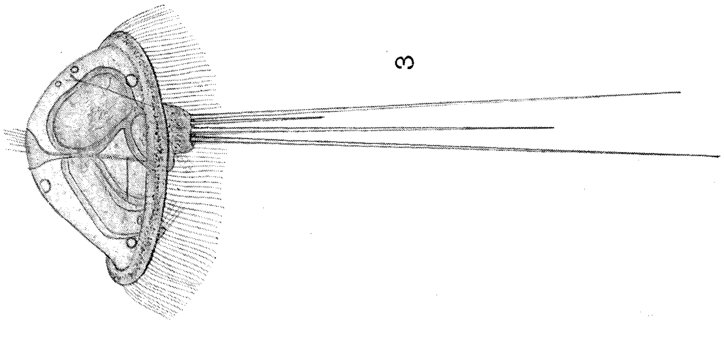

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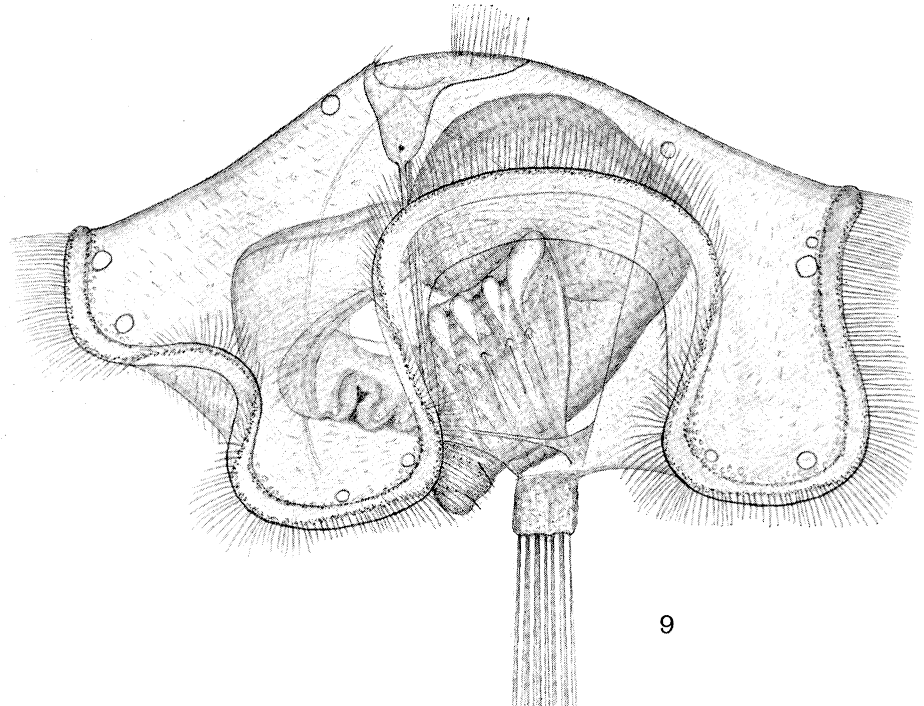

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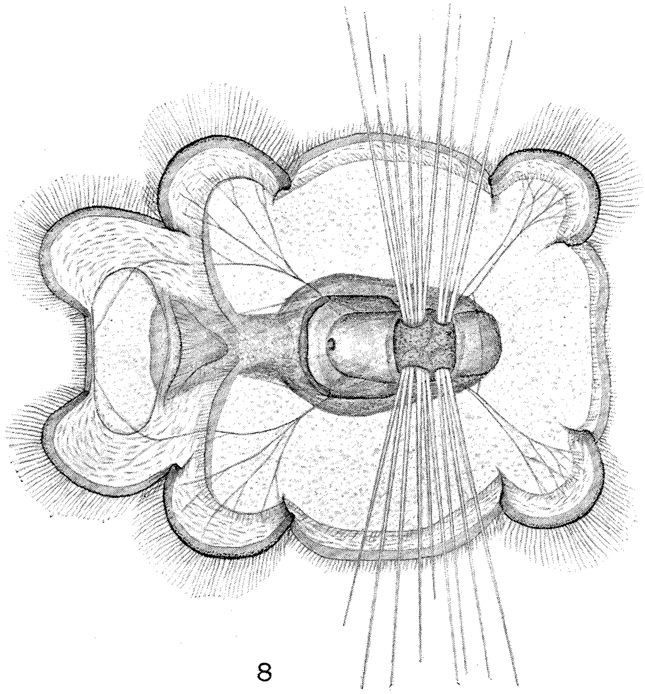

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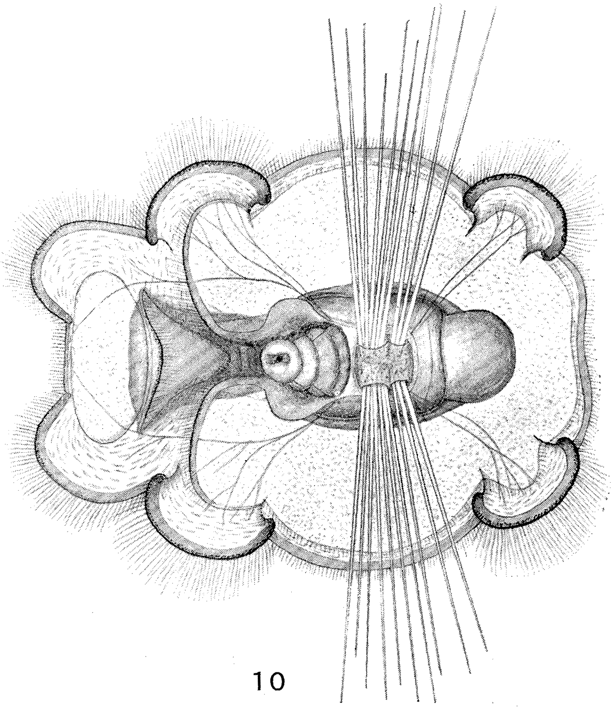

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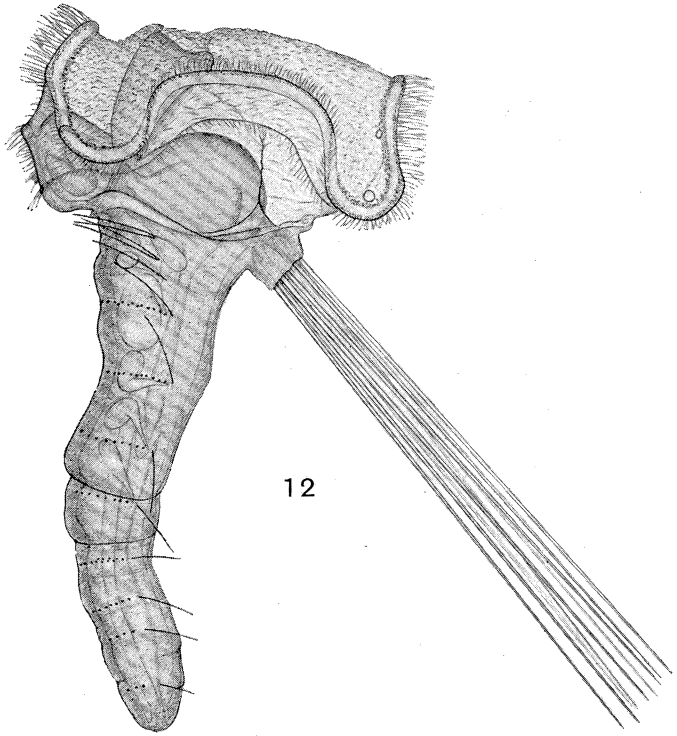

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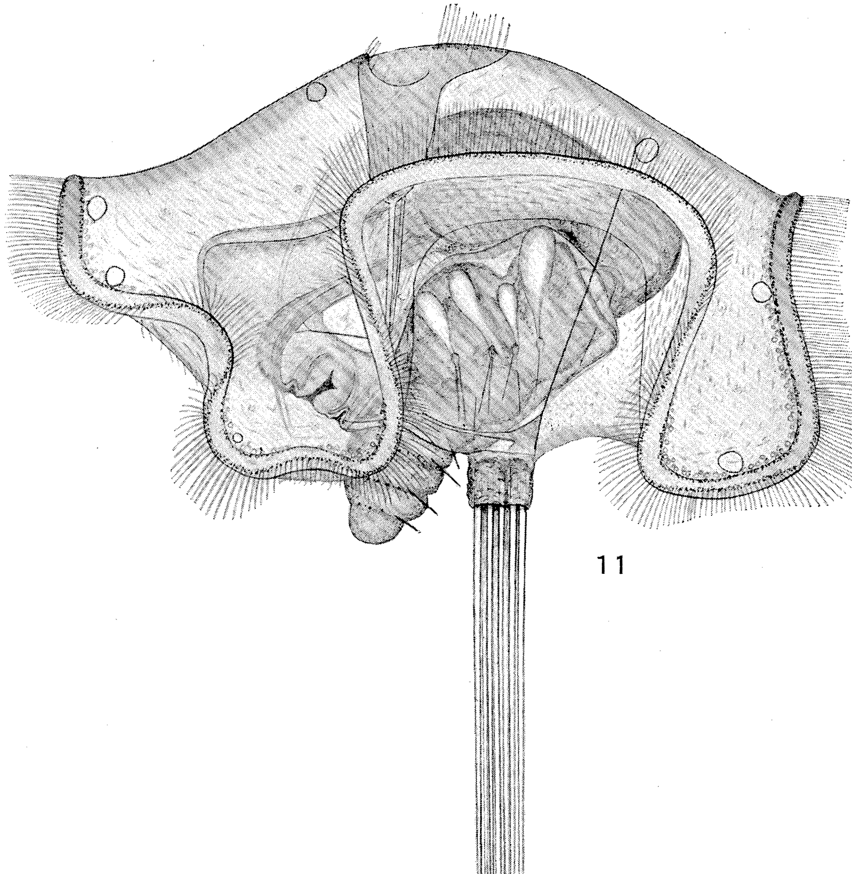

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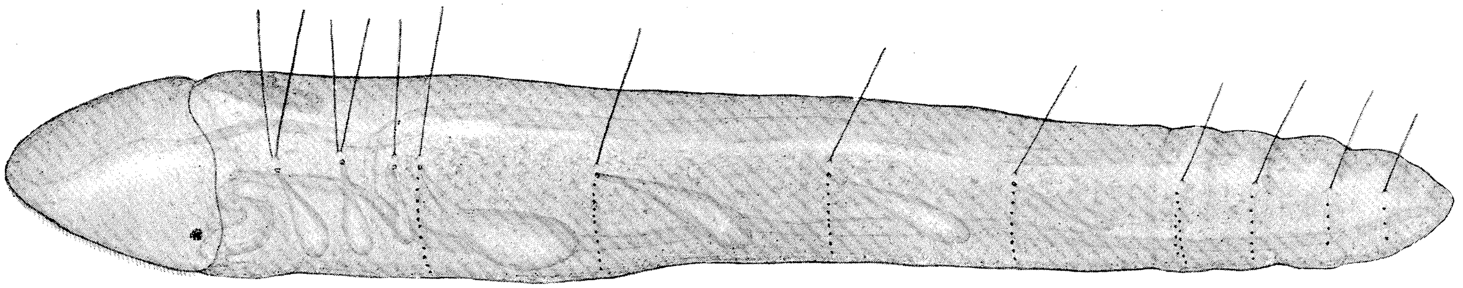

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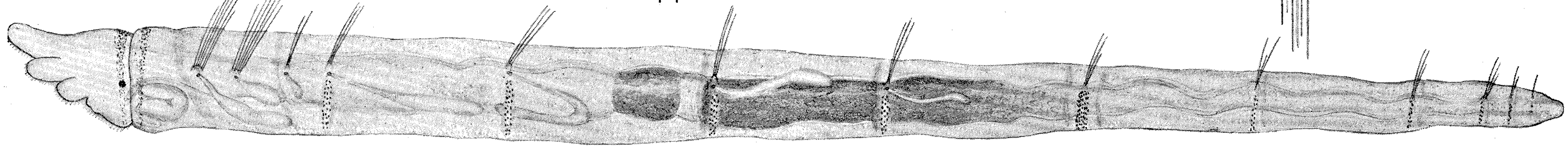

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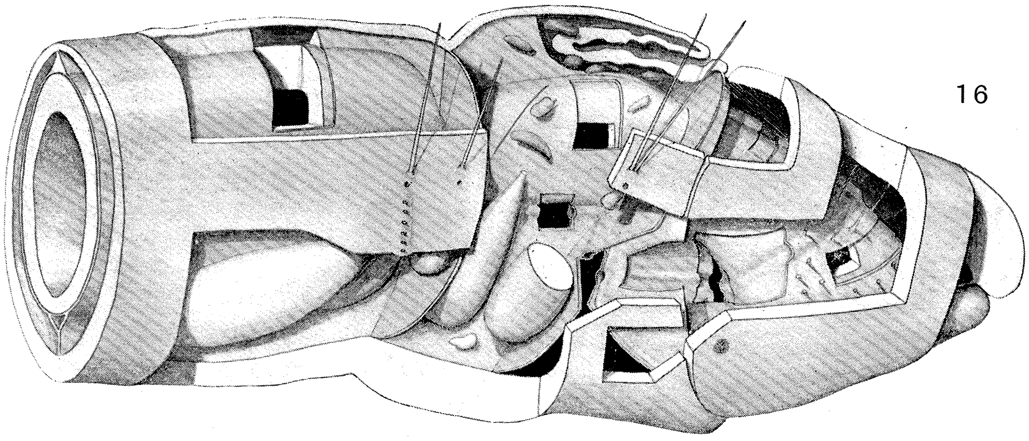

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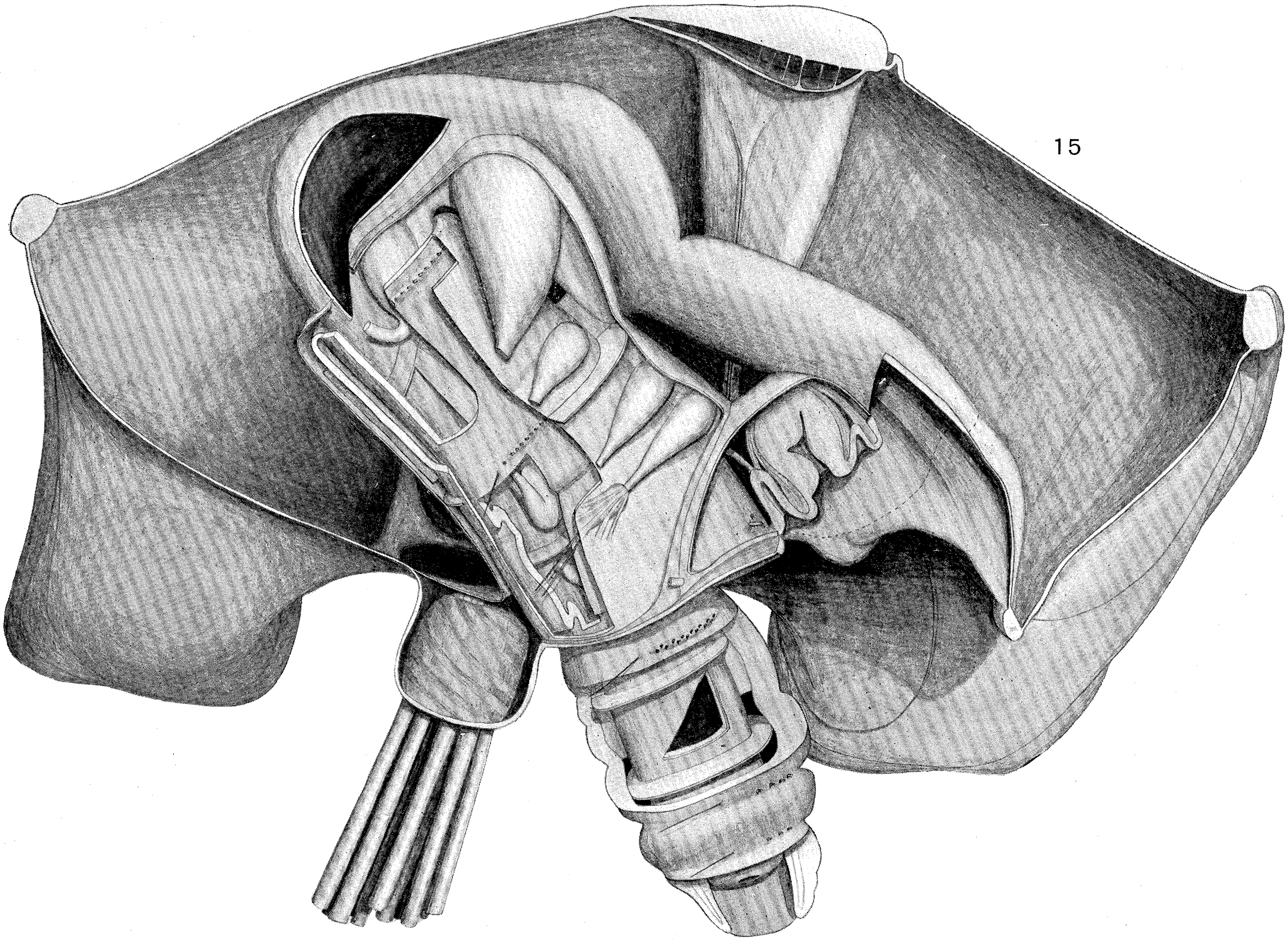

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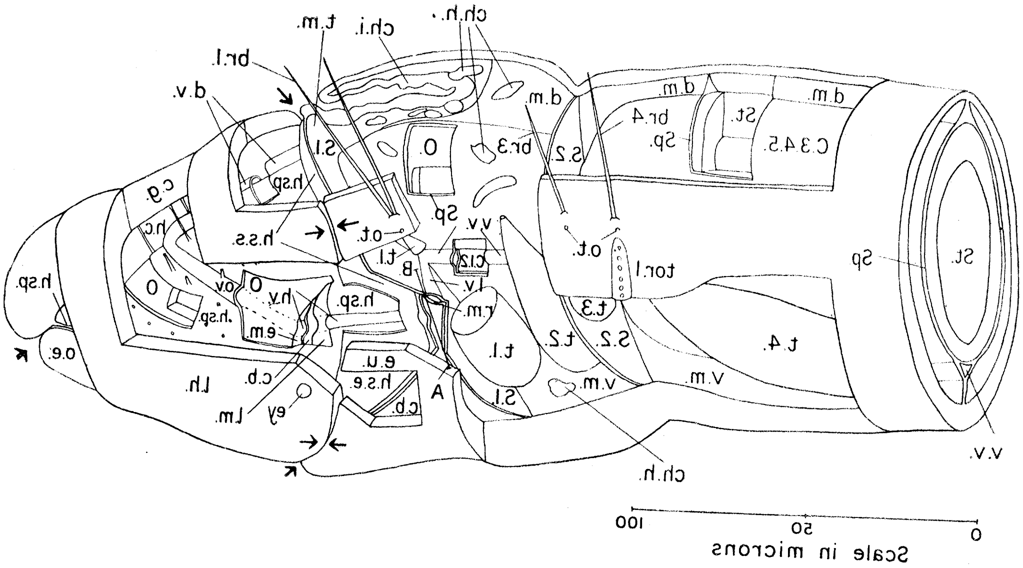
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S.2.
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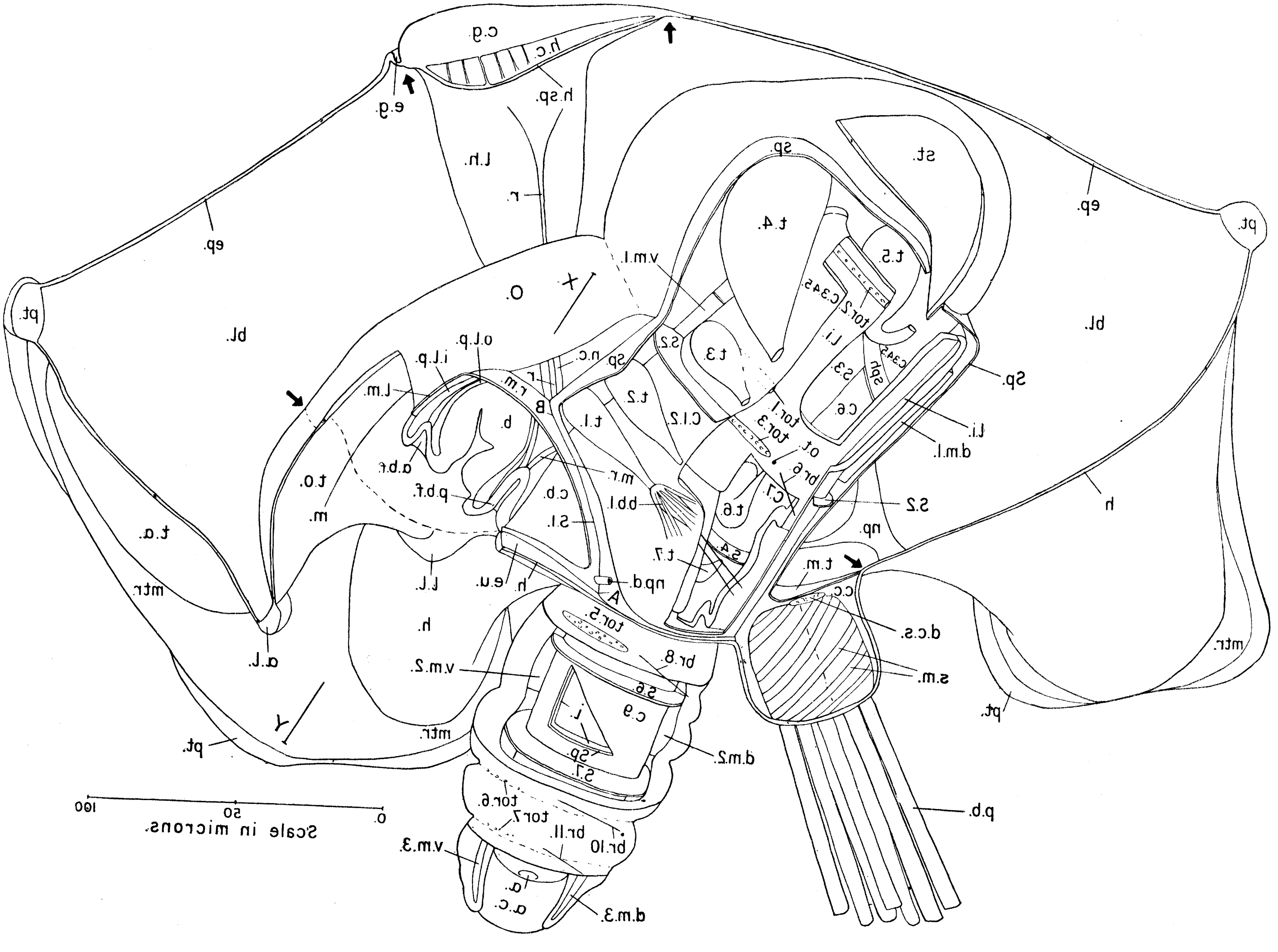
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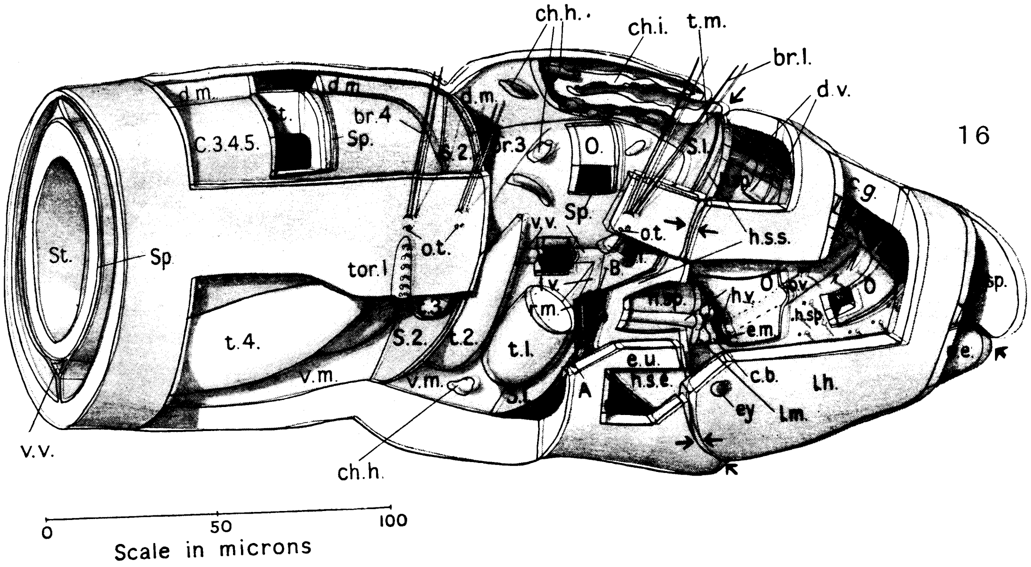
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Scale in microns

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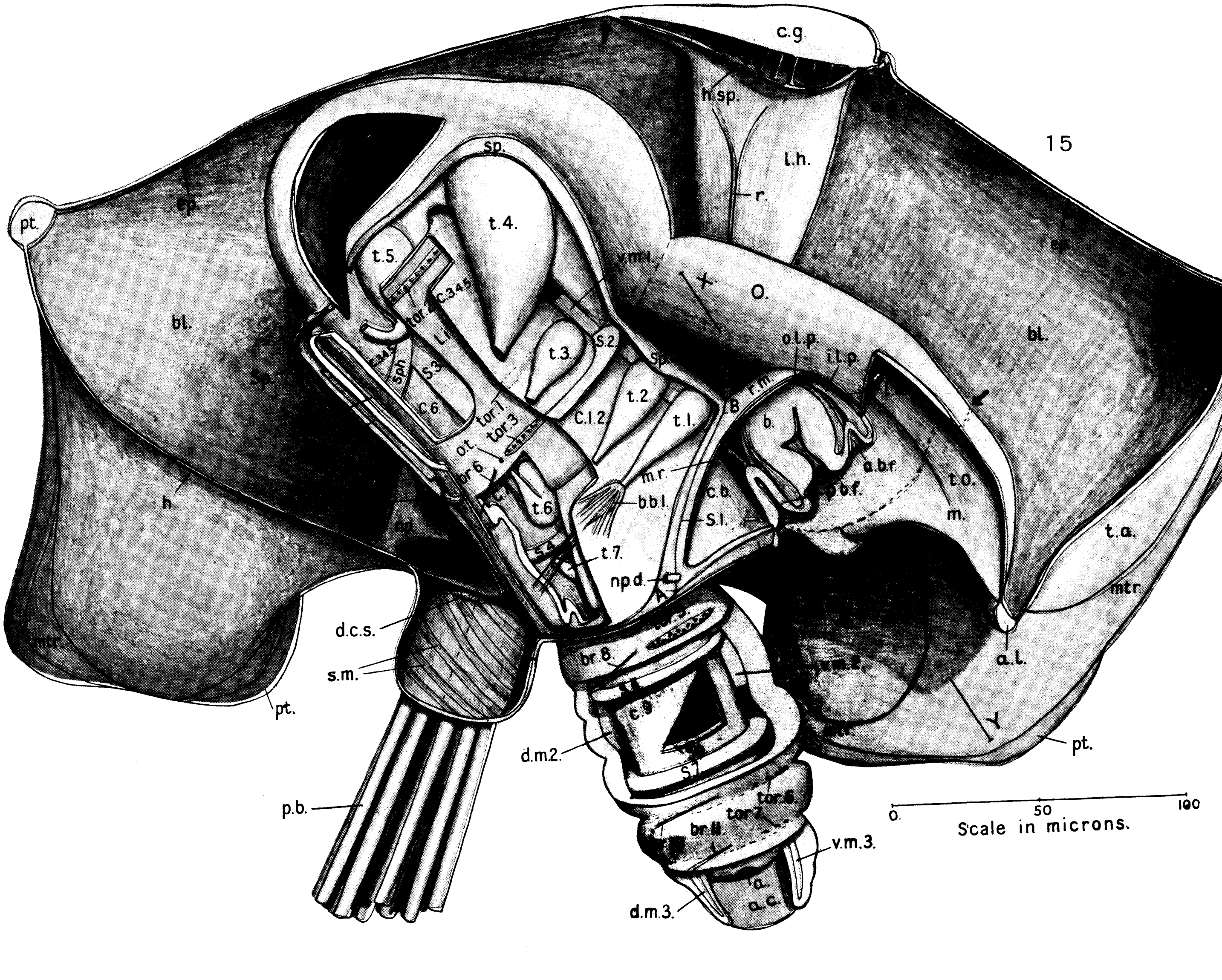
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tor.7.
p.b.
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v.m.3.
d.m.3.
a.c.
0.
50
100
Scale in microns.

15

Fig. 12.—Larva about the same age as that shown in fig. 1 metamorphosing, 15–20 seconds after metamorphosis began. × 130. View from left side. Actual length from head to anus, approximately 710 μ. (For description see p. 288.)

This drawing is from a fixed and mounted specimen. It is in an almost exactly naturally expanded state. The third adult septum has been drawn as though it were continuous all round. Actually it could not be seen over the gut and therefore may or may not have a lateral gap. Efforts to settle this point have failed.

The position of the seventh thread gland is interesting. It must either be projecting through a lateral gap in the fourth adult septum (such a gap was suspected before this slide was studied, see p. 307) into the previous segment, or the septum has been broken by the strain (very unlikely). A third alternative is that the septum has been stretched out into a very thin membrane over the gland. Unfortunately a fold of the body wall occurs just where this septum should be visible and practically nothing can be seen of it, and it has therefore not been indicated in the drawing. It should be mentioned that the seventh gland of the right side is in its normal position behind the seventh parapodium.

Fig. 13.—Young worm 15 or 20 minutes after metamorphosis began. × 216. View of left side. Actual length approximately 870 μ. The remains of the histolysed prototroch and other larval tissues can be seen in the gut. The worm has been removed from its mucus tube. (For description see p. 289.)

Fig. 14.—Young worm about a month after metamorphosis. × 95. View of left side. Actual length approximately 2560 μ. (For description see p. 302.)

Plate 32.

Fig. 15.—Stereogram of Mitraria larva of *Owenia fusiformis* ready to metamorphose. × 450.

This diagrammatic figure is a mental reconstruction made after study of living larvæ, whole mounts and sections. The scale has been obtained from the latter, but the dorso-ventral diameter of the prototroch (left to right across the figure) is shown a little shorter than it actually is in order to keep the figure within bounds. The tissues are shown as though they were opaque and the lesser curves and wrinkles smoothed out. No cilia are indicated. The whole of the right side of the prototroch, episphere, hyposphere, and head have been removed and the larval trunk has been dissected (mentally) by cutting away portions of the body wall and other organs. The transparency provides a key to the parts and a full description of this stage appears on pages 281–285.

Key to stereogram, fig. 15, Plate 32. Arrows show breaking places at metamorphosis. Line XY indicates approximate plane of section shown in text-fig. 23. *b.*, buccal organ, right half cut away. *b.b.*1, chæta-sac of first bristle bundle and associated muscles. *c.g.*, position of cerebral ganglion. *d.c.s.*, divaricator of larval chæta-sacs, cut across. *d.m.*1, dorsal mesentery, anterior and posterior portions cut away. *d.m.*2, dorsal mesentery, posterior portion cut away. *d.m.*3, dorsal mesentery in wall of anal chamber. *ep.*, cut edge of episphere. *e.u.*, anterior lateral portion of trunk ectoderm which is not covered by mesoderm. The ventralmost part is cut away. *m.r.*, left median retractor of buccal organ. *np.d.*, nephridial duct. *o.t.*, external opening of thread gland. *s.m.*, spiral muscles for spreading provisional bristles. *sph.*, sphincter in splanchnopleure anterior to third adult septum. *st.*, stomach, part of right side removed. *t.*1, *t.*2, etc., first, second, etc., thread gland. *t.m.*, thin membrane separating cœlom of larval chæta-sacs from blastocœl. This after metamorphosis forms part of first adult septum. *tor.*1, *tor.*2, etc., first, second, etc., torus. *v.m.*1, *v.m.*2, ventral mesentery cut away posteriorly. *v.m.*3, ventral mesentery in wall of anal chamber.

FIG. 16.—Stereogram of the head end of an *Owenia* worm immediately after metamorphosis and before the head has fused with the trunk. × 450.

This figure was constructed in a similar manner to fig. 15. It is more hypothetical in certain parts, particularly as to the mode of formation of the dorsal blood vessel and the branches connecting it to the horizontal vessels (see p. 298). The transparency provides a key to the parts and the figure is referred to in the text on pp. 290–298.

Key to stereogram, fig. 16, Plate 32. Arrows show places where fusion of the head to the trunk and to the œsophagus will take place. Line AB is referred to in the text on p. 296, *br*.1, *br*.3, and *br*.4, bristles of first, third and fourth parapodia. *c*.1. 2, combined cœlom of first and second chætigerous segments. The letters are placed where a hole has been cut in the ventral mesentery separating the right and left halves of this cœlom. *c*.3. 4. 5, combined cœlom of third, fourth and fifth chætigerous segments. *d.m.*, dorsal mesentery. The exact anterior limits in the region of the invaginated larval chæta-sacs are not quite certain (see p. 293). *d.v.*, dorsal blood vessel. Mode of formation as shown here somewhat hypothetical. *e.u.*, anterior lateral portion of trunk ectoderm which is not coated internally by trunk mesoderm. *h.s.e.*, the part of the head splanchnopleure which lines *e.u.* internally. *h.s.s.*, the part of the head splanchnopleure which fuses with splanchnopleure of the first chætigerous segment and the thin membrane (*t.m.*) to form the dorsal part of the first adult septum. *h.v.*, horizontal blood vessel in horizontal septum. *l.m.*, mesodermal sheet which in the late larva forms the lateral wall of the buccal organ cœlom, and here forms part of the horizontal septum. *l.v.*, lateral blood vessel connecting horizontal vessel to ventral vessel. *o.*, œsophagus. This is, of course, covered externally with œsophageal mesoderm fused with it. *o.e.*, turned up anterior edge of œsophagus which is about to fuse with the anterior border of the head. *o.v.*, oblique blood vessel connecting horizontal vessel to dorsal vessel. Mode of formation as shown here somewhat hypothetical. *r.m.*, mesodermal sheet which in the late larva forms the roof of the buccal organ cœlom, *t*.1, *t*.2, etc., first, second, etc., thread gland. *t.m.*, portion of first adult septum which forms the thin membrane (*t.m.* in key to Plate 32) of the late larva. This membrane apparently becomes much thicker and of smaller area at metamorphosis and can no longer be distinguished from the splanchnopleure of the first chætigerous segment, with which it merges. *tor*.1, first torus.

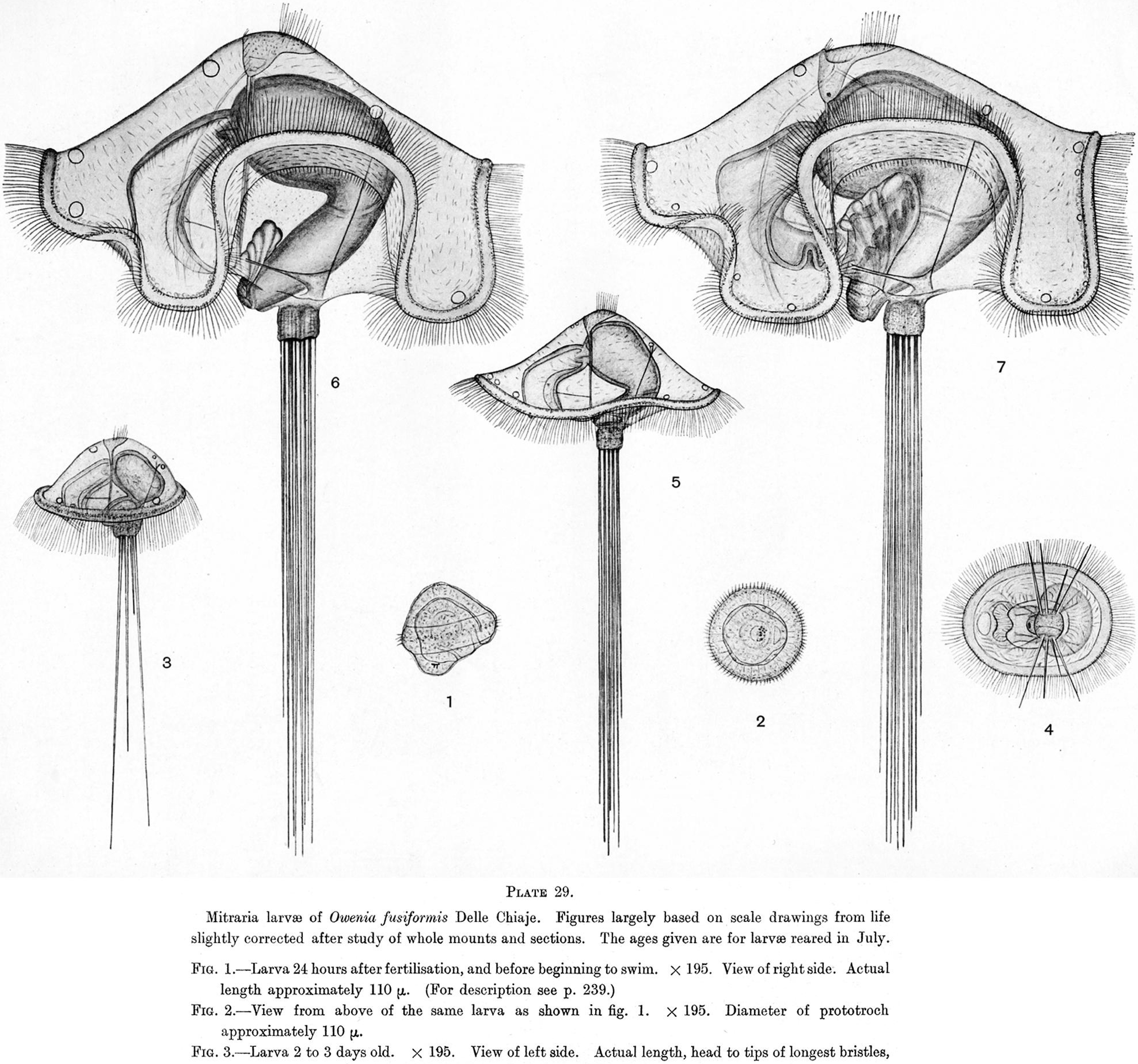


PLATE 29.

Mitraria larvæ of *Owenia fusiformis* Delle Chiaje. Figures largely based on scale drawings from life slightly corrected after study of whole mounts and sections. The ages given are for larvæ reared in July.

FIG. 1.—Larva 24 hours after fertilisation, and before beginning to swim. × 195. View of right side. Actual length approximately 110 μ. (For description see p. 239.)

FIG. 2.—View from above of the same larva as shown in fig. 1. × 195. Diameter of prototroch approximately 110 μ.

FIG. 3.—Larva 2 to 3 days old. × 195. View of left side. Actual length, head to tips of longest bristles, approximately 450 μ. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 166 μ. (For description see p. 239.)

FIG. 4.—View from below of a larva at the same stage as that shown in fig. 3. × 195. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 166 μ, lateral diameter approximately 135 μ.

FIG. 5.—Larva about 12 days old. × 180. View of left side. Actual length, head to tips of longest bristles, approximately 640 μ. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 300 μ. (For description see p. 242.)

FIG. 6.—Larva about 17 days old. × 200. View of left side. Actual length, head to tips of longest bristles, approximately 900 μ Dorso-ventral diameter (*i.e.*, left to right across figure) approximately 500 μ. (For description see p. 243.)

FIG. 7.—Larva about 21 days old. × 185. View of left side. Actual length, head to tips of longest bristles, approximately 950 μ. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 560 μ. (For description see p. 243.)

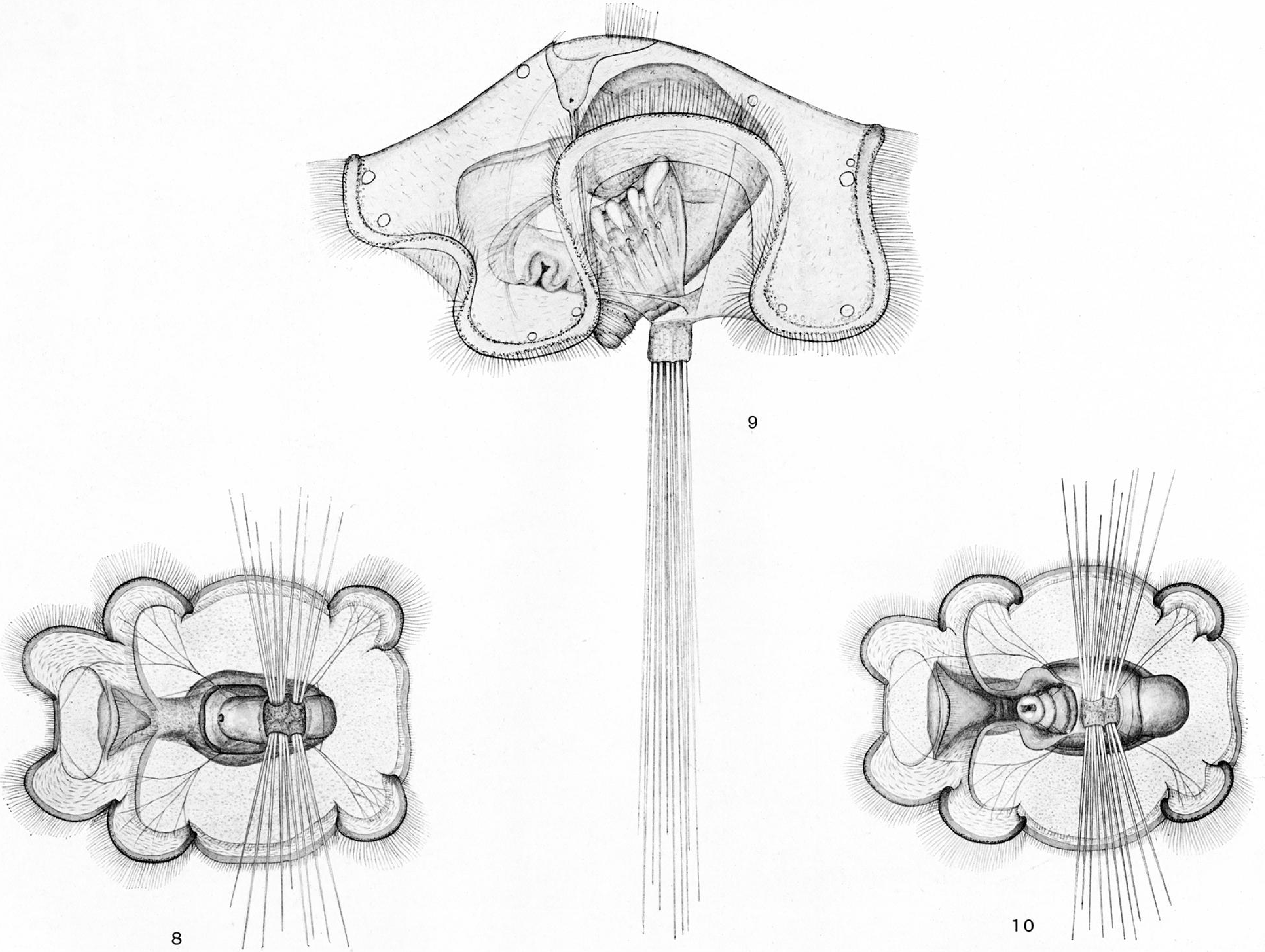


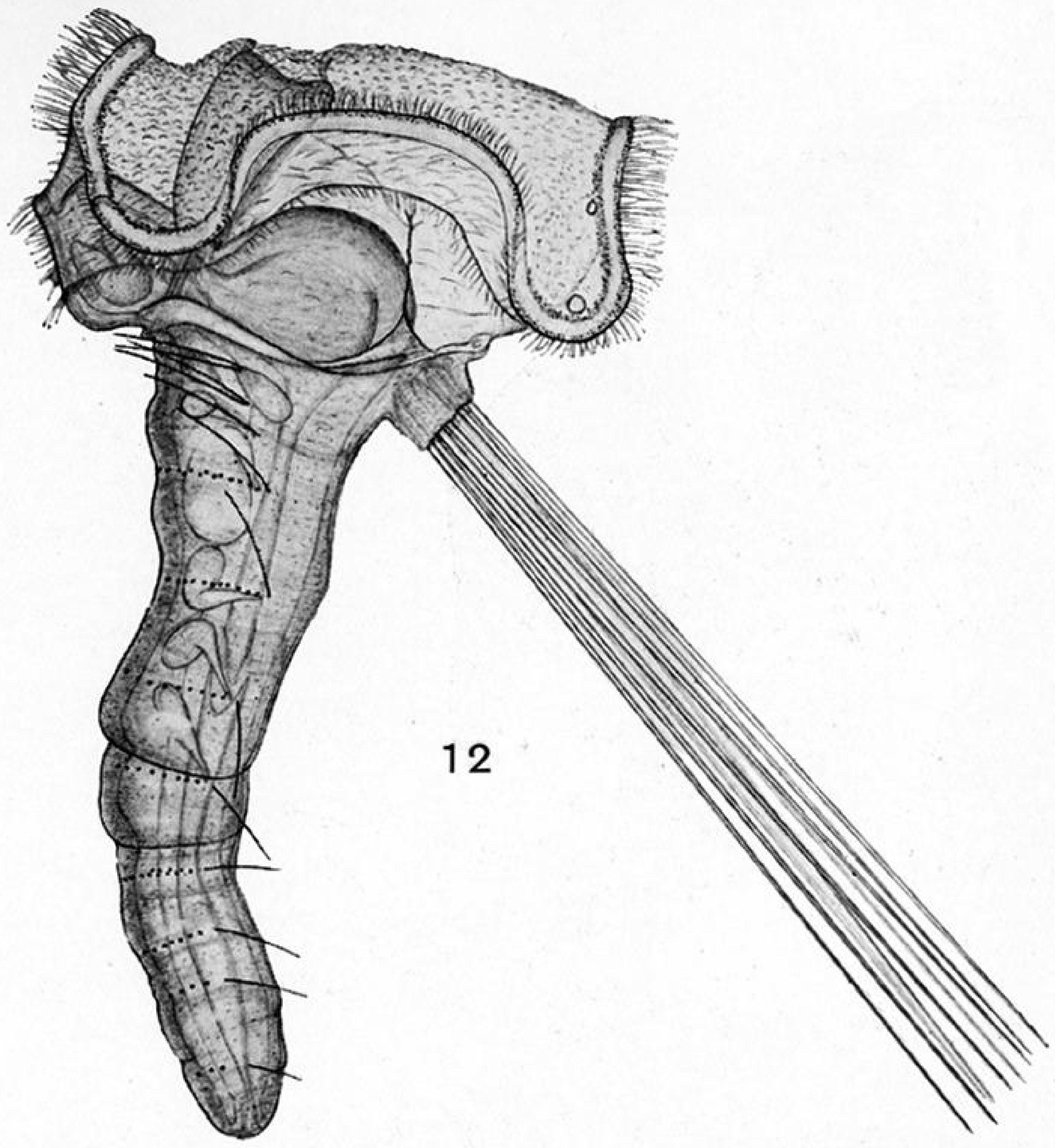
Plate 30.

Mitraria larvæ of *Owenia fusiformis* Delle Chiaje. Figures largely based on scale drawings from life slightly corrected after study of whole mounts and sections. The ages given are for larvæ reared in July.

Fig. 8.—Larva a little earlier than that shown in Plate 29, fig. 7, viewed from below. × 132. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 560 μ. Lateral diameter of prototroch approximately 410 μ. (For description see p. 243.)

Fig. 9.—Larva about 23 days old. × 180. View of left side. Actual length, head to tips of longest bristles, approximately 950 μ. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 560 μ. (For description see p. 243.)

Fig. 10.—Larva the same age as that shown in fig. 11, Plate 32, viewed from below. × 132. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 560 μ. Lateral diameter of prototroch approximately 410 μ. (For description see p. 243.)



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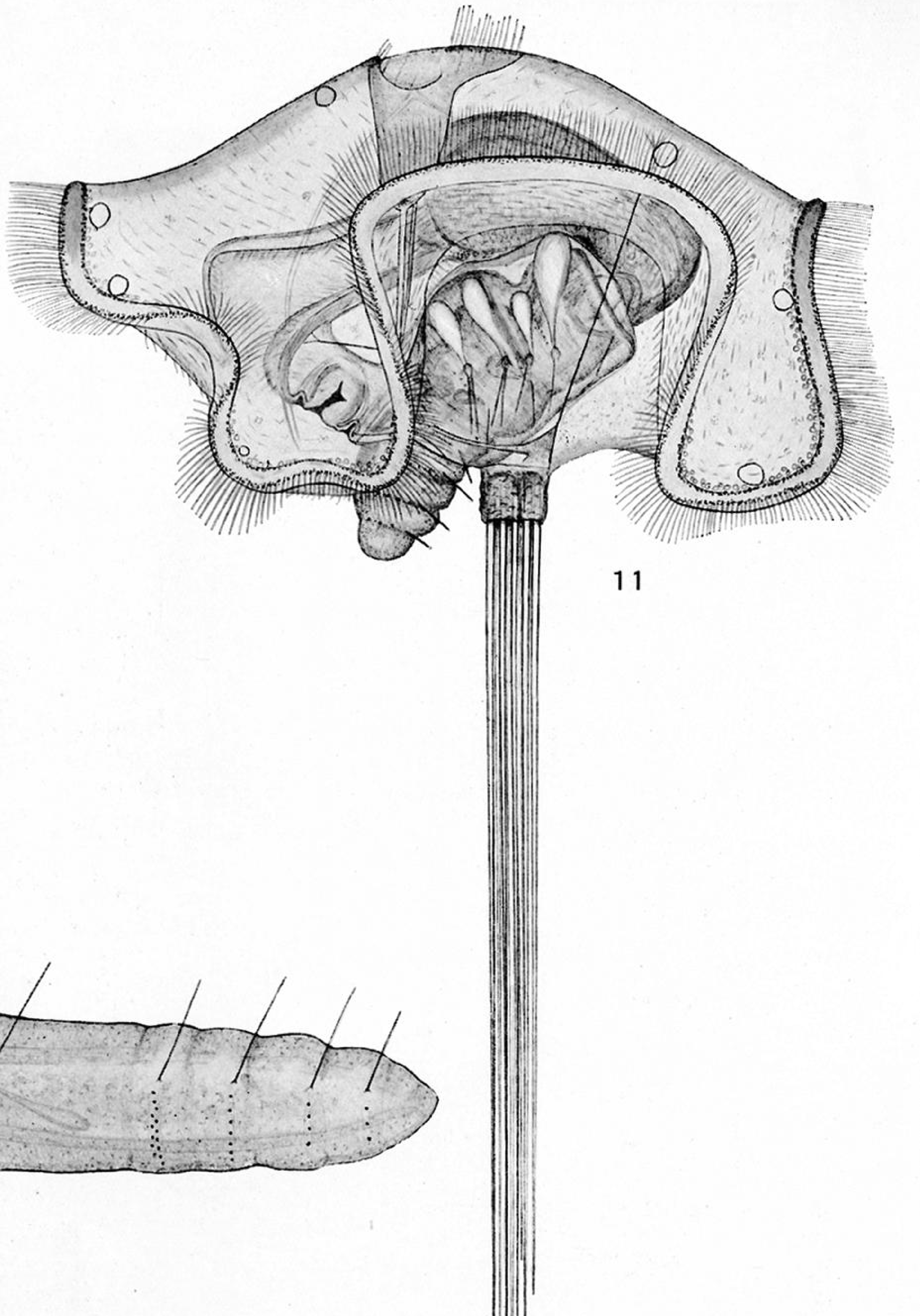

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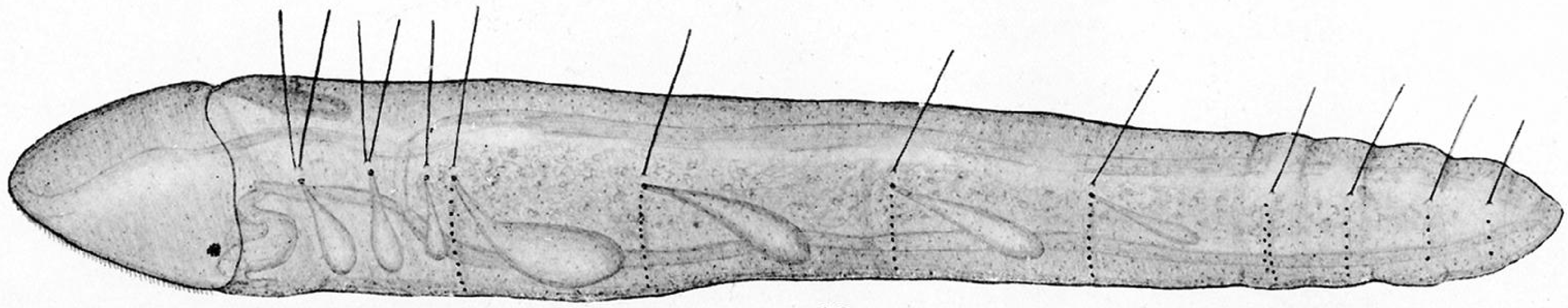

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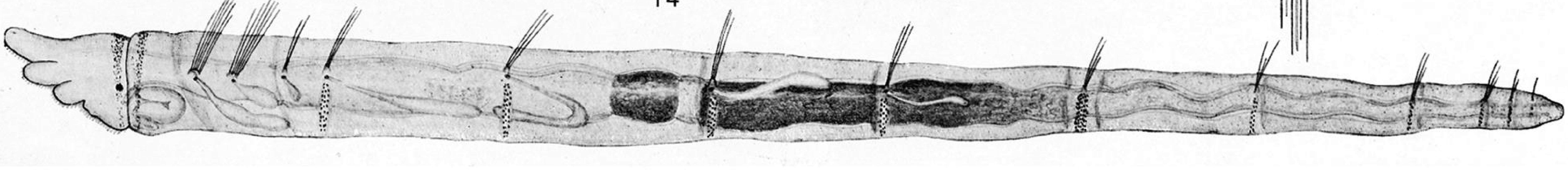

Plate 31.

Mitraria larvæ and young worms of *Owenia fusiformis* Delle Chiaje. All figures except fig. 2 are largely based on scale drawings from life slightly corrected after study of whole mounts and sections. The ages given are for larvæ reared in July.

Fig. 11.—Larva about 27 days old and ready to metamorphose. × 175. View of left side. Actual length, from head to tips of longest bristles, approximately 950 μ. Dorso-ventral diameter of prototroch (*i.e.*, left to right across figure) approximately 560 μ. (For description see p. 243.)

Fig. 12.—Larva about the same age as that shown in fig. 1 metamorphosing, 15–20 seconds after metamorphosis began. × 130. View from left side. Actual length from head to anus, approximately 710 μ. (For description see p. 288.)

This drawing is from a fixed and mounted specimen. It is in an almost exactly naturally expanded state. The third adult septum has been drawn as though it were continuous all round. Actually it could not be seen over the gut and therefore may or may not have a lateral gap. Efforts to settle this point have failed.

The position of the seventh thread gland is interesting. It must either be projecting through a lateral gap in the fourth adult septum (such a gap was suspected before this slide was studied, see p. 307) into the previous segment, or the septum has been broken by the strain (very unlikely). A third alternative is that the septum has been stretched out into a very thin membrane over the gland. Unfortunately a fold of the body wall occurs just where this septum should be visible and practically nothing can be seen of it, and it has therefore not been indicated in the drawing. It should be mentioned that the seventh gland of the right side is in its normal position behind the seventh parapodium.

Fig. 13.—Young worm 15 or 20 minutes after metamorphosis began. × 216. View of left side. Actual length approximately 870 μ. The remains of the histolysed prototroch and other larval tissues can be seen in the gut. The worm has been removed from its mucus tube. (For description see p. 289.)

Fig. 14.—Young worm about a month after metamorphosis. × 95. View of left side. Actual length approximately 2560 μ. (For description see p. 302.)

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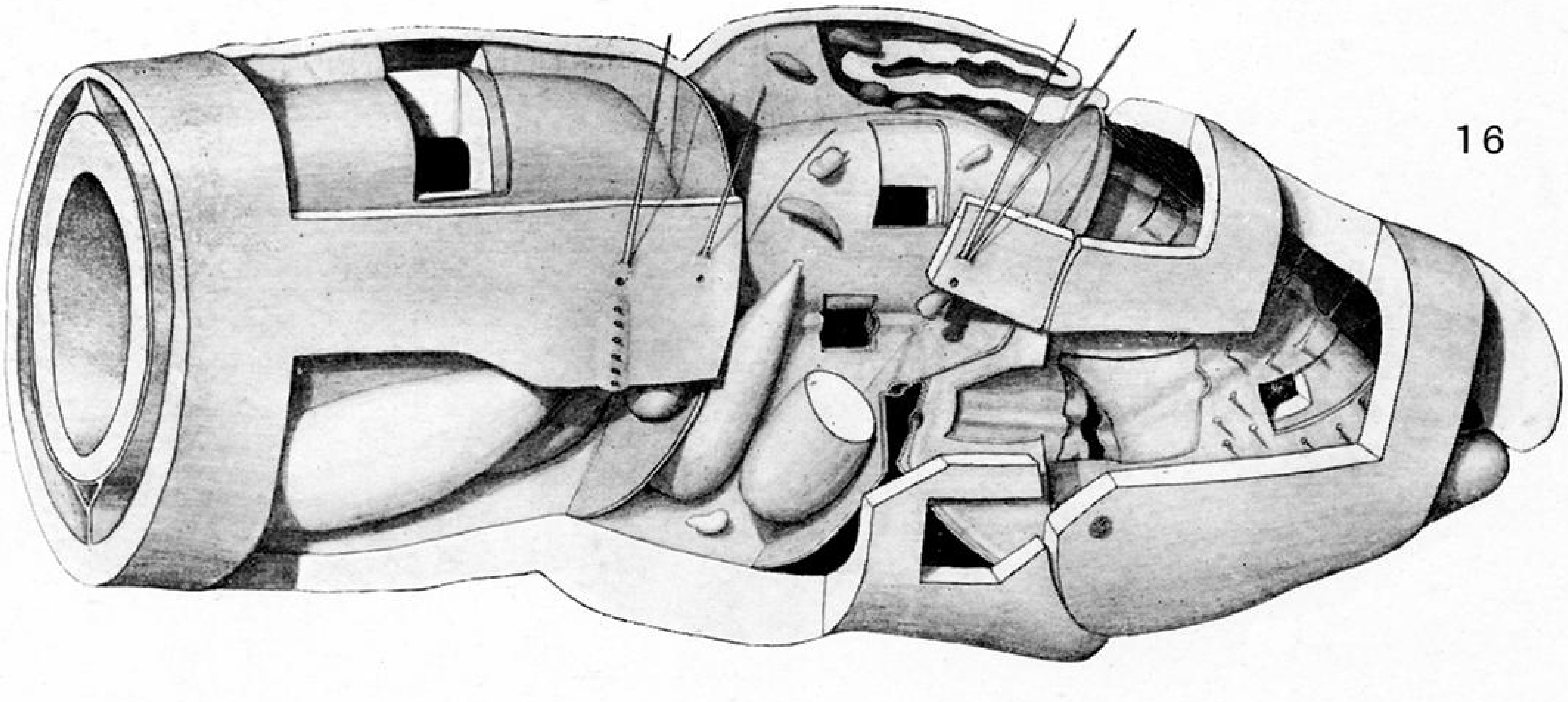

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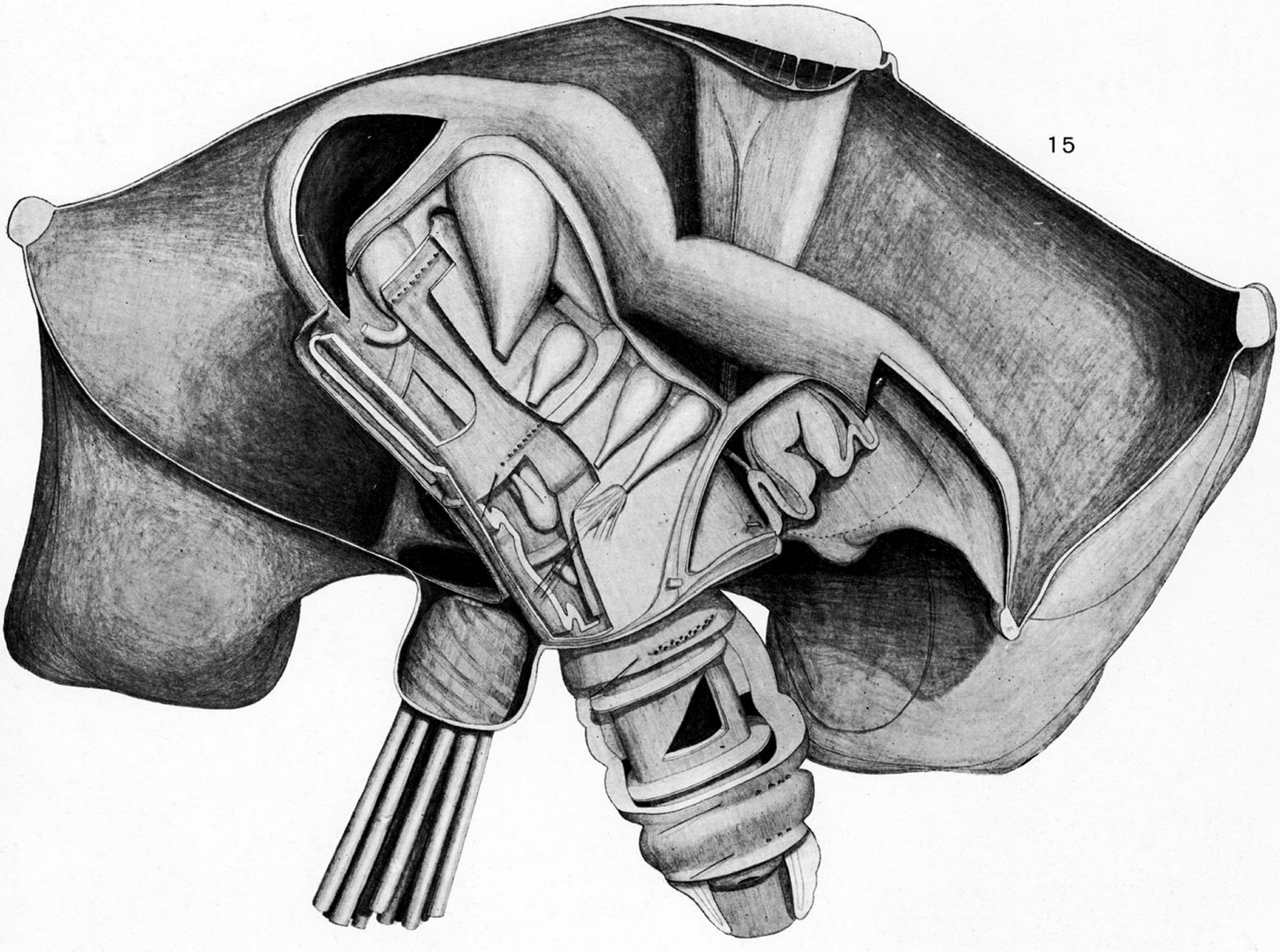

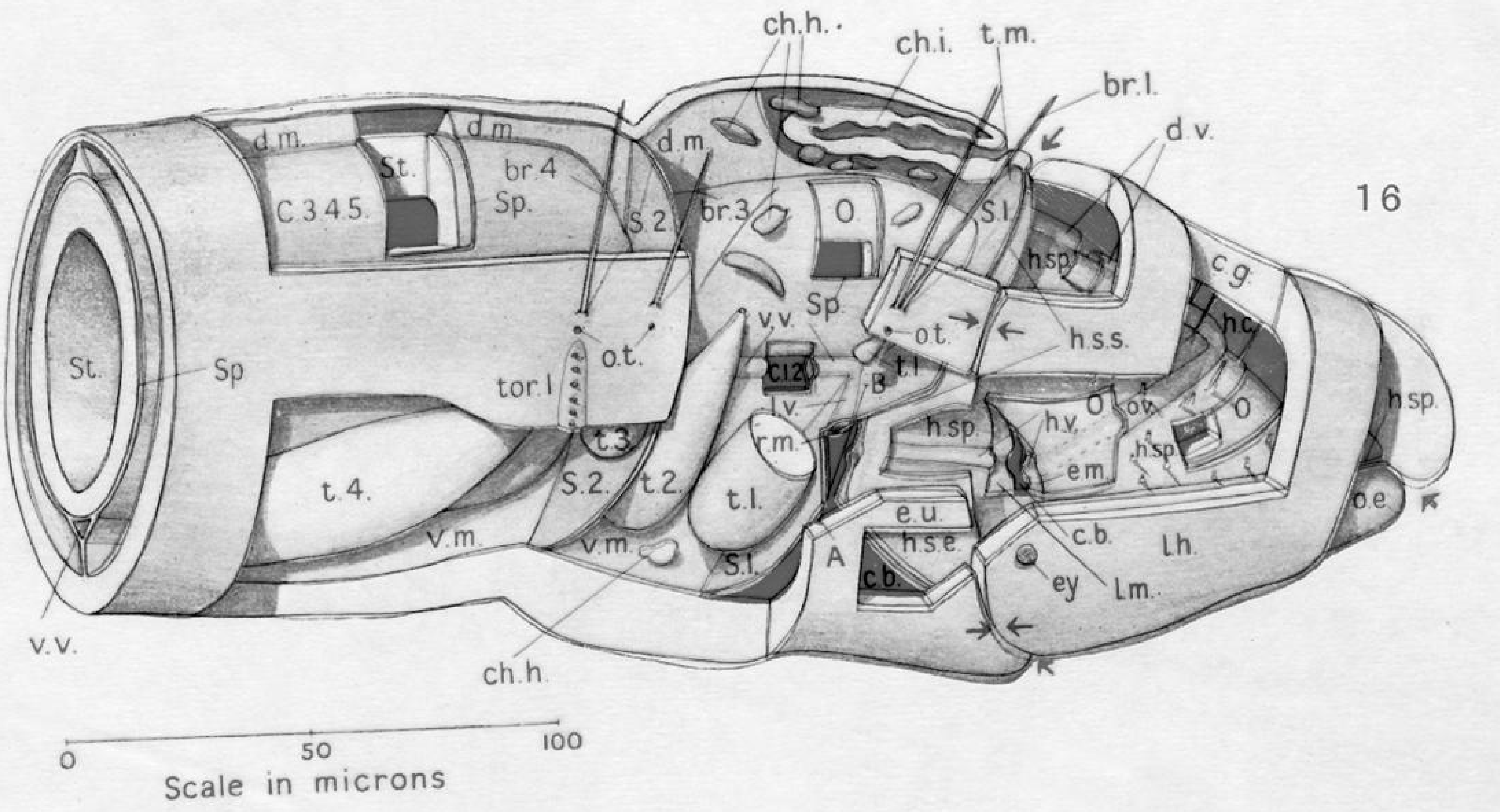

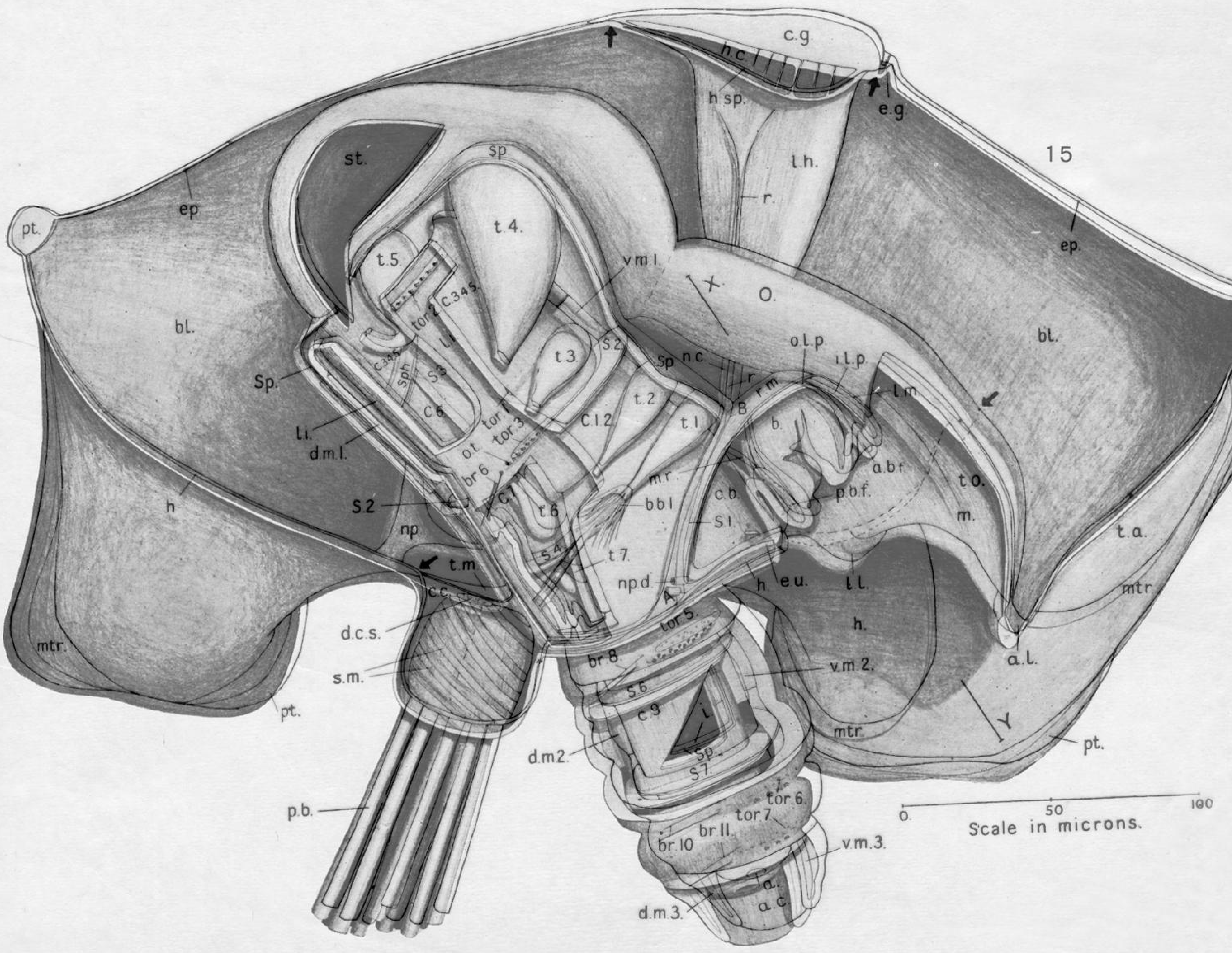

PLATE 32.

FIG. 15.—Stereogram of Mitraria larva of *Owenia fusiformis* ready to metamorphose. × 450.

This diagrammatic figure is a mental reconstruction made after study of living larvæ, whole mounts and sections. The scale has been obtained from the latter, but the dorso-ventral diameter of the prototroch (left to right across the figure) is shown a little shorter than it actually is in order to keep the figure within bounds. The tissues are shown as though they were opaque and the lesser curves and wrinkles smoothed out. No cilia are indicated. The whole of the right side of the prototroch, episphere, hyposphere, and head have been removed and the larval trunk has been dissected (mentally) by cutting away portions of the body wall and other organs. The transparency provides a key to the parts and a full description of this stage appears on pages 281–285.

Key to stereogram, fig. 15, Plate 32. Arrows show breaking places at metamorphosis. Line XY indicates approximate plane of section shown in text-fig. 23. *b.*, buccal organ, right half cut away. *b.b.*1, chæta-sac of first bristle bundle and associated muscles. *c.g.*, position of cerebral ganglion. *d.c.s.*, divaricator of larval chæta-sacs, cut across. *d.m.*1, dorsal mesentery, anterior and posterior portions cut away. *d.m.*2, dorsal mesentery, posterior portion cut away. *d.m.*3, dorsal mesentery in wall of anal chamber. *ep.*, cut edge of episphere. *e.u.*, anterior lateral portion of trunk ectoderm which is not covered by mesoderm. The ventralmost part is cut away. *m.r.*, left median retractor of buccal organ. *np.d.*, nephridial duct. *o.t.*, external opening of thread gland. *s.m.*, spiral muscles for spreading provisional bristles. *sph.*, sphincter in splanchnopleure anterior to third adult septum. *st.*, stomach, part of right side removed. *t.*1, *t.*2, etc., first, second, etc., thread gland. *t.m.*, thin membrane separating cœlom of larval chæta-sacs from blastocœl. This after metamorphosis forms part of first adult septum. *tor.*1, *tor.*2, etc., first, second, etc., torus. *v.m.*1, *v.m.*2, ventral mesentery cut away posteriorly. *v.m.*3, ventral mesentery in wall of anal chamber.

FIG. 16.—Stereogram of the head end of an *Owenia* worm immediately after metamorphosis and before the head has fused with the trunk. × 450.

This figure was constructed in a similar manner to fig. 15. It is more hypothetical in certain parts, particularly as to the mode of formation of the dorsal blood vessel and the branches connecting it to the horizontal vessels (see p. 298). The transparency provides a key to the parts and the figure is referred to in the text on pp. 290–298.

Key to stereogram, fig. 16, Plate 32. Arrows show places where fusion of the head to the trunk and to the œsophagus will take place. Line AB is referred to in the text on p. 296, *br.*1, *br.*3, and *br.*4, bristles of first, third and fourth parapodia. *c.*1. 2, combined cœlom of first and second chætigerous segments. The letters are placed where a hole has been cut in the ventral mesentery separating the right and left halves of this cœlom. *c.*3. 4. 5, combined cœlom of third, fourth and fifth chætigerous segments. *d.m.*, dorsal mesentery. The exact anterior limits in the region of the invaginated larval chæta-sacs are not quite certain (see p. 293). *d.v.*, dorsal blood vessel. Mode of formation as shown here somewhat hypothetical. *e.u.*, anterior lateral portion of trunk ectoderm which is not coated internally by trunk mesoderm. *h.s.e.*, the part of the head splanchnopleure which lines *e.u.* internally. *h.s.s.*, the part of the head splanchnopleure which fuses with splanchnopleure of the first chætigerous segment and the thin membrane (*t.m.*) to form the dorsal part of the first adult septum. *h.v.*, horizontal blood vessel in horizontal septum. *l.m.*, mesodermal sheet which in the late larva forms the lateral wall of the buccal organ cœlom, and here forms part of the horizontal septum. *l.v.*, lateral blood vessel connecting horizontal vessel to ventral vessel. *o.*, œsophagus. This is, of course, covered externally with œsophageal mesoderm fused with it. *o.e.*, turned up anterior edge of œsophagus which is about to fuse with the anterior border of the head. *o.v.*, oblique blood vessel connecting horizontal vessel to dorsal vessel. Mode of formation as shown here somewhat hypothetical. *r.m.*, mesodermal sheet which in the late larva forms the roof of the buccal organ cœlom, *t.*1, *t.*2, etc., first, second, etc., thread gland. *t.m.*, portion of first adult septum which forms the thin membrane (*t.m.* in key to Plate 32) of the late larva. This membrane apparently becomes much thicker and of smaller area at metamorphosis and can no longer be distinguished from the splanchnopleure of the first chætigerous segment, with which it merges. *tor.*1, first torus.