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# No. 3. — *On Nectonema agile, Verrill.* By Henry B. Ward.[1]

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## I. Introduction.

For more than twenty years an interesting pelagic worm has been frequently captured at the Newport Marine Laboratory. It was probably first seen by Dr. Alexander Agassiz, who encountered it as early as 1870. His first recorded observations, made in 1871, as well as his subsequent studies, have remained, however, in the form of unpublished notes and drawings, which were placed at my disposal in June, 1891, when I began the present study. Two of these drawings, which illustrate most clearly the external appearance, and show also some of the internal organs, are reproduced on Plate I. Figs. 3, 6.

In 1873 Professor A. E. Verrill published, under the title "*gen. indet.*," a short description of two specimens captured in towing near Wood's

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy, under the direction of E. L Mark, No. XXXII.

Holl, Massachusetts, and in 1879 he established a new genus *Nectonema* with the type species *N. agile*,[1] regarding it as a Nematode of uncertain systematic position.

Dr. J. W. Fewkes ('83, p. 201) was the first to figure this form; he also gave a short account of its external anatomy. But unfortunately both text and figures are somewhat inexact.

Last summer there appeared a paper by Dr. O. Bürger ('91) on the anatomy and histology of Nectonema, which placed beyond question its affinity to the Nematodes, and for the first time furnished evidence as to the details of internal structure. His work was based on material collected at Newport in 1885, but, as he himself says, it consisted of only a few specimens, and these were not in good histological condition. The gaps in his description, as well as the errors, which were largely due to insufficient and poorly preserved material, influenced me to carry out my work, which was already well advanced before the receipt of his paper. For the sake of comparison it will be more advantageous to consider under the appropriate topic the various points of structure which he describes, rather than to give a connected *résumé* of his paper at this place.

The material at command for the following study consisted of fourteen specimens, collected and preserved with great care at Newport last summer, besides those received from other persons. From Dr. E. A. Andrews I received five, which he had collected at Wood's Holl in 1890, and from Dr. W. M. Woodworth one, which was obtained at the same place in 1888. Professor Verrill, at the solicitation of Dr. Agassiz, very kindly sent me his entire collection, consisting of thirty-five specimens taken in Vineyard Sound between 1875 and 1883. For all these kindnesses, and especially to Professor Verrill for his courtesy in supplying me with personal information on numerous questions addressed to him, I desire to return my sincere thanks. To Dr. Agassiz I am deeply indebted for the hospitality of the Newport Marine Laboratory last summer, and for permission to use his notes and drawings, as well as for many other favors. To Professor E. L. Mark I owe much for valuable suggestions, and for his continued personal interest in the progress of the work.

## Methods.

Nectonema is certainly an animal which it is difficult to preserve well. This is largely due to its resistent cuticula, which hinders the

[1] *N. agilis*, Verrill, '79, p. 187.

passage of most fluids. It shows, further, a strong tendency to curl in the killing fluid, thus rendering it less serviceable for section cutting. There is no reagent which does not in some cases produce a collapse of the body wall and consequent distortion or maceration of the internal organs. No reagent gave uniformly good results; the best were (1) a saturated aqueous solution of corrosive sublimate, and (2) Perenyi's fluid, heated to a temperature of about 60° C. Picro-nitric acid gave nearly as good results. The curling of the specimens may be largely prevented by straightening the worm gently with the fingers, and dropping it suddenly into the warm killing reagent. Flemming's chrom-osmic-acetic mixture made the material very brittle, even after subsequent treatment with Merkel's fluid, and is not to be recommended. Material preserved simply in alcohol, however carefully, is useful for little more than topographical work.

Bürger mentions the difficulty experienced in staining the material satisfactorily, and I agree with him fully. I experimented more than a month before obtaining a really satisfactory method of preparation; it may therefore be advisable to review the methods employed. The only carmine solution of those (8) tried which will stain it at all is Mayer's hydrochloric acid carmine, and this only after prolonged immersion. All hæmatoxylin solutions stain it fairly well, but require more time than usual. Böhmer's and Ehrlich's give brilliant results, but on the whole the latter is more reliable and can be highly recommended. The results obtained by Pfitzner's safranin are also good, and various aniline dyes are nearly as satisfactory.

In embedding in paraffin it is necessary to keep the temperature low. Series cut in paraffin of 50°–52° C. were in all respects most successful. The infiltration must be complete, but a long immersion in paraffin renders the objects very brittle.

Maceration was tried on preserved material with little success.

Great assistance was derived from the study of portions of the body cleared in clove oil before staining. Only in this way was it possible to obtain a clear idea of the structure of the two ends.

## II. Systematic.

Since none of the previous observers have given an accurate description of the female, if indeed it has been seen at all, and since a more extended study has modified some of the points given in the original description of the genus and species, I have determined to restate here

the characters in synoptic form. The original description (Verrill, '79) has been followed as closely as was compatible with the changes necessitated by the discovery of the female, and by a more perfect acquaintance with the anatomy of the male.

*Nectonema*, Verrill, *char. emend.* — Body long, slender, nearly round. Cuticula finely ringed, on the median lines often deeply infolded and bearing on each line two rows of hair-like bristles. Bristles hollow, superficial and unconnected with each other. Head without appendages, obtusely rounded or bluntly conical with a shallow dorsiventral furrow on its anterior aspect. Mouth-opening in the centre of this furrow, minute. In the male the tail is curved ventrad, and terminates in a small conical intromittent organ. Female smaller, the posterior end slightly enlarged, abruptly truncate, with terminal vaginal (?) opening. Alimentary tract rudimentary and anus wanting in both sexes.

*Nectonema agile*, type species. — A long, slender, and exceedingly active round worm, resembling in form and motions a Gordius, found swimming at the surface of the sea with a rapid undulatory motion. Integument firm, opaque, smooth, except for many minute circular ridges interrupted at the median lines, which are themselves often thrown into larger, deeper folds, locally very prominent. Body in life round, of nearly uniform size throughout, tapering slightly close to the head, and somewhat more towards the posterior end in the male. Each median line is distinguished by two narrow longitudinal bands of minute dots between which stand two longitudinal rows of hair-like bristles. The worm undergoes torsion in the anterior third of the body, so that the median lines appear lateral in the posterior two thirds of the body. The double row of bristles extends from a point 1 or 1.5 mm. behind the apex of the head to about the same distance from the posterior extremity of the body. The bristles are 0.3 mm. in length, opposite to each other, hollow, unconnected by any web, entirely superficial, and hence easily detached and often injured or lost over considerable stretches of the body. The head is marked anteriorly by the presence of a shallow median dorsiventral furrow, on each lateral edge of which are one, sometimes two, low rounded papillæ. The anterior portion of the body is semi-transparent, externally not separated by any constriction from the rest of the body; but internally an anterior chamber is divided from the general body cavity by a partition which is concave anteriorly. The anterior chamber is traversed by the œsophagus, and contains ventrally the brain, while the dorsal space is filled by four large conical cells which send processes down into the nervous matter of the brain. The œsopha-

gus is lined by a minute chitinous tube, which is intracellular in position, and after forming a loop opens out into an intercellular intestine that is highly degenerate posteriorly and in the adult lacks a terminal opening. The posterior end of the male is a ventrally curved conical intromittent organ with a terminal opening. The female is provided with a terminal bulb having a central cloacal (?) opening. The small spherical eggs are filled with large refractive yolk granules and protected by a shell, the thickenings of which become long and pointed spines on coming in contact with the water. The eggs measure 36–40 $\mu$ in diameter without the spines, which are 8 to 10 $\mu$ long. Color of the animal in life grayish to yellowish white with transparent anterior end. The median lines each show two narrow longitudinal bands of dark slate color.

Length of the male, 50 to 200 mm.; of the female, 30 to 60 mm. Diameter, 0.3 to 1 mm.

Habitat: Narragansett Bay, R. I., A. Agassiz (1870–90), J. W. Fewkes (1883); Vineyard Sound, Mass., S. I. Smith (1871), A. E. Verrill (1879); Wood's Holl, Mass., W. M. Woodworth (1888), E. A. Andrews (1890).

## III. Biology.

Up to the present time Nectonema has been reported from two places only, Newport, R. I., and Wood's Holl, Mass., and the south shore of New England may fairly be considered as its home. Here it is not so rare as has been supposed, for by systematic search fifteen specimens were secured in one summer. The dates of capture of some sixty-five specimens show that it may be found from the last of June to the first of October,[1] with two maxima, one in July and a second in September, more than two thirds however having been caught at the earlier date. It is noteworthy that, of the fifteen individuals captured at Newport last summer, all were taken while the tide was going out, and on evenings when there was no moon, the ordinary time of towing being between 8 and 10 P. M. The latter circumstance seems to indicate that the worms are susceptible to light; the possible significance of the former will be discussed later. It is also an interesting fact, that they were caught in towing near the shore, two in fact having been dipped up one evening in July when filling a pail with water at the landing of the

[1] I do not believe that towing has been done in this region with any regularity at other times of the year, so that these dates cannot be accepted as fixing the time of its occurrence.

laboratory, which is located on a small cove near the mouth of Narragansett Bay.

When first caught they were very active, swimming vigorously from side to side of the vessel into which the tow was emptied, and trying alternately the surface and the bottom of the water. Their motions are of two kinds; first, a rhythmical movement, evidently caused by progressing waves of muscular contraction alternating on the two sides of the animal; and, secondly, a more violent motion, which consists in first coiling the body into two large successive loops, and then straightening it suddenly and coiling it at once in the opposite direction. In this way the worm assumes much the general appearance of a figure ∞. By the first kind of motion it makes rapid and definite progress; but the purpose of the latter did not seem to be *locomotion*; it was rather relief from some irritation, which on one occasion was apparently a mass of foreign matter which had accumulated on the bristles. These, together constituting what has been called the lateral fins, can ordinarily be easily seen even during the motion of the animal, and evidently are not actively concerned in its movements.

Nectonema always swims with the translucent end, which, as will be seen later, is the head, in advance. How long the activity exhibited at first persists, I do not know. The animals captured in the evening were usually found on the following morning resting on the bottom of the dish, and exhibited only occasional fits of activity. This may have been due to the effects of light or of captivity. I am inclined to consider it to be the result of the latter, since usually before noon of the first day after capture the worms voided into the water masses of eggs or spermatozoa which were often unripe, and then became more and more sluggish. But the material was too valuable to warrant the risk of its becoming injured for histological purposes by longer delay, and observations were therefore terminated at this point by killing the animals.

## IV. General Morphology.

### 1. External.

Nectonema is in life of an opaque grayish white with semi-transparent ends. The body is perfectly round and the median lines show no trace of the flattening described by Verrill and Fewkes, and so often seen in the preserved specimen. This condition is unquestionably the result of collapse. The general surface of the body is smooth, except for the many

fine circular striations which are so characteristic of Nematodes in general. Here however the striations are interrupted by the median "lines,"[1] whose smooth surface is marked off more or less regularly by deep furrows which extend transversely, yet only across the line itself. These lines may be seen to start near the anterior end of the body on its dorsal and ventral surfaces. Owing to a gradual torsion of the anterior third of the body, they are brought at the end of this portion into a lateral position, which they preserve throughout the rest of their course, i. e. up to a short distance from the posterior end. This apparent change in position is diagrammatically represented in Figure 1[a]. They are really the median lines, though their position throughout the greater part of the body caused them to be described at first as the lateral lines. Each of them is limited on either side by a narrow dark-colored border which under a high power resolves itself into a crowded mass of deep-seated dots (Fig. 7). Between these two marginal bands, on the lighter portion of each median line, are located in a double row the characteristic bristles. Their arrangement and structure will be considered later.

It is the deep furrows in the median lines together with the dark borders they transsect which produce the "squares marked in outline by black pigment" described by Fewkes ('83, p. 201). The transverse furrows appear and disappear with the movements of the animal, while the squares vary both in form and size (Fig. 7) and are due merely to the folding of the lines necessitated by the contraction of the adjacent muscular areas, as Bürger ('91, p. 636) has shown. They have, then, nothing to do with internal segmentation, but are purely mechanical in their origin.

The length of the males that I have examined varies from 32 to 130 mm.[2] Of the seventeen which were measured exactly, only three were less than 55 mm. long, and the same number were over 100 mm., the most of the rest being close to the average, 68 mm. The diameter of the male varies at the head from 0.32 to 0.65 mm., at the middle from 0.4 to 0.75 mm., and just in front of the terminal papilla from 0.2 to 0.4 mm. The three females caught measured 34, 38, and 40 mm. in length, and at the regions of the body mentioned above on the average 0.35, 0.45, and 0.25 mm. in diameter. Thus, excepting the imperfect specimen men-

[1] The name "line" seems to be peculiarly inappropriate as a designation for these broad bands, but I have used it in the technical sense in which it has been employed for Nematodes in general.

[2] There was but one male less than 45 mm. in length, and this one must also have been nearly as long, since the head and a portion of the body were gone.

tioned above, they are shorter than any of the males, though in diameter intermediate between the extremes of the latter. The only female among the specimens sent by Professor Verrill measured 60 mm., which still is shorter than the average male, and far below some of the large males in that collection, one of which reached even 200 mm. in length.

Not only is the female smaller than the male, but also far less plentiful. Among the sixty-five specimens which I have examined, there were but four females, a proportion so small as to suggest that it is due in part to other causes than the greater number of males produced. Possibly the female, being the less active of the two, is not so often at the surface, and consequently is less frequently caught.

From the general external appearance one easily recognizes three main parts of the body, a short semi-transparent anterior portion, a very long opaque middle region, and a terminal part, which resembles in transparency and length the anterior portion. The anterior region goes over into the body proper without any external demarcation. There is no constriction at this point in the living animal, and although one is usually found here in alcoholic specimens, it is surely the result of contraction or collapse. If, however, the living animal be studied under a compressor (Fig. 2), or still more clearly if the anterior part of the body of an alcoholic specimen be examined in clove oil, this clear portion is seen to be cut off from the general body cavity by a transverse partition (Plate I. Fig. 8), thus forming an anterior chamber, which will be considered in detail later.

Following this the body proper is uniformly opaque in appearance, and constitutes the greater part of the entire length, passing over insensibly near the posterior end of the body into the posterior translucent region, which is however by no means so clear as the anterior part. The body terminates in the female abruptly, but in the male it is prolonged into a ventrally curved conical organ with a terminal opening. The general appearance of the worm is shown in Figure 1, which represents in its natural size one of the largest specimens captured. The difference between the posterior end of the male and that of the female is easily seen by comparing views of the two as seen under a dissecting miscroscope (Plate I. Figs. 4, 5). The female is represented in the act of discharging eggs. The end of the body of the male differs greatly in appearance in different individuals. It may be nearly straight with only the intromittent organ turned slightly ventrad (Fig. 89), or for a greater or less distance anterior to this point it may be flexed ventrally or even coiled (Fig. 1). The end of the female is on the other hand nearly straight,

slightly enlarged, and blunt. The centre of the blunt surface shows the terminal orifice, from which the eggs are being voided.

The general external surface, more particularly on the lines and about the rows of bristles, is often covered with minute algæ and dirt. A similar mass frequently envelops the posterior end, making the determination of its character a difficult matter, especially in the case of the female, if an egg mass be protruding from the opening. It may have been such an appearance which caused Verrill ('79, p. 187) to describe the female as possessing a small terminal papilla.

### 2. Internal.

The general plan of the internal anatomy may be easily understood from any cross section (Fig. 11). Passing from the surface inward, the thick cuticula is followed by a thin hypodermis, beneath which is the highly refractive muscular layer. The protoplasmic ends of the muscle cells, together with other elements, form the layer immediately surrounding the body cavity. Dorsally and ventrally the muscular layer is broadly interrupted by the prominent median lines. The body cavity contains in a varying position the alimentary canal, which is strikingly small and sometimes wanting. A sac-like organ varying in size hangs from the dorsal line into the body cavity, which may also be filled with generative products. The ventral line encloses the ventral nerve cord. A comparison of the various figures will show the variation in the proportions of the ventral line at different points in the body and in the two sexes.

## V. Anatomy and Histology.

### 1. Body Wall.

In treating of the finer anatomy I shall consider first the structure of the part under consideration in the male only, and at the end of each section give a comparative account of its character in the female.

#### *a. Cuticula.*

The cuticula, which covers the entire body and is continuous with the lining of the œsophagus and of the terminal sexual opening, is of nearly equal thickness throughout, averaging 3 $\mu$ on the side of the body, being however perceptibly thicker (about 4 $\mu$) over the median lines. On top of the anterior chamber it is only 2 $\mu$ thick, which partially explains the transparency of that region. At the front of the head, on the other

hand, it measures from 5 to 10 $\mu$ in thickness. It is highly refractive, and similar to chitin, although not identical in composition with that substance, since it may be easily dissolved in boiling KOH. Occasionally one notes a fibrous or lamellar structure, the layers being parallel to the surface. In most places an outer extremely thin layer may be easily distinguished from the subjacent portion by its higher refractive power. The inner surface of the cuticula is not always even and clearly marked off from the hypodermis, but frequently shows a jagged outline with underlying granules, which decrease rapidly in size toward the muscular layer.

On the front and upper surface of the head one finds occasional fine pore canals, and in total preparations short hairs were seen, but no connection between the two could be established. In the hollow produced by the ventral flexion of the posterior end, the cuticula displays a curious peculiarity. The highly refractive outer layer remains intact, but the inner layer is, as it were, bored with conical holes, into which the hypodermal tissue projects. These probably represent sensory organs, but in spite of the proximity of the anal ganglion a nervous supply could not be demonstrated.

There are present as structures of undoubted cuticular origin the hairs or bristles of the lines and peculiar scales found along the posterior portion of the body of the male.

The *bristles* (Fig. 72) form a double row along the dorsal and ventral lines, beginning only 0.2 mm. behind the transverse partition which cuts off the anterior chamber, and extending to within 0.5 mm. of the posterior end of the body. The two rows are only 15 to 25 $\mu$ apart, and the bristles stand opposite each other (Plate II. Fig. 12) at regular intervals of 10 $\mu$. Normally they are entirely unconnected by any web of tissue or mucus. They are, moreover, but lightly attached to the cuticula, and hence easily broken off, so that even in the living animal it is rare to find any considerable tract perfect. One can usually see the scars that have been left, and apprehend from these the normal relation of the bristles. Each bristle is, when perfect, about 0.3 mm. long and hollow, having in cross section (Fig. 13) an external diameter of 5 $\mu$ and an internal one of 2 $\mu$. The base is slightly enlarged, and rests on the cuticula (Fig. 13), from which in sections it is separated by a definite line of demarcation. From the base the bristle tapers very gradually to a fine point. Its cavity is simply rounded off at the base, being separated from the cuticula by a thin layer; toward the point its cavity gradually disappears. These structures are, then, entirely super-

ficial, and evidently cannot be actively moved by the animal. They are in all respects carefully to be distinguished from the setæ of Annelids, with which they have nothing in common. Bürger ('91, p. 634) called attention to the fact that the hairs are hollow, and are purely cuticular structures.

*Scales.* — One finds on the sides of the male near the posterior end numerous scale-like cuticular outgrowths. My attention was first attracted to them in transverse sections, where they present the appearance of a tooth (Plate II. Fig. 14). Seen from the surface (Fig. 18) they have much the shape of a clam shell. They are found scattered over both lateral aspects of the animal, in spots so thickly that from one to seven are cut in each transverse section (Fig. 14). They vary much in size, the smallest occurring near the beginning and end of the area, whereas they are interspersed with larger ones at the centre. The area which they occupy begins about 1.2 mm. from the posterior end of the body, and extends over 5 to 10 mm. In general such a scale may be said to resemble a narrow clam shell attached along the hinge side (Fig. 18). The line of attachment is always parallel to the long axis of the body, but the concavity of the scale is directed indiscriminately dorsad or ventrad (Fig. 14). The length of the scale is about 40 $\mu$, its height averages 15 $\mu$, and the thickness varies from 7 to 8 $\mu$.

In most transverse sections the external layer of the cuticula is continuous over the entire surface of the scale (Fig. 15), and only in certain cases can one see that it is interrupted by a minute opening (Fig. 16), which is connected with a fine canal. On account of its minute size this canal can be traced through its entire length only in exceptional cases, and usually appears as a groove at the outer or inner margin of the scale (Fig. 17). I was unable to find either gland cell connected with the canal or sensory filament passing through it.

The core of the scale is formed of a substance which stains like the internal layer of the cuticula, but which is in nearly all sections well marked off from that layer. If the scales be treated with caustic potash, the core is broken up into lamellæ by lines which radiate from the apex of the scale. In transverse sections, however, the core is marked by fibres parallel to the central canal, and thus nearly perpendicular to the fibres of the internal layer of the cuticula elsewhere. This difference in the direction of the component fibres serves to separate the core of the scale from the internal layer of the cuticula in and near the plane of the central canal (Fig. 17), whereas elsewhere one finds no definite line of demarcation between the two (Fig. 15)

It is difficult to believe that this canal represents merely the central protoplasmic core upon which the scale was formed, since, if this were the case, it would be closed terminally by at least a thin layer of cuticular substance. But this is not the case. The opening is always larger and plainer than the canal itself, which is too narrow to be measured.

These structures are entirely lacking in the female. Their occurrence in the male alone, and over only a limited area near the posterior end, suggests connection in some way with the sexual act. If it be the case here, as in Gordius, that the male grasps the female during copulation by winding itself about the posterior portion of her body, the use of these scales in holding on to the cuticula, which differs from that of Gordius in being smooth, is at once suggested. The canal may then be either the duct of a gland or a tactile organ.

The cuticula of the female is nowhere more than 1 $\mu$ thick, and precludes thus any profitable study of its structure. The bristles are present, and do not differ materially from those of the male. They are, however, somewhat more slender and shorter. The scales of the male are entirely lacking, and no analogous structures were found.

### *b. Hypodermis.*

The hypodermis, or subcuticula, as it is often called in Nematodes, forms immediately under the cuticula a layer of comparatively uniform thickness and structure, being, however, peculiarly modified in the anterior chamber, in the median lines, and in the terminal organ of the male. Its modifications will be considered under the organs in question. Sometimes no trace of this layer can be found, but in the majority of sections it can be demonstrated in some places.

The hypodermis (Fig. 20) normally appears as a narrow granular layer 7 $\mu$ in thickness, without cell walls, but containing numerous prominent nuclei arranged somewhat regularly, and characterized particularly by the indefinite distribution of their chromatic matter and the faint, uncertain way in which they are stained. It is separated from the underlying muscular layer by a delicate basement membrane, which ordinarily cannot be demonstrated, but which is easily seen where the muscle cells are shrunk apart or torn away.

*Median Lines.* — The hypodermis appears to be highly differentiated in two regions, the dorsal and ventral lines, where it becomes so much thicker as to cut down into the muscular layer and separate it into two lateral areas. These lines were regarded by Verrill and Fewkes as lateral; but, as Bürger has clearly shown, they undoubtedly correspond

to the median lines of other Nematodes. The curious torsion, by which in the normal position of the body they come to lie laterally for the larger portion of its length, has already been described.

The two lines, dorsal and ventral, are very similar in form and structure (Plate VIII. Figs. 101, 102), except that the ventral line contains the prominent ventral nerve cord, which will be described in connection with the other portions of the nervous system.

The *dorsal line* can first be seen distinctly immediately behind the partition which cuts off the anterior chamber. In front of this I find no dorsal differentiation of the hypodermis, and consequently no dorsal line. At its anterior end the dorsal line has a thickness of 20 $\mu$; passing posteriad, this gradually increases to 40 $\mu$, and this thickness remains nearly constant until within a short distance from the end of the body, where it becomes gradually reduced and finally disappears. The line is separated from the body cavity by a prominent basement membrane, the direct continuation of that which separates hypoderm and muscular layer. The elements which make up the dorsal line (Fig. 102) appear both in longitudinal and in transverse sections as a row of elongated cells, the walls of which are usually first visible a short distance below the cuticula, although in one specimen preserved in Flemming's mixture they could be traced even up to the lower surface of the cuticula itself. The nuclei are oval, poor in chromatic substance, hence pale and not at all prominent. They lie with the long axis perpendicular to the surface of the body, nearly or quite filling the entire diameter of the cell. In most cases they are found at about the same level in the different cells, which thus form a regular epithelial layer. The deep ends of the cells are prolonged into processes which extend down to the basement membrane through a mass of fibres which cross in every direction. Among this net-work of fibres in the lower portion of the line one finds occasional cells with branching processes (*, Fig. 102), which may be nervous. There is however no definite nerve cord extending through the line, and no evidence was found of the connection of these cells with other parts of the nervous system.

The *ventral line* is similar in structure to the dorsal line, in that it consists likewise of a layer of high cells of an epithelial character immediately underlying the cuticula (Fig. 101). Their deep ends are also prolonged into processes, which are here bent around the ventral nerve cord, which lies in the centre of the line. Between the cord and the basement membrane below is seen again a confused mass of fibres, into which, as in the case of the dorsal line, the deep ends of the cells

pass. The boundaries of the epithelial cells extend in this case also only to within a short distance of the cuticula ; they cannot ordinarily be traced up to it, except, as in the case of the dorsal line, in material preserved in Flemming's mixture. The entire ventral line is separated from the body cavity dorsally, and from the muscularis on both sides, by a basement membrane.

In the adult female the lines do not exceed 8 $\mu$ in width, and are consequently difficult to study, but I think the same elements can be seen, though not so clearly as in the case of the male.

Bürger ('91, p. 636) believed the collection of long cells at the apex of the head to be a part of the dorsal line. I can find no special connection between the two regions, and no striking similarity in structure. The shallow groove which he believed characteristic of the external surface of the dorsal line is not present in the living animal. It is undoubtedly the effect of collapse, since it is found only in preserved specimens. I did not find the large cells which he says occur at regular intervals in the dorsal line. Perhaps they are found only in individuals of a certain age, or they may be connected with the formation of the hairs. Proof of the existence of a columnar epithelium, which he conjectured to be present, has been given above.

### *c. Muscular Layer.*

In cross sections of the body the muscular layer presents two sharply marked portions, a peripheral, radially striated zone (Plate I. Fig. 11) and a deep protoplasmic region. Along the line of union of the two lies a double or triple row of thickly crowded nuclei, and in some regions, or under certain conditions, other nuclei are found scattered through the protoplasmic portion. The relative thickness of the two zones varies greatly. In the most of the specimens which I cut they were of nearly equal breadth, but in some the protoplasmic zone was more than twice as wide as the striated portion, and in other cases not half so wide. These conditions are represented somewhat diagrammatically in a number of transverse sections (Plate II. Figs. 23 to 26), which are taken from different individuals.

The general meaning of the two zones is at once apparent. The striated portion is made up of the contractile fibres of the muscle cells ; the protoplasmic area represents the non-differentiated portions of the same cells together with certain other elements. I met with indifferent success in attempting to make macerations of this region and I am not able to affirm definitely what proportion of the protoplasmic zone is

made up of the ends of muscle cells and to what extent it is formed of other elements. Certain it is that this zone contributes to the formation of at least a certain number of other elements: this may be by a differentiation from the protoplasmic body of the muscle cell, or it may be that the elements have no genetic connection with the muscle cells. In order to discuss this question it will be necessary first to consider carefully the structure of the individual muscle cell.

For this study the region near the dorsal or ventral line is very favorable, since here the cells are shorter and broader than elsewhere, and thus it is easier to trace the cell walls. Cross sections of this region (Plate II. Fig. 21) show with perfect clearness that each muscle cell is composed of two portions, corresponding in appearance and position to the two zones of the muscular layer. The highly refractive peripheral portion is seen in longitudinal section (Fig. 22) to consist chiefly of fibrillæ; these are, however, developed only at the periphery of this portion of the cell, the core of which is composed of a finely granular protoplasm. The latter is directly continuous with the granular protoplasm of which the deep-seated part of the cell is exclusively composed and in which the nucleus is located. This is the condition of the typical muscle cell of the "Cœlomyaria." In the centre of the muscular layer, i. e. in the lateral walls of the body, the cells diffe. only in being much deeper and more flattened. From maceration preparations (Fig. 22) it may be seen that the inner or deep margin of the band of fibrillæ is bounded by a very thin layer of protoplasm which at intervals is continued downward into the elongated cell body. This is also seen in transverse sections (Figs. 28–30). It would seem (Fig. 22) as if each muscle cell had more than one protoplasmic prolongation, but since I was unable to ascertain the length of the individual cells this cannot be positively asserted.

The nuclei usually lie just below the contractile portion of the cell. They are oval, and each has a thick nuclear membrane, which stains deeply, but encloses very little stainable substance, the numerous nucleoli being minute and faint. There are also at times unquestionably as many as two nuclei in each protoplasmic projection, and in certain specimens it was common to find nuclei far down toward the deep end of the cell.

I must call attention in this connection to some very peculiar nuclei which were found among the nuclei of the muscle cells, but which differ from them strikingly (Fig. 27). They were usually located rather more distally than the others, and each showed a tail of varying length, which

projected far up among the contractile fibrillæ. Tailed nuclei have been found by various observers, and sometimes the form has been attributed to mechanical injury in cutting. But this cannot be the case here, since they were plainest in sections 20 $\mu$ thick at a level distinctly between the two surfaces of the section. From the general appearance of the sections I doubt the probability of the form being due to pressure in killing or preparing, and am inclined to regard their form as normal. Such nuclei have been explained as nervous; the only argument which can be said to favor that view in this case is their position, and the absence of other known nervous terminations in the muscular layer.

The striated zone is narrow in the anterior chamber and at the posterior end of the body, but is elsewhere of nearly uniform width in any one specimen. It remains entirely colorless in carmine stains, but takes up hæmatoxylin with avidity and does not give it up in acid fluids.

The protoplasmic portion of the muscular cell is highly granular and ordinarily does not stain at all. In the protoplasmic zone, however, there are cells which stain in eosin much deeper than the remaining elements (Fig. 29, *x*). The contents of these cells are so finely granular as to appear almost homogeneous. Lying in the body cavity near these cells are corpuscles, which in general appearance and affinity for stain are identical with them. It will appear probable, I think, from the figures given (Figs. 28–30), that these corpuscles are derived by abstriction from the deeply staining cells of the protoplasmic zone. They are found of all sizes, but never in very great numbers. There is some evidence to show that the deeply staining cells are the proximal ends of certain muscle cells, the contents of which are perhaps chemically altered; the corpuscles, however, never contain nuclei, so far as I have seen. In view of the evident correlation between the thickness of the muscular wall of the body and the sexual maturity of the animal, it is possible that the function of these corpuscles is nutritive. This will be discussed in connection with the description of the sexual organs.

Evidence which goes to prove the formation of true cells from this layer is obtained from the study of the female in which the eggs were in the most immature condition of any which I had. Here (Plate IV. Fig. 59) sections show the body wall to be composed of the layers already described, except that the protoplasmic portion of the muscle cells is much shorter, and there seem to be proportionally fewer nuclei and fewer cells than in the sections previously described. In addition

to these there is, however, the remnant of a deeper layer. Certain cells (*, Fig. 59) project into the body cavity; they are homogeneous and lightly stained, in opposition to the muscle cells which remain unstained, and they contain nuclei at the proximal end of the cell. In the body cavity of this specimen were found, in addition to the eggs, cells of a very similar appearance to these, and at points along the wall of the body cavity flattened cells had arranged themselves in the form of an epithelium. All these points naturally suggest that this layer is concerned in the production or nourishment of the sexual cells, and that the remnant of the layer is in process of forming itself into a secondary epithelium. The evidence is however too incomplete to justify more than a suggestion; but it points strongly to the existence of more than one kind of histological element in the protoplasmic zone of the muscular layer.

The foregoing description of the muscularis differs essentially from that given by Bürger ('91, p. 635). Especial attention must be called to the fact that the relative thickness of protoplasmic and contractile portions as he gives it, namely, 2 : 1, is true in only one of the sections he figures (Taf. XXXVIII. Fig. 5), whereas others of his sections (Figs. 3, 4) represent exactly the opposite extreme.

## 2. Alimentary Canal.

### *a. Œsophagus.*

Attention has already been called to the fact that one finds a shallow dorsiventral groove (Fig. 2) at the front of the head, and that the minute mouth opening is located at the centre of this groove (Plate V. Fig. 63). The cuticula, which is extremely thick at this point, is here infolded; the deep layer extends but a short distance, while the external layer is continued backward to form the œsophageal tube. Here as elsewhere this layer is highly refractive, and has walls 2 $\mu$ thick, enclosing a lumen only 3 $\mu$ in diameter. The deep layer, which surrounds the beginning of the œsophageal tube, measures 9 $\mu$ in thickness. After this layer stops, the chitinous œsophageal tube, which is the continuation of the external layer, becomes somewhat thicker, and it is seen that the entire tube is contained within a cell of small diameter (Plate III. Fig. 32). From longitudinal sections it is seen that the cell is coextensive with the tube; at least there are no transverse cell boundaries, though throughout its length one finds many nuclei which lie closely packed together. In transverse sections the nuclear matter

appears fragmented, and usually has a more or less concentric form surrounding the tube (Fig. 50). That face of the nucleus which is turned toward the tube is less regular than the other, and usually shows a broken and less sharply marked contour than elsewhere. The amount of chromatic substance, large in proportion to the somewhat meagre supply of protoplasm in the cell, is also noticeable.

The œsophageal cell with its contained tube traverses the anterior chamber; from the mouth opening it first passes through the hypoderm, then it lies in a groove on the dorsal surface of the brain (Plate VI. Fig. 73), and later is spanned by the dorsal commissure (Fig. 80), directly behind which (Fig. 81) it extends a short distance free until it reaches and pierces the transverse partition (Plate I. Fig. 8). It is enveloped throughout its course by the same peritoneal membrane which lines the anterior chamber. The occasional flattened nuclei of this membrane may be seen at intervals on the outside of the œsophageal cell, even where the latter is surmounted by the dorsal commissure of the brain.

Before tracing the further modifications of the œsophageal cell, it is interesting to note one or two points of variation in the portion already described. Although the tube is commonly of uniform caliber and open from end to end, this is not always the case. Figure 50 (Plate III.) shows a cross section of the œsophageal cell 0.1 mm. from the apex of the head. Here the tube is of the usual appearance, but a few sections farther back not only lumen, but tube as well, has disappeared (Fig. 51). Some distance farther posteriad the tube appears again, but as a solid cord, which, however, acquires a lumen at a point 0.4 mm. from the apex of the head, and from this place on preserves its ordinary character. Furthermore, variations in the diameter of the lumen are common. The important physiological bearing of these features will be discussed subsequently.

The œsophageal tube and cell enter the partition and pass through with only a slight expansion in the size of the cell (Plate V. Fig. 63). Bürger has figured and called attention ('91, p. 643) to the presence of a strong dorsal bend of the tube within the partition. This is assuredly abnormal, since it is found but rarely. It is entirely wanting in the other individual figured by him (Taf. XXXVIII. Fig. 1). When present it is probably *on*, not *in*, the partition, and is evidently due to the ventral flexion of the œsophageal cell and tube resulting from the forcing forward of the partition in preservation. This wall is in life concave anteriorly, and it will be clear at once, from a glance at Figure 3, that, if at

any time in the course of manipulation a change to a denser fluid be made too suddenly, the result will be to force the partition forward, and consequently to bend the œsophagus in the space between the brain and the partition, where it is free from supporting tissue. Exactly this is shown to have happened, only in a less degree, in my Figure 8.

Following now the course of the œsophageal cell after it emerges from the partition into the general body cavity, one finds a second cell alongside of it (Plate III. Fig. 33, *cl. in.* I.), which resembles it in the entire absence of transverse cell boundaries and in the presence of many nuclei, but is unlike it in the highly granular condition of the protoplasm and in the shape and appearance of the nuclei. Since the intestine hangs free in the body cavity, the position of the cells can be determined only in a general way. The new cell, which may be named the first intestinal cell, lies approximately lateral to the œsophageal cell (Fig. 33). It begins at about 0.8 mm. from the apex of the head; about 0.4 mm. farther back, a second intestinal cell (*cl. in.* II., Fig. 34) is added. This lies nearly ventral. A third cell (*cl. in.* III., Fig. 35) begins 1.3 mm. from the apex of the head, and a fourth (*cl. in.* IV., Fig. 36) 0.1 mm. farther posteriad. This completes the number. The œsophageal cell, which may be recognized by the presence in it of the cross section of the chitinous tube, now lies lateral to the four intestinal cells (Fig. 36), but soon wedges itself in between two of them until it reaches the centre of the group (Fig. 43), and then suddenly ends, leaving a cavity (Fig. 44) surrounded by the four intestinal cells which have accompanied it a longer or shorter distance from their origin.

It is now necessary to ask how the chitinous tube is concerned in these changes. Up to the point where the fourth intestinal cell is added, it remains a straight simple tube. Shortly beyond that point it makes a complete turn upon itself (Plate I. Fig. 8), and from a lateral position with reference to the four intestinal cells it reaches a median one (Plate III. Figs. 37–43). Hence the loop lies in that portion of the œsophageal cell which is wedged in between two of the intestinal cells (Fig. 38), and almost completely fills the space. The tube proceeds a short distance farther, 60 $\mu$ only, tapers to an exceedingly fine point, and opens out into the space which has arisen between the four intestinal cells (Figs. 8 and 44). This space is the intestine proper, and justifies the application of the name "intestinal cells" to those elements which, though originating farther forward, were destined to bound it. These relations, which are evident in every complete series through this region, are represented in a succession of figures taken from one series of

transverse sections at short intervals (Figs. 31–44). The general form of the tube may also be seen in the optical section represented in Figure 8.

The œsophagus varies from 0.75 to 1.5 mm. in length, being from $\frac{1}{40}$ to $\frac{1}{90}$ of the total length of the worm. The loop which occurs near its posterior termination measures from 50 to 100 $\mu$ in length, and from 20 to 30 $\mu$ in width. It lies nearly in the sagittal plane, and ventral to the general course of the œsophageal tube. The absolute uniformity of its occurrence and the normal appearance of the adjoining intestinal cells preclude the idea that this is an accidental fold. It must be regarded as a normal yet very curious feature of the œsophagus.

### *b. Intestine.*

The intestinal cells, which, as has been shown, are first encountered on the œsophageal cell just behind the partition, are four in number at the point where the intestinal cavity is formed and the œsophagus opens into it. These four however clearly constitute two pairs which are unlike (Fig. 37). The contents of one pair is a coarsely granular plasma, whereas that of the other pair is finer. The first remains unstained in hæmatoxylin, but takes up enough hydrochloric acid carmine to give the plasma a reddish tinge. The reverse is true of the other pair of cells. Occasionally the granules in the first pair of cells become very coarse, and then appear like excretory secretions. As already mentioned, there are no transverse partitions dividing the cell (Fig. 52), although a very large number of nuclei are present, usually several in each section (Fig. 39). Only two of the four cells are represented in Figure 52, which is a surface view. The differences in the character of the nuclei are well shown in the figure.

The walls of the intestinal cells are very strong, perhaps even cuticular, since they remain intact long after the cell contents have been completely macerated out. There is however, no special chitinous lir ing for the intestine, such as Bürger has figured ('91, Taf. XXXVIII. Figs. 25, 29). This appearance is probably due to the partly macerated and detached membranes of the adjacent cells.

The portion of the intestine bounded by four cells is relatively short. One of the finely granular cells[1] dwindles down to a point (Fig. 45) and a new one takes its place. This pushes itself obliquely under the adjacent coarsely granular cell on one side, so that the latter is excluded

[1] Owing to a break in the series figured, I am unable to state positively which one of the original four is the first to disappear.

from participating in the boundary of the lumen, which is now limited by only three cells, one dorsal and two ventral. The cell, thus forced back from the lumen, dwindles away, and is not replaced by another.

A lumen bounded by three cells persists for some distance backward, but finally another cell disappears and the lumen lies between two cells (Fig. 49). I did not succeed in finding the exact spot where the disappearance of the third cell takes place, and am hence unable to give the details of the process. From this place posteriad all further change is of degree and not of kind, since the intestine simply grows smaller, the lumen all but disappears (Figs. 46–48), and finally the whole structure vanishes (Fig. 9) shortly before the end of the body is reached. In no case, either in entire preparations or in sections, was I able to trace it nearer than within a few millimeters of the posterior end of the body; and since it was entirely free at its termination, no clue was given as to its relation to the terminal orifice of the body. Since this orifice is clearly connected with the sexual organs, as will be demonstrated later, it remains doubtful whether it is a cloacal opening, or whether the end of the intestine is to be found elsewhere. Certain it is that in the intestine we have a highly degenerate organ, so that from a study of the adult alone no light can be gained as to its termination. It is interesting to note that the œsophagus is intracellular, the intestine however clearly intercellular.

No mesenteries were found binding the intestine to the body wall, and consequently its position varies in different individuals. It was more often ventral than dorsal, and lateral than median. In the female (Plate IV. Fig. 58), however, it extends directly through the middle of the mass of nearly ripe eggs.

The description of the structure of the alimentary canal already given for the male, holds good for the female as well, except that the lengths of the various parts are somewhat less than those of the male. The anterior chamber is much smaller, and the parts contained in it more compressed.

Bürger ('91, p. 643) described in general the four intestinal cells under the somewhat inappropriate name of œsophageal cells. He failed to recognize the cellular nature of the real œsophageal cell, since he speaks only of the tube and of a fibrous envelope. He seems to have entirely overlooked the loop in the tube, which probably existed in sections between those represented in his Figures 22 and 23, and naturally was able to give but little on histological structure. I do not believe it is advantageous to speak, as he does, of the intestinal cells as

cell-rows. There certainly are no transverse cell walls and no very regular distribution of nuclei; and while they may be potentially equivalent to rows of cells, they certainly are not the same as the structures in the Trichotrachelidæ known as cell-rows. To use the expression, then, is to emphasize a morphological relationship which, if it exist, is much more distant than the use of this word would lead one to suppose. Bürger also described the regions of the intestine bounded successively by four, three, and two cells; but in spite of his strongly expressed doubts on the subject, he was led to regard the terminal orifice of the papilla as the anus, following the description of Verrill and Fewkes. It must have been poorly preserved material which gave the appearances shown in his Figures 25 and 29, for I am convinced that the supposed cuticular lining of the intestine does not exist. I have found nothing which supports his claim, set forth at length, that the intestinal lumen when apparently bounded by three cells is really formed at the expense of only one, and belongs to that cell alone.

### 3. Anterior Chamber.

The anterior chamber is a prominent and characteristic feature of the anatomy of Nectonema. Even in the living animal one can usually distinguish its main features (Fig. 3) under a compressor. The semi-transparent area extends as far as the transverse partition which, at about 0.3 to 0.4 mm. from the apex of the head, cuts off this portion from the general body cavity. In the living animal this partition is concave anteriorly, and apparently slightly thicker at the centre; on sections it is seen to be covered on its anterior face by a thin peritoneal membrane, whose flattened nuclei (Plate VII. Fig. 95) may be easily discerned at intervals. I am not sure that this same peritoneal membrane lines the entire anterior chamber. It can easily be demonstrated over the lateral surfaces and around the œsophageal cell, where similar nuclei may be demonstrated even under the dorsal commissure of the brain and farther forward. On the dorsal surface of the brain I have searched in vain for the nuclei or the membrane; yet it is equally impossible to find where it stops, if it does not line the entire chamber.

In alcoholic specimens much of the regular character of the partition is lost, and it is usually found to be more or less distorted, as the effect of the various processes through which the material has passed. The fibres of which it is composed run in all directions, chiefly radiating from the centre toward the body wall. They show frequent pale nuclei (Fig. 95). The partition is pierced by the œsophagus alone, and it

encloses the œsophageal cell, so that no space is left on any side. The dorsal line does not extend as far forward as the partition, but the ventral line, which takes its origin from the brain, passes under the partition, the fibres of which spread out above it.

The anterior wall of the chamber is grooved externally, and this median groove, already mentioned, is supplied with one, or often two, low papillar elevations on each side. The cuticula on the anterior face of the head has about twice the thickness of that over the body in general. The underlying hypodermis from the mouth opening upward over the anterior dorsal aspect of the head is composed of high narrow cells, which are continued basally into one or more processes that are probably connected with nerve fibres. These relations are represented in Figure 92 (Plate VII.), which shows a somewhat oblique section near the apex of the head. It is the dorsal and lateral cells that are in question, and they show in some places very clearly the basal processes. Two such cells more highly enlarged are shown in Figure 93. The fibrous masses on either side into which the processes pass are the anterior prolongations of the fibrous mass of the brain. Bürger ('91, p. 637) has described these cells as rounded at the deep end, and he did not find their connection with the nervous system.

Along this part of the head and farther ventrad on the anterior face minute pore canals in the cuticula are by no means uncommon, and once or twice in total preparations fine hairs were seen in this region. Without having demonstrated any connection between the canals and hairs, I believe they are really united, and that the mass of cells which is here connected with the brain is sensory in function. Just dorsad to the mouth opening there was found in three specimens a small perfectly regular cuticular pocket about 30 $\mu$ in diameter. Its nature and value could not be determined, but, if at all significant, it is probably the remnant of a larval organ.

The striking transparency of this region in the living animal is due to the thinness of its walls. Everywhere but at the extreme anterior end the cuticula is thin, and, although the muscular layer begins in this region, it is insignificant. Only on the ventral surface does one find a mass of tissue, the brain with its capsule. The dark streak which in the living animal crosses the anterior chamber just in and above this mass is the œsophagus already described. The chamber is filled with a fluid in which float small scattered corpuscles of great transparency. Two such are shown in Figure 95, immediately below the ganglionic cell, *cl. gn.* V.

The most prominent objects in the anterior chamber, however, are the four big cells which, in two pairs, an anterior and a posterior, fill almost the entire space above the brain, and send their processes ventrad into its substance. They are the cells which Fewkes ('85, Expl. of Plates, p. 208) designates as "ova (?)," and which Bürger ('91, p. 646) supposed to be salivary glands. Neither hypothesis has much in its favor, and I shall present evidence which I believe shows them to be clearly nervous, i. e. ganglion cells. Accordingly, the description of their structure and relations will be deferred until the consideration of the ganglion cells in the brain.

## 4. Body Cavity.

The main body cavity extends from the posterior face of the partition which cuts off the anterior chamber to the extreme posterior end of the body. It varies much in size in different individuals (Plate II. Figs. 23–26, Plate IV. Fig. 58, and Plate VIII. Fig. 96) and can hardly be said to have a definite form. It is smallest in immature individuals, and most capacious after sexual maturity. It differs somewhat from the body cavity of the anterior chamber. The latter, as has been shown already, is lined, in great part at least, by a peritoneal membrane, but the general body cavity shows no trace of such a lining. The protoplasmic ends of the muscle cells terminate at variable depths, thus giving it an irregular boundary, which shows no sign of an endothelium. In the body cavity one finds neither dissepiments nor mesenteries; the intestine floats free, or at regular intervals in its course is grown fast to cells in some part of the body wall.

In the male one always finds a sac more or less developed hanging from the dorsal line, and varying in form and structure. This will be more fully described under the sexual organs, to which it unquestionably belongs.

There is often a small amount of coagulated substance in the body cavity which contains scattered corpuscles similar to those of the anterior chamber. They are very pale, entirely unstained, and of a spongy texture. One finds various sizes, and their origin from the protoplasmic ends of the muscle cells has already been maintained. They are by no means abundant, and the amount of coagulum found in the body cavity is also small. In addition to these one always finds in the body cavity of the male free spermatozoa in greater or less numbers. In all of the females obtained the body cavity was nearly or quite filled with eggs.

## 5. Nervous System.

### *a. Brain.*

The anterior ganglionic mass, or brain, forms the larger portion of the floor of the anterior chamber (Plate V. Fig. 63). In general it is somewhat wider than long, being from 0.16 to 0.28 mm. in width, and from 0.12 to 0.2 mm. in length, and has an average thickness of only 0.14 mm. (Figs. 3, 8, 63, 72–88). Its anterior limit is the one most difficult to make out, since the brain substance goes over gradually into the tissue in front of it, from which it is not separated by any prominent capsule. The middle of the dorsal surface is marked by a longitudinal groove, in which the œsophagus lies. Behind the narrow meagre cerebral commissure the œsophagus is separated from the brain by a considerable space, and here the dorsal groove in the latter is wider and less defined than farther forward. (Cf. Plate VI. Figs. 72–88.) Laterally the limits of the ganglionic mass are more distinct, although no envelope of connective tissue separates it plainly from the adjacent cells. In fact, there does not seem to be a definite capsule anywhere, even on the dorsal surface. In places the limits of the mass are so sharp as to suggest a covering membrane, but I was unable to find any corresponding nuclei. The connective-tissue fibres which bound the ventral nerve cord dorsally are first apparent behind the last pair of large ganglion cells of the brain.

*α. Ganglion Cells.* — On the whole the brain is poorly supplied with ganglion cells; of those found, one can nevertheless distinguish two kinds, which represent extremes in size. The first and smaller kind is only moderately abundant, but they far exceed in number the second. No appreciable amount of cell protoplasm can be seen about them, but they appear everywhere simply as small oval nuclei (Plate V. Fig. 68) only 4–5 $\mu$ in diameter. These nuclei stain deeply, and show a thick nuclear membrane with numerous chromatic granules, of which one, or occasionally two, are very prominent. In general they correspond closely to the nervous nuclei (Nervenkerne) described by various observers for different groups of animals. A further point of resemblance is found in their position, for they lie embedded in a mass of fibres, and, although it is difficult to decide whether certain of the fibres are connected with them, appearances decidedly favor this view. Cells of this kind are most abundant on the anterior face of the ganglionic mass, and around the stalks of the dorsal cells. In the fibrous mass of the brain they occur ordinarily only at the ventral surface, and in one or

two definite lines whose significance will be considered later. (Cf. Plate VI. Figs. 72–88.)

The large ganglion cells so far surpass in size those of the first kind that they might well be called giant cells were it not that the name implies a homology which I do not wish to affirm. There are in all five pairs of these large cells, which are nearly constant both in position and in size. Figure 94 (Plate VIII.) represents them diagrammatically and from a comparison of this with Figure 63 (Plate V.) the different cells may be recognized at once. It will be convenient in the description to designate them by numerals, beginning with the most anterior pair.

Since the chief characteristic of the cells of the second class taken as a whole is the nucleus, I shall begin with a description of this structure, which is relatively very large and somewhat irregular in form (Plate V. Fig. 69). It never stains deeply, and shows one or more clear vacuolated areas. The nuclear membrane is delicate, and the chromatic substance finely distributed in lines or rows of dots. The nucleoli vary; sometimes (Pl. VII. Fig. 95, *cl. gn.* III.) none are present, and again there are (Fig. 95, *cl. gn.* V.) one or two very prominent ones, or in other cases (Pl. V. Fig. 69) a number of smaller ones. Frequently, one finds within the nucleus structures (Fig. 69) of an irregular appearance surrounded by a clear space of varying width, and bounded externally from the surrounding nuclear matter by a very definite line. It appears as if the irregular bodies had originally filled the clear space or vacuole, and had shrunken away from the enveloping nuclear matter in the process of preservation. Exactly similar structures occur in the nuclei of the dorsal cells to be described, as well as in the nuclei of the large cells in the anal ganglion: whatever the nature of these enclosures may be, they seem to be characteristic at least of the larger ganglion cells. I do not know that similar bodies have been found in ganglionic cells of other animals.

The amount of protoplasm which surrounds the nucleus in the five pairs of large ganglion cells varies, somewhat in relation to the position occupied by the cells. The cells of the most posterior pair (Fig. 95, *cl. gn.* V.), which protrude above the mass of the brain, have a considerable amount of cell protoplasm; those of the third pair (Fig. 95, *cl. gn.* III.), which are only partly surrounded by fibres, show a lesser quantity, while the others, which are deeply embedded in the fibrous substance, have merely a thin mantle of protoplasm surrounding the nucleus.

Although numerous fine processes pass off from the cell body in various directions, each cell has one prominent process, which may usually be followed without difficulty. They are, hence, really unipolar cells. Of these cells the first and fifth pairs (Fig. 94) are much larger than the others, the third is intermediate in size, and the second and fourth are considerably smaller, though nearly equal in size to each other. All things considered, each of these cells has such a characteristic appearance that after study it is possible to recognize at once a cell from any pair.

One also finds a few cells about half as large as those of the second class; they vary in position and seemingly in number in different specimens. A pair of these are shown in Figure 84 (Plate VI.) between the nuclei of the fifth pair of large cells. These cells are too indefinite in number and position to be regarded as constituting a third class. They resemble the cells of the second class in general appearance, differing from the latter only in size. At most one finds two pairs of such cells ventral to the fourth pair (Fig. 84), and another pair anterior to the third pair of large ganglion cells (Fig. 76). They constitute perhaps an appendix to the cells of the second class.

It is necessary now to ascertain the exact position and relation of these cells to other parts of the nervous system. Figures 72 to 88 (Plate VI.) represent a series of successive transverse sections including the entire brain. By comparing them with Figures 63 and 94, one may determine the exact position of the large cells, and follow their processes. In the cross sections only the nuclei are represented, since the cell body is too small and too poorly marked off from the surrounding tissue to be seen under this power.

The first pair (Plate V. Fig. 63, *cl. gn.* I.) lie farthest anterior, as well as most ventral of all. They usually approach the median plane of the body very closely, being separated from each other by only a narrow space. Occasionally one of them lies a little higher than the other in the fibrous mass of the brain. These cells are pear-shaped (Plate VII. Fig. 94), with the long diameter parallel with the chief axis of the animal. Each possesses a single large process, which passes directly backward. As the two processes from these cells pass posteriad they approach each other and rise slightly, by which they come to lie in the central V-shaped portion of the ventral nerve cord (Plate VI. Fig. 88).

The second of the five pairs of large cells is somewhat smaller than the first, and its position varies within narrow limits (Plate VII. Fig. 94).

In Figure 75 (Plate VI.) the left cell of this pair is represented; the corresponding right cell has fallen out or is aborted in this series. The general position of these cells may be said to be midway between the first and third pairs, as well in height as in antero-posterior relation. They may in some cases lie nearer the median plane than the following pair, as well as farther from it in other cases. The extremes of variation in both directions are shown in Figure 94 (compare the right with the left). It was very difficult to follow the processes of this (second) pair, and I can only say I think they pass into the dorsal commissure, and through that to the opposite side of the body; but their further course could not be made out. It is close to this pair of cells that the stalks of the anterior dorsal cells enter the brain. (Compare Figure 94, *pd. cl. d.*, the anterior of the two stalks.)

The third pair of large cells may properly be called the commissural cells, on account of their intimate connection with the dorsal commissure. They are pear-shaped cells, and lie on the extreme upper surface of the brain, and near the median plane, as may be seen in cross sections (Fig. 77). Their position with reference to the commissure is somewhat variable. Sometimes they are located well to one side in the brain mass (left side, Fig. 78), but again they are found well up on the commissure, even so far that the apex of the cell reaches the median plane, and the entire cell is dorsal to the œsophagus (Plate VIII. Fig. 99, *cl. coms.*). As has already been intimated, the processes of these cells cross through the commissure to the opposite side of the body. After leaving the commissure, they bend at once sharply to the rear, and may be followed some distance. They were ultimately lost to view near or alongside of the fifth pair; not because they are in any way connected with those cells, but rather because the size of the latter tends to obscure the neighboring processes. I believe that the processes pass one into each lateral bundle of the ventral nerve cord, but this point could not be established with absolute certainty.

The fourth pair of cells (Figs. 63 and 94, *cl. gn.* IV.) makes its appearance several sections back of the third. They are intermediate in size between those already described, and possess nearly spherical nuclei (Plate VI. Fig. 82, *nl. gn.* IV.). They occupy the dorsal portion of the ganglionic mass near its posterior end (Fig. 63), and are situated only a short distance from the median plane. Their processes pass sharply ventrad and toward the median plane, where they ultimately come to lie near the processes of the first pair of cells in the central unpaired portion of the ventral nerve cord. In spite of the difference in size between

the first and fourth pairs of cells, their processes cannot be distinguished from each other in size (*pr'c. gn.*, Fig. 88).

The fifth pair of cells lies farthest lateral and dorsal of all (Plate V. Fig. 63), forming as it were the posterior outer corners of the ganglionic mass. They are the largest of the five pairs of cells, on account of the larger amount of protoplasm which surrounds their nuclei, and they lie wholly without the fibrous mass of the ganglion (Fig. 63); in fact, they often project above the general level of the brain (Plate VI. Fig. 85). The processes of these cells are the most prominent of all, having a diameter twice as great as those from any other pair of cells. They pass directly backward into the corresponding lateral bundle of the ventral nerve cord, and for a long distance occupy the centre of this portion; but farther back they cannot be distinguished among the numerous processes which occupy this portion of the cord.

β. *Dorsal Cells.* — The probable nervous nature of the dorsal cells has already been referred to, and to make this clear it is necessary to consider in detail their structure and relations to the brain. In life they appear spherical when viewed from above (Fig. 2), but when seen from the side (Fig. 3) they are evidently conical. The two constituting the anterior pair lie in juxtaposition at the median plane, the posterior ones farther apart and in contact with the posterior and lateral portions of the first. No particular structure can be made out in the living cell further than the presence near the stalk of a dark body, presumably the nucleus. The stalks pass ventrad and slightly posteriad into the substance of the brain, where they are seen to bend decidedly backwards (Fig. 3), and are then lost to view. Nothing further was determined from the living animal, since the possibility that they might be nerve cells did not force itself upon me until much later.

In preserved specimens the shape of the cells is much altered. They are usually shrivelled and distorted; or, again, they often contain a huge vacuole on one side (Plate VIII. Fig. 98). Unstained specimens cleared in clove oil serve only to confirm what is seen in the living animal, and show quite distinctly that the stalks of the cells are not connected either with the œsophagus or the external cuticula at any point. There is also clearly apparent in the cell a fine network, which takes its origin from the stalk (compare Fig. 99), and fills the whole cell with a mass of minute meshes. If now we examine sections through this region, the peculiar character of the cells becomes more apparent (Fig. 99). Each is surrounded by an extremely fine membrane, which is continued on to the stalk as a delicate superficial layer hardly recog-

nizable. The dorsal end of the stalk projects a perceptible distance (Figs. 99 and 67) within the membrane into the cell, and seems to be resolved into a number of fine branches, which make their way in all directions through the cell, and give off still finer processes, which anastomose to form a network of finest fibrils. These are highly refractive, and, like the stalk, take up staining fluids slightly, so that the coarser branches assume a decided tint in well stained specimens.

Near the stalk in the lower portion of the cell lies a stainable body, the nucleus (Plate VI. Fig. 78, *nl. d.*) ; this is of such a peculiar character as to make its right to the name nucleus appear at first sight questionable. It is irregular in form, and often has a very indistinct contour (Plate V. Figs. 64, 65), since a nuclear membrane can be seen only in places. The larger branches of the network already described connect directly with the projecting angles of the nucleus, so that the latter often seems to be prolonged some distance out into the cell. In the ground substance of the nucleus, when lightly stained, one sees a network similar to that already described as existing in the cell plasma, and with which it seems to be connected. There are, besides the network, at least two distinct sorts of enclosures in the nucleus : first, comparatively regular bodies (Fig. 64, *nll.*), nearly spherical in shape and about 5 $\mu$ in diameter, which are uniformly and deeply stained, and which in every respect resemble nucleoli ; secondly, irregular bodies (Fig. 64, $x$), which are always surrounded by a lighter area of varying width, and which thus have the appearance of being shrunken. These do not stain either like the first-mentioned bodies or like the rest of the nucleus itself, but in depth of color are half-way between the two. What these enclosures may be I do not know, but I believe the larger mass itself to be the nucleus, despite its peculiarities, and I regard the dark round bodies enclosed within it as nucleoli. It may be urged in this connection, that the very irregular form of the nucleus makes it impossible to cut the surface perpendicularly for more than a short distance, and that an oblique cut would make the membrane very indistinct. This probably accounts for its apparent absence in places.

It is possible, I think, to furnish at least a partial explanation of these peculiarities. Evidently the cells contain a highly fluid plasma. This is shown by the small quantity of solid matter found in those that have been "fixed," and by their variation in size. This condition might indeed be expected on purely physical grounds, since the cells float entirely free in the fluid which fills the anterior chamber. If however cell and nucleus contain more fluid than ordinarily, the curious appear-

ance, especially of the latter, can be easily understood : the nucleus has been shrunk by dehydration in the course of preservation. This shrinkage was prevented in a measure at certain points where the strong threads of the network were connected with it. It is important in this connection to call attention to the fact, that in those cases where the cells were unusually small the nucleus was most nearly regular in shape (Fig. 66). No particular attention was paid to this point when studying the living animal, but in the sketches made at the time I find that the nucleus, which in general size and position agrees with this structure, was drawn with a regular oval outline (Fig. 2). This may well be its shape in life.

The stalks of these four cells present a uniform appearance. They, or at least their initial portions, stain more deeply than any other tissue both in hæmatoxylin and in carmine solutions, and hence are easily traced so far as the stained portion extends. Under a high power the stalk exhibits in places a faint longitudinal striation, and sometimes shows lines of minute vacuoles between the striations. The method of termination in the cell has already been described. From the cells the stalks pass directly into the brain, those of the first pair entering just lateral to the second large ganglion cell on either side, and those of the second pair just antero-lateral to the fifth pair of large ganglion cells in the brain (Plate V. Fig. 63, *pd. cl. d.*). The processes, which in transverse sections appear to be directed toward the median plane, show in longitudinal section (Plate VII. Fig. 95, *pd. cl. d.*) a backward tendency also. They may in this way be followed for a very few sections ; in the last, in which they are prominent, one sees a splitting or branching of the process in various directions, but beyond this the parts can be traced at most a couple of sections. The more compact character of the mass and the large number of other fibres make it difficult to say whether any part of the process extends farther, or whether the whole is split up at this point into fine fibrillæ. The place where the splitting begins is surrounded by a considerable number of small ganglionic cells (Plate VI. Figs. 78, 82).

Bürger ('91, p. 639) describes two pairs of large ganglion cells and two subordinate pairs in the brain. His description is not in all points clear, and to judge from appearances the figures do not correspond to his interpretation. Of the structures which he calls "giant cells," the anterior pair is my third or commissural pair ; his posterior pair corresponds to my fifth pair. Of his subordinate cells, the pair which lies close to the commissure corresponds perhaps to my second pair, and

those described as lying between the two pairs of giant cells are my fourth pair. His figures do not fully agree with this, however. His Figure 2, *Gz*, shows the "anterior giant cells," which are clearly my commissural cells; Figure 11, *Gz*, which he regards as one of the same pair, has more of the appearance and position of a cell of the fifth pair. The dorsal cells are described as salivary glands (*Spdz*, Figs. 1, 2, 11), and it is curious that he has nowhere represented the prominent processes (stalks) of these cells, unless, indeed, they are the structures labelled *Gz F* (Fig. 12). Although the position is somewhat peculiar, they certainly look more like the stalks of the dorsal cells than like processes of the cells marked *Gz* in the same figure, which is his interpretation, since the latter usually extend directly backward.

Bürger expresses a doubt that the posterior giant cells (i. e. my fifth pair) lie opposite each other. If not, it was because of some deformity or twisting of the head, as they evidently are opposite each other in my preparations (Figs. 63, 85). Instead of being nearer together than the other cells, as he maintains (p. 640), they are certainly farther apart than the components of any other pair (see my Figs. 63, 85).

γ. *Fibrous Mass.* — The central fibrous portion of the brain shows few definite points of structure. The fibres run in every direction; one finds few commissural bands, and in general no fixed arrangement. At two points, however, one notices (Fig. 99) vertical bands of fibres which divide the brain into three parts, a central and two lateral portions, which in position correspond to the three divisions of the ventral nerve cord, which will be described later. The dorsal commissure (Plate VI. Fig. 80, *coms. æ.*) is very meagre, being cut in only two or three sections. The number of fibres in it is consequently small, but there are at least four large nerve processes; two belong to the third or commissural pair of cells, the other two perhaps to the second pair. A few finer fibrils accompany these.

In the female the brain measures only 0.08 mm. in length, and 0.1 mm. in width. In consequence of its more compressed form, the cells stand closer together, and are more difficult to study. One finds exactly the same number of large ganglion cells, and they occupy corresponding locations. It may, then, be fairly assumed that the processes are distributed in the same manner, although I was unable to follow them as clearly as in the male.

In total preparations (Fig. 63) one often sees groups of fibres passing anteriad from the brain into the front wall of the anterior chamber. As has been shown from sections, however, this is all solid tissue in front of

the brain, and these groups of fibres are apparent merely by virtue of a different refractive power. They are somewhat irregularly arranged, and yet correspond very nearly on the two sides of the body. I regard them as groups of nerve fibres. They may be seen to turn dorsad (Plate VII. Fig. 92, *n. a.*) in the tissue of the wall, and probably innervate the numerous sensory cells found in this wall.

### *b. Ventral Nerve Cord.*

The ventral nerve cord extends directly posteriad from the brain through the entire body. It is located in the ventral line just above the epithelial layer, and appears in cross sections near the middle of its length as a roughly cordiform mass (Plate VIII. Fig. 101), which is separated by internal divisions into three areas. These areas represent the three nerves of which the cord is composed. The median area (Plate VIII. Fig. 101, *n. m.*) is triangular, with its apex directed ventrad, and is, so to speak, wedged in between the two oval lateral areas. Near the brain the form of the ventral nerve cord is somewhat different, and gives a hint as to its relation to the brain, which can be easily traced in any series of sections which includes the brain and the following portion of the body. The first trace of a partition in the fibrous mass is found well forward in the brain, and is shown in the arrangement of the small nervous nuclei, and of the dorsoventral groups of nerve fibres already mentioned; these indicate a division of the brain into a central mass quadrangular in cross section, and two lateral masses more or less rounded off on the outer side (Plate VI. Fig. 86). At the position of the fourth pair of large cells a row of small cells, already mentioned, makes this division more apparent, and even before reaching the ventral nerve cord one sees the separation of the three portions by fibrous bands which cross the brain vertically. At the beginning of the nerve cord the three portions are of about equal size; gradually the lateral areas push themselves in under the central portion until the latter has been compressed into a triangular shape, with the lateral areas almost touching in the median plane beneath it. This relation, with slight modifications, is preserved throughout the entire length of the animal, and I do not find, as Bürger (p. 641) has maintained, that the median portion is more prominent in the anal ganglion (Plate VIII. Fig. 96, *n. m.*). The central and lateral portions seem to be, so far as I can find, alike in structure. The number of faintly stained homogeneous processes in the three portions is nearly equal; in the posterior part of the body they are perhaps more numer-

ous in the lateral portions of the cord. Each part also contains nerve fibrillæ, and the relation of the ganglion cells to each appears to be the same.

There are, moreover, ganglion cells in the ventral nerve cord; they may be conveniently treated of in two groups, which correspond in general to those of the brain. The first are simple nervous nuclei, distinguished from the nuclei of the surrounding connective tissue especially by the intensity with which they take up stains. They are small oval nuclei, measuring 4–5 $\mu$ by 6–8 $\mu$ in diameter, and possessing a prominent nuclear membrane, but not provided with any appreciable amount of surrounding protoplasm. They are found along the dividing lines between the areas of the cord (Plate VIII. Fig. 101) and also on the external boundary of the latter, usually closely crowded together; in cross sections they appear as a single or double row; in longitudinal sections they are collected into a certain area (Fig. 97). They are about equally distributed throughout the length of the cord, and produce the dark dotted rows seen on the ventral line in the living animal (Fig. 7).

The large cells of the ventral cord form the second class, and in many cases are equal in size to those of the brain. Though not plentiful, they are scattered along the whole length of the cord. I was unable to find, however, any regularity of distribution, since the interspaces vary considerably in extent. Furthermore, they are not plainly paired except in rare cases. Usually the successive cells are separated from one another by a distance equal to the thickness of ten, or even twenty, cross sections (100 to 200 $\mu$). Bürger (p. 641) has described these cells under the name of median cells. I do not think that they begin, as he maintains, in the brain; but I agree with him in regarding them as unpaired. These big cells are ordinarily found wedged in between the two lateral areas and immediately below the ventral portion of the cord (Plate VIII. Fig. 101). So far as I have seen, these cells possess each but a single process, which passes dorsad between the median and one of the lateral areas, but its ultimate fate I was unable to determine. Rarely one finds a large cell below the lateral area on one side or the other. In this case the nucleus is much flattened dorsoventrally. Bürger regards these cells as bipolar. I have seen appearances such as he represents in his Figure 13, but do not regard this as decisive, since the two processes are not shown, so that, while I have no positive contradictory evidence, I am also unable to confirm his statement.

The form of the ventral nerve cord may be much altered by collapse of the body, which flattens the cord between the two lateral muscular

areas. By this process the elements of the ventral line, as well as those of the cord itself, are so changed as to give rise to abnormal appearances even in otherwise well preserved specimens. Such a crushed condition of the cord is figured by Bürger (Fig. 19).

Strictly speaking there are no nerves arising from the cord. The fibres which branch from it are nowhere collected into a group worthy the name of nerve. In every second or third section one finds a few delicate fibrils arising from the nerve cord; some emerge from the dorsal surface and some from the median ventral cleft, and in both cases they pass off towards the lateral hypodermis (Fig. 101). They may be traced as far as the beginning of the hypodermis proper, but their ultimate fate is unknown. If these are not nerve fibres, I am at a loss to explain them, or to find other branches which may be nervous. Only once did I find any evidence of a large process leaving the cord; in one cross section a process like those found cut transversely in the cord was cut longitudinally; it passed out from above the lateral area and followed the course of the fibres already described, as far as the hypodermis.

### *c. Anal Ganglion.*

Bürger has shown (p. 638) that the anal ganglion far exceeds the brain in size, and is in no sense a small local thickening of the ventral nerve cord, but that it is a gradual differentiation of its posterior portion. In a total view (Plate VI. Fig. 89) one is unable to see any definite line of demarcation between the ventral nerve cord and the ganglionic enlargement which terminates it. There is seen to be rather a gradual increase in the size of the cord extending over a distance of about 1.2 mm., and culminating at the posterior end, where the ganglion is abruptly rounded off. In some specimens the differentiated portion of the cord is deeply cut by cross furrows which give it a metameric appearance. Although these may be present at times when no external folding of the cuticula can be found (Bürger, '91, p. 638), they certainly do not indicate any metameric condition of this organ, since they vary in size and since there is no corresponding structure in them. The furrows are entirely wanting in most well preserved specimens (Fig. 9), and when present are simply due to a folding of the cord, such as occurs in other portions of its length as well, but is more prominent here on account of the thickness of the organ.

A cross section through the anal ganglion (Plate VIII. Fig. 96) shows at once that the increase in size is due chiefly to the addition of a peripheral layer of cells above the cord proper. On account of this increase

in size it is no longer possible for the ventral line with the entire nervous mass to be retained in its usual position. It is therefore forced upward into the body cavity, and fills a considerable portion of it (Fig. 9). In fact, near the end its diameter is half as large as that of the body at that point.

Seen in cross section (Fig. 96) the nerve cord itself presents little here that is different from its general character throughout the body. It is the peripheral layer which demands particular attention. This is crescent-shaped, and composed of a dense matrix in which are embedded many nuclei. Along its periphery the matrix is finely striated perpendicularly to the surface, and is separated from the underlying nerve cord by a narrow space filled with loose fibrous tissue (Fig. 97). The horns of the crescent are turned ventrad around the nerve cord, and are connected with the tissue lying below it. In these horns one sees a fine longitudinal striation; occasionally more plainly marked fibres, coming from the cells above, may be traced into them. The matrix, which usually takes a faint stain, contains numerous oval nuclei (Plate V. Fig. 71) with a sharply defined nuclear membrane. The nucleus in general does not take up the stain, so that the one or two large nucleoli stand out in strong contrast to the rest. One can neither find any cell boundaries in the matrix, nor determine how much, if any, of the surrounding protoplasm belongs to each nucleus. In fact, the fibres which one finds often appear to extend from the nucleus itself around into the horn of the crescent.

In addition to this thick peripheral layer, which I regard as nervous, there are in the anal ganglion a few large cells. Some are wedged in below the middle portion of the cord like those which have already been described. Others may be found in the space between the peripheral layer and the cord (Fig. 96), with the process directed ventrad. These cells do not as a rule appear to be paired.

At the extreme posterior end of the cord one finds somewhat different conditions. Here there is a mass of large ganglionic cells of varied size, closely crowded together, and with their processes (Plate VII. Fig. 90) mostly directed forward into the ventral nerve cord. The space between the cord and the peripheral layer does not exist, and, curiously, the ganglion cells of the latter have nearly always two nucleoli instead of one as is usual elsewhere. The relation of the cells and processes is very complicated here, and the gradations of size are so fine that with the material at my command I was unable to determine the exact number of cells, or the plan on which they are arranged. The nuclei of these

cells recall those of the brain. One finds (Plate V. Fig. 70) the same distribution of chromatic substance, but with more numerous nucleoli, and the same sort of enclosed bodies. One edge of the nucleus shown in the figure is very indistinct, because it was cut obliquely. It recalls the appearance presented by the nucleus of the dorsal cells. The same is true of the nuclei of the large ganglion cells in the ventral nerve cord seen in longitudinal section.

The female does not show any such extreme modification of the posterior end of the ventral nerve cord as was found in the anal ganglion of the male. The only specimen favorable for the study of these relations shows (Plate IV. Fig. 57) a slight swelling in the ventral nerve cord just at its end, which lies below the terminal bulb. There appear to be a very few large ganglion cells at this point, and yet it is an unimportant modification as compared with that of the male. The peripheral layer of ganglion cells, so characteristic of the anal ganglion in the male, seems to be entirely lacking in the female.

## 6. Sexual Organs.

### *a. Male.*

In all males one finds a sac suspended from the dorsal line and filling a larger or smaller portion of the body cavity. It shows the character of its walls best when almost empty. Then one sees a fine outer boundary (Plate IV. Fig. 54), with occasional elongated deeply stained nuclei. From this fibres radiate through the cavity of the sac to form a delicate large-meshed network, or the sac may in places be entirely empty. These details are all hidden when the organ is filled; even the walls cannot be demonstrated, although their presence may be inferred from the regular outline of the mass. In this case the sac is enlarged so as to fill a considerable portion of the body cavity. This is true of the anterior or middle region of the body; farther posteriad the sack seems to become crescentic in cross section, the two horns of the crescent being fastened to the lateral body walls. When the sac here is filled, it occupies the entire space dorsal to the anal ganglion (Plate VII. Fig. 90).

In most cases the sac is filled with minute oval bodies of uniform size, only 1 $\mu$ in diameter. No other structures are constantly present, so that their abundance, minuteness, and uniformity in size and appearance render it practically certain that they are spermatozoa, and that the sac is the male generative organ.

In some specimens cross sections through the middle or anterior region of the body show that the sac is moderately filled with cells whose nuclei stand close together and are in a kinetic condition. Here the ventral boundary of the dorsal line seems to be wanting in places, as if the cells in the sac were directly connected with those of the dorsal line, while the wall of the sac is laterally directly continuous with the basement membrane, which covers the line ventrally. One can sometimes find among the cells in this region stages which seem to show a transition between the kinetic nuclei and the groups of spermatozoa found among them; but this condition was encountered in only a single specimen, and the material was not in sufficiently perfect histological condition to allow a study of spermatogenesis. This anterior portion of the sac I regard therefore as testis, and the posterior crescent-shaped portion as at once receptaculum and vas deferens. In the stage in which the kinetic nuclei were found in the anterior portion of the sac, the walls of the posterior portion were collapsed, and hardly a single spermatic element was to be found in it. This is the youngest stage which I have studied.

In another, older stage the sac was filled from end to end with the deeply stained highly refractive spermatozoa, and so enlarged that it occupied nearly the entire body cavity. Finally, in the oldest stage found the sac (Fig. 11) appears in the anterior part of the body as a mere remnant with collapsed walls containing an occasional spermatozoön. At the tail, however, a small quantity of spermatozoa was collected near the terminal orifice. The diminished thickness of the protoplasmic zone in the body wall shows this individual to have been comparatively old. In one specimen in which the sac was thus collapsed, however, the body wall was moderately thick. In these cases copulation seems to have taken place, and the few spermatozoa are merely remnants of the original contents of the sac.

The organ described by Bürger on pages 646, 647, is evidently the same as this, and his belief that it was a testis rather than an ovary is confirmed by the preceding account. The description he gives of the organ either shows that the specimen studied by him was intermediate between the first and second stages here described, or else was based upon different individuals and represents different stages.

In one of my individuals which, to judge from the thickness of the body wall, must have been young, there were clusters of polyhedral cells here and there in the anterior portion of the body cavity, and these clusters were crowded full of spermatozoa in small bunches, as if they

had originated there. The dorsal sac was moderately large, and contained numerous spermatozoa, which were, however, scattered, and not in groups. I do not know how to explain this case, unless indeed it be due to a rupture of the dorsal sac in places, and the consequent evacuation into the body cavity of a part of its contents. Although I did not find any point at which this could be shown to be unmistakably true, yet there were many places where the wall could not be distinguished; furthermore the body was in this case much distorted in killing. Even when the outlines of the sac are plainest, one always finds spermatozoa in the body cavity in greater or less numbers; so, for example, in the cavity of the terminal organ (Plate IV. Fig. 53). This, so far as I know, is the only fact which favors the view that their place of origin is in the body cavity; aside from this, the evidence points to the dorsal sac as testis. A further study of additional material is necessary to determine finally this point, as well as many others.

The external sexual organ of the male consists of the terminal papilla to which reference has often been made. It has much the shape of a slightly curved truncated cone (Fig. 53) with an opening at the smaller base, and with the larger base joined to the body obliquely, so that it naturally turns ventrad. The length and curvature of the organ vary a little, as can be seen from the different figures (Figs. 4, 9, 53, 89, 90). The essential features of its structure can be made out from a total preparation in clove oil (Fig. 53). The muscular layer of the body wall, which for some distance has been growing thinner, stops suddenly along a well defined line. Beyond this only the hypodermis lies between the cuticula and the body cavity. The cuticula, which is here a little thicker than usual (see also Plate VII. Fig. 90) is infolded at the end of the organ and runs forward as the lining of the cavity for a variable distance. I was at first inclined to believe that this infolded portion could be to a limited extent extruded and then drawn in; but further study seems to show that it cannot. The thick cuticula is too stiff to be rolled in or out without being folded somewhere, yet on sections it is always smooth; moreover, there is no muscular provision for moving the organ in this way. At its anterior end the cuticular infolding is continous with a sac (*va. df.*) having delicate walls, and this is in turn connected with the dorsal sac previously described (Fig. 53 and Plate VI. Fig. 89, *va. df.*). Although I plainly saw and drew in several cases the walls of this connecting portion from clove oil preparations, yet they are so delicate that in sections they were not once preserved except as loose shreds of tissue. I was consequently unable to ascertain whether there was

anywhere the trace of a connection with the intestine. On general grounds one would be inclined to believe that the single terminal opening was that of a cloaca; but no evidence of any connection between the end of the intestine and this thin-walled portion communicating with the dorsal sac was obtained. It must then remain for future investigations to decide whether this is morphologically a cloaca or merely a vas deferens.

The hypodermis in the terminal papilla is composed of a single layer of approximately cubical cells (Plate VII. Fig. 90). This is the only portion of the body wall in which the boundaries of the hypodermal cells can be seen.

The spermatozoa are usually found in such enormous numbers that it is difficult to make out their true shape. They appear much like micrococci, and when seen alone (Plate IV. Fig. 55) are slightly oval, highly refractive bodies 1 $\mu$ in diameter. The merest indication of a protoplasmic envelope surrounding them is found in the shape of a very narrow light peripheral zone. They stain very deeply, and their minute size renders it impossible to recognize any structure in them. It is probable that, as in other Nematodes, the spermatozoa undergo some metamorphosis after being introduced into the body of the female. From one individual spermatic masses were voided into the sea-water in which it was kept. There was no sign of motion in the mass when flattened under a cover glass, and when dried on a cover glass and stained nothing besides the oval spermatozoa could be seen, except a certain amount of coagulated fluid.

There may be found in the dorsal sac as well as in the body cavity of certain male specimens peculiar pale bodies, not easily stainable and varying greatly in form. They are probably the same as those which Bürger (p. 647) speaks of as "ovale Gebilde von mattem Glanze, an denen nicht zu errathen war, ob sie gleichfalls Kerne oder Einlagerungen bedeuten." Macerations show that they are probably parasitic Gregarinida, the various appearances obtained from sections being due to their having been cut in different planes (Plate VII. Fig. 91).

### *b. Female.*

I do not believe that any of the previous observers have had a female. Verrill ('79) described the posterior end in the "female" as "subtruncate with a small terminal papilla." This applies exactly to some males, and, as Figure 56 (Plate IV.) shows, is very unlike the

female.[1] Furthermore, in the entire collection which Professor Verrill kindly placed at my disposal there was only one female, that one being found coiled up in a mass of twenty specimens.

Bürger (p. 647) describes one form which differs materially in structure from all others studied by him; he was inclined to regard it as a female. He found a sac with flattened walls hanging from the dorsal line. The description and figures given by him resemble strongly an immature testis, — certainly it cannot be an *empty* ovary. But the termination which he describes for it is so extraordinary that one must doubt the normal nature of the specimen or the accuracy of the observations. Certainly neither in male nor female does one find anything like the tube and cells which he describes as lying on the ventral cord, except the œsophagus. It is impossible, however, that he has mistaken the anterior for the posterior end of the worm, because he mentions the head of this specimen. There are certain points in his description of this individual, especially the lack of an anal ganglion, which recall the female, yet in view of the many problematic points which cannot be referred to either sex, I am of the opinion that this must have been a very abnormal specimen. I shall give a description of the sexual organs of the female without any further reference to his work, describing only those conditions which I believe to be normal.

The three females obtained present three stages in the growth of the egg, but unfortunately all are too far advanced to give any clue as to the place or method of origin of the egg cells. In the first stage the body cavity is already half filled with well developed eggs, and no trace of ovaries or of the walls confining the ova is present, but the ova seem to lie free in the body cavity. Each egg (Plate IV. Fig. 60) has a firm outer membrane, highly granular protoplasmic contents, and a large irregular nucleus, which has a very thin nuclear membrane and is strikingly poor in chromatic substance. Between and around the eggs one finds a granular substance, and more rarely small nuclei.

In the next older stage the body cavity is more nearly filled, and the eggs are very similar except that the nucleus is smaller and more deeply stained. One finds also around each egg an external covering of minute quadratic blocks, which seem to be easily separable from the egg and from one another.

The oldest stage observed differs from that just described in some

[1] I should not neglect to mention that a female with protruding egg mass (see Fig. 10) would correspond generally to this description; but such a state would hardly be available for identification.

small particulars. The nucleus (Fig. 61) is now so small as to be found with difficulty among the large opaque yolk granules, the external membrane is firmer, and the blocks with which it is covered are more prominent. In this stage the eggs fill the entire body cavity (Fig. 58), being roughly arranged in nearly concentric layers. It was from this individual that eggs were discharged into the water and later preserved. At first sight (Fig. 62) such discharged eggs appear very different from those previously described, being armed with a thick covering of long conical spines. An examination of the posterior end of this animal, which was killed while the eggs were being discharged, showed that the eggs which were still in the body and those in the mass outside possessed not a trace of these spines, but simply the blocks on the external membrane, as already described. Further investigation showed that *not all* the eggs which had been laid were already provided with long spines. In some cases the spines were very short and thick; indeed, all stages were found from this condition up to the one first described. The probable explanation of this phenomenon is, that the block-like thickenings on the membrane of the immature egg are swollen by the sea-water, first into shorter, then into longer spines, which at the beginning are probably soft and become rigid later. Certainly in alcoholic specimens they are rigid.

In almost every transverse section one finds a delicate membrane stretching from the ventral line to the egg mass (Fig. 58). This may represent a mesentery, as it is too uniform to be merely accidental. In only one case (Fig. 57) was there anything present in the body of the female which had the appearance of spermatic elements, but the poor histological condition of this specimen prevented an accurate determination of the matter.

The body of the female ends (Fig. 10, and Plate IV. Figs. 56, 57), as already mentioned, in a slight bulbous enlargement with a central terminal opening. The cuticula turns inward for a short distance, as in the male, but in the specimens at my command there were no internal organs connecting with this opening. The mass of eggs filled the body cavity up to the tissue of the terminal bulb. The same question recurs here which suggested itself in the case of the male, as to the morphological value of this opening, — whether it is or is not a cloacal orifice; but I have no evidence to present on either side of the question. The bulb is made up of elongated cells containing pale nuclei, and passing off at right angles to the infolded cuticula. These cells have much the appearance of unstriped muscle cells, and seem to be able to affect the

caliber of the opening (Figs. 10, 56), and thus to facilitate the passage of the egg mass. The hypodermis can hardly be followed around the bend under the infolded cuticula. If it exists there, it is certainly a very much attenuated layer. I do not think that the elongated cells of the bulb can be regarded as modified hypodermal cells.

## VI. Discussion.

### 1. Dorsal Cells.

The nature of the dorsal cells is not definitely determined, yet I have little doubt as to their nervous character. The interpretation of them as gland cells (Bürger, '91) seems to me untenable for many reasons. The cells do not have at all the appearance of gland cells, there is no trace of any secretion in the cell or its process, nor anything in this process which suggests even remotely a duct, and finally one finds no connection of the stalk with the intestine or with the exterior. Against the possibility that they may be *degenerate* gland cells, functional in larval life, it may be urged that there is absolutely no evidence of degeneration in the appearance of the cells.

If the positive evidence on the other side be examined, it will be found to be almost equally strong, and in favor of their nervous character. In the first place, their nuclei are like those of the large ganglion cells in affinity for stains, in the possession of one or two large homogeneous nucleoli, and in the curious unstained enclosures already described. On the other hand, it cannot be denied that the nuclear membrane is more irregular, and seems to be connected with the fibres of the cell substance, a condition which was not seen in the large ganglion cells.

The stalks of these cells certainly resemble nervous processes optically, as well as in their relation to the cell body, and in their termination, which has been considered in detail. Unless one regards the stalk as differentiated into a stainable and a non-stainable portion on account of some unknown difference in the chemical nature of the parts, it must be granted, I think, from the evidence previously produced, that the process branches at or near its termination in the brain. This branching seems to me to be an insuperable objection to the interpretation of these cells as glandular, and indeed to render it almost certain that they are nervous. Their enormous size and extremely fluid contents may be due to the freedom for growth which they enjoy in an unrestricted space, and, in part at least, to osmotic conditions.

Since in the ripe individual the mass of sexual products whch fills the body cavity would exert a dangerous or even fatal pressure on cells so delicate as these, it is evident that the partition is absolutely necessary for their development, and may be so for the protection of the brain. While the presence of the partition is essential to the existence of these cells as they are, it is impossible from the evidence at hand to form any idea of the cause which led to its development. However, the partition being formed, I believe it is possible to understand how the large cells may have attained their size and position.

It has already been mentioned that the dorsal cells vary considerably in size; an early sketch of a living animal, made before the structure was well understood, shows in a dorsal view, not two, but three successive pairs of large cells. There were, however, in this case, actually only two pairs of dorsal cells, and the supposed third pair was the most posterior pair of large ganglion cells in the brain. It has already been said of these that they are located, not *in* the mass of the brain, but in great part *above* it. Now, given two pairs of lateral cells located on the upper surface of the brain anterior to the fifth pair of ganglion cells near the place where the processes of the dorsal cells enter the brain mass, it is easy to conceive how they may have become larger and larger, and finally may have risen entirely above the brain into the free space dorsal to the œsophagus, where no obstacle is offered to their further increase in size. At the same time, the stalk would be produced as a mere mechanical result of the lengthening of the cell process to accompany this migration. Once free in this cavity there is every reason to believe that the cell might continue to develop in size until, with its companions, it should occupy the entire space, which is approximately the present condition of affairs. The fifth pair of large ganglion cells in the brain, half projecting, as they do, above its surface, would then represent the first stage in the migration which the dorsal cells have already accomplished. Attention must be called to the fact that the three pairs of cells which have been compared in this hypothetical statement of the case are not similar in one important particular; for whereas the processes of the fifth pair of ganglion cells are unbranched and may be traced far posteriad through the ventral nerve cord, the processes of the dorsal cells are branched and can be traced only a comparatively short distance. I do not see, however, that this difference greatly affects my explanation, — which is purely mechanical, — since the cell and not the process is directly concerned. The nuclei of both kinds of cells are nearly equal in size and alike in structure. The great difference be-

tween the two is due to the preponderance of the cell body in the dorsal cells, which I have assumed to increase by virtue of its changed surroundings. A similar difference in the size of the cell body is found between the fifth pair of ganglion cells, half projecting above the surface of the brain, and the first pair, which is deeply embedded in its substance.

One further point of interest suggests itself. It has already been mentioned that the commissural cells vary in position, being in many cases higher on the commissure than in others. If there be a tendency toward a more dorsal position in this case as well, — which on the basis of free space for development is more probable than the opposite movement, — then there are two distinct ways in which originally ventral elements may reach a dorsal position; first by the independent dorsal migration of superficially located ganglion cells, and secondly by a movement of commissural cells dorsad along the commissure. The first method is illustrated in the case of the dorsal cells, the second in the case of the commissural cells (the third pair). In both instances the cause of the migration may well be gain in nourishment and vigor as such cells advance more and more into the free space above the brain mass. If such a change in position involve a gain in vigor on the part of the cells concerned, then the origin of a dorsal ganglion from a simple commissure by the dorsal migration of elements either independently or along the commissure may be easily conceived, since around the ganglion cells which have acquired this position other nervous elements will collect with the increase in the amount of nervous matter accompanying the development of the nervous system. In this way, then, lateral and dorsal ganglia may arise.

If the brain of Nectonema shows distantly how the higher development may be reached, it shows still more clearly its immediate relation to the ventral nerve cord. The anterior ganglionic mass may be viewed as a differentiation of the anterior portion of the cord. The agreement between the portions of the brain and the three bundles of the cord has already been emphasized. It remains to call attention to the correspondence in the location of ganglion cells. As it was true of the cord that ganglion cells were found on the borders of the three fibrous tracts, so it is correct to say of the brain that the ganglion cells are developed on the edges of the corresponding tracts. This will be easily seen if, in a comparison of Figures 72–88 (Plate VI), one proceeds from behind forward; and it is still more evident when the vertical bands of fibres are taken into account; the fibres are, however, not represented in these figures.

### 2. Large Ganglion Cells.

The large ganglionic cells of the brain and ventral nerve cord were called by Bürger "giant cells." So far as mere size is concerned, the name is well chosen, but it has been pre-empted for the neurochord cells of higher groups. To avoid the confusion which has entered into other divisions of morphology owing to the use of a single name for a multiplicity of organs, this designation should not be employed here unless there is some reason for regarding them as homologous with the cells of Annelids and Crustacea which first received the name. In comparing these large cells with the neurochord cells of Annelids, the first point of difference to be noted is the number of the former. There are, as we have seen, at least five pairs of such cells in the brain and others along the ventral nerve cord. In the Nemertines, as in the Annelids, there is only a single pair of neurochord cells in the brain; and those in the ventral nerve cord are distributed in pairs and at regular intervals, which does not seem to be the case in Nectonema.

The second prominent point of dissimilarity has to do with the processes. The fibres of these large cells vary somewhat in size, and do not possess any very definite shape, being now nearly round, now angular, with a variety of form which may, however, be in part due to the effect of reagents. In optical appearance and in reaction toward staining fluids they recall strongly the neurochords or giant fibres of higher groups. They also extend for long distances, perhaps the entire length of the worm, in an unbranched condition. But they differ from the giant fibres in one striking respect, — they have no sheath; in fact, it is very difficult to say that they are even enclosed by a delicate membrane, so fine is the boundary between them and the surrounding tissue. On the other hand, the sheath of the giant fibres is the most striking peculiarity which they possess, and often exceeds in prominence the fibre itself.

These seem to me sufficient reasons for regarding the cells in question as not homologous with the giant cells of other groups. I have therefore avoided using the expression "giant cells" to designate them, in order not to suggest a false homology. It cannot be denied that these may represent the primitive form of the giant cells, in which the fibres have not yet acquired the highly differentiated sheath; but until this becomes more probable by reason of evidence as yet lacking, it is better to use the non-committal term, and to designate them as large ganglion cells.

### 3. Rows of Hairs.

The lateral rows of hairs are evidently developed in connection with the free life of Nectonema. Moreover, they are not structures without a parallel among the Nematodes. Many forms have been described with hairs distributed irregularly or regularly — sometimes in rows (Trichoderma) — over the surface of the body. Unfortunately, in such cases little or no idea has been given of the size and structure of the "hairs" by the authors who have mentioned them. In one form at least, the peculiar free-living marine genus Chætosoma (Giard et Barrois, '75), there is found a double row of hairs along a portion of the ventral line. The setæ are hollow and entirely superficial, thus agreeing in several points with those of Nectonema; they are not, however, so extended in their distribution as in the latter form.

### 4. Muscular Layer.

The complete degeneration of the posterior portion of the alimentary canal in the adult, as well as its minute size in comparison with the body of the worm, makes it at once evident that this organ cannot be functional in the adult. The question then suggests itself as to the source of nourishment during this period of life. As has been already noted, the protoplasmic zone of the muscular layer is thicker in the immature individual, and diminishes in thickness with the attainment of sexual maturity. This decrease in volume may take place in two ways, — by the formation of corpuscles directly from the cells of the layer, and by the giving up of food matter to neighboring cells or to the cœlomic fluid and thus to all tissues of the body.

As has been shown, the corpuscles of the body cavity probably originate from the cells of this layer by a process of abstriction. This process is never very extensive, so far as I have been able to judge, and hence will hardly serve to explain entirely the decrease in the volume of the layer. One is, therefore, compelled to accept the second method suggested, that of the indirect transmission of food matter either through neighboring cells to remote tissues, or by means of the fluid in the body cavity. The unusually large size of the protoplasmic portion of the muscle cells, and its granular condition, are well explained on the supposition that these cells have secondarily acquired the function of storing up nourishment for the support of the body during the period of adult life.

### 5. Parasitic Nature.

Nectonema possesses neither eye spots nor sense organs, such as are present in practically all cases of free-living, and especially of pelagic forms. The general structure of the alimentary tract, its diminutive size as compared with that of the animal, its occasional closure anteriorly, the complete degeneration of its posterior portion, and the absence of any functional anus, speak even more strongly against the possibility of regarding Nectonema as primarily a free form, and practically force one to the conclusion that it is a parasite, which passes its larval life in some unknown animal, wandering out of its host at sexual maturity and passing the final stage of its life history in a free condition, in which alone it is at present known. On the analogy of Gordius, the host may be surmised to be some fish or crustacean, and, since Nectonema is not so rare as has been supposed, it ought not to be difficult in its proper home to discover its host.

There are certain facts which should be mentioned in this connection. As has already been said, Nectonema was caught only on an ebbing tide and in the bay near shore, not in open water. And although a large amount of truly pelagic material was obtained in the same manner, yet numerous Annelids which are by no means truly pelagic were found in the same towing. The latter form part of the bay or shore fauna which in towing near the land is habitually found in the net. In the same way, Nectonema, which is probably set free from some one or more of the small fish or Crustacea which inhabit the shores of the bays or shallow water in general, will live normally in the little coves and quiet places along shore, but may be carried out by the tidal currents even to some distance. It is probably found at or near the surface at night only, and at the bottom during the day. The greater prominence of the contractile portion of the muscular layer in the male would seem to indicate that it is the more active of the two, and to this may be due in part the much larger number of males captured.

## VII. Comparison with other Forms.

Numerous possible relationships have been suggested for Nectonema, many of which rest upon resemblances of a superficial character, such as the comparison with Sagitta on the ground that both possess lateral fins. Bürger ('91, p. 650) has sufficiently shown the fallacy of any comparison with Eubostrichus, which resembles Nectonema at most in

possessing external hairs! It is an interesting and at once a significant fact that Chætosoma possesses a double row of hollow hairs or bristles on a portion of the ventral line. These hairs strongly resemble those of Nectonema, but it is apparent at once from a comparison of internal organs that the resemblance is purely superficial, since Chætosoma is as like the Nematodes *s. str.* as Nectonema is different from them; this is simply an interesting case of the development of like structures in widely different forms, which may be traced perhaps to similarity in their conditions of life.

In much the same way the resemblance to the Trichotrachelidæ emphasized by Bürger ('91, p. 649) is at most an instance of the convergence of parasitic types. The resemblance is indeed close in the muscular and digestive systems. The latter is, however, the system most immediately and directly affected by parasitism, and such resemblances may easily have arisen independently in any number of animals. The peculiar structure of the œsophagus is shared by the Mermithidæ as well; and so far as the muscles are concerned this type is common to an entire group of Nematodes, the Cœlomyaria. On the other hand, the reproductive and nervous systems of Nectonema and the Mermithidæ represent opposite extremes in the class Nematoda.

There is one comparison, however, which deserves more detailed consideration. Verrill ('73, p. 632) said of Nectonema, "In general appearance when living and moving, it resembles Gordius"; and again ('79, p. 187) he calls attention to the external similarity of the living animals. Bürger ('91, p. 649) enumerates the points of agreement between the two as the absence of lateral lines and the position of the nervous system in the ventral line, and emphasizes the difference in the digestive system and in the structure of the muscles. This is not a sufficiently broad and accurate comparison, and it will be valuable to enumerate here more exactly the points of agreement and difference for the various systems of organs in order.

The cuticula differs both in thickness and in the possession of rows of bristles and scales in the one form, and of scattered papillæ and sensory bristles in the other. The subcuticula has in both the characteristic Nematode nature. The muscular elements show at first sight a considerable difference in structure, yet I am convinced that this is more apparent than real. The muscle cells of Nectonema are those of the typical Cœlomyarian, in which the muscle fibrillæ are arranged in a peripheral ∩-shaped layer about the distal edge of the muscle cell. Into the hollow of this contractile portion extends a projection from the plasmatic portion of the cell which is found at the inner border of the

contractile portion. If now we conceive this plasmatic portion to be reduced to a minimum, the form of muscle cell characteristic of Gordius will be reached; for in this genus the projecting protoplasmic portion is entirely lacking, the layer of contractile fibrillæ surrounds the entire cell, and the nucleus is found in the thin strip of plasma which occupies the centre. Not only do we find in a typical Cœlomyarian cells in which the plasmatic cell body hardly projects beyond the contractile layer, but I have also been able to find in cross sections of Gordius *sp. ?* certain regions where the fibrillar layer in the proximal portion of the cell differs in thickness and in refractive power from that in the distal portion. I do not believe, therefore, that the difference in the muscular systems is so great as has been maintained.

To consider the second objection urged by Bürger against the relationship of Gordius and Nectonema, namely, the structure of the intestine, it will be necessary to make a short digression to consider the structure of the alimentary canal, and especially of the œsophagus, in Nematodes. Most text-books recognize only one type of œsophagus in this group, a muscular organ with a more or less triangular lumen lined with chitin, from which muscle fibres radiate perpendicularly to the long axis of the tube. This organ evidently acts like a suction pump in taking up nourishment.

If, however, one examines the literature on the group, it is evident that there are a number of families to which this description will not apply, and that there is really a second well marked type of œsophagus. This consists of a minute chitinous tube extending through a cell, or row of cells, with which no muscle fibres are connected. Evidently there is here no means of varying the size of the lumen. I believe the œsophagus in every family of Nematodes may be reduced to the one type or the other. The larger number of forms show the first, but in the Trichotrachelidæ and Mermithidæ the œsophagus is constructed on the second type, as is also the case in Nectonema. In Gordius this organ is found to be highly degenerate, and in certain species, or in specimens of a certain age, has entirely disappeared. Its condition appears to be different according to the descriptions given; but in a specimen collected in Cambridge there is absolutely no trace of an œsophagus in a perfect series of transverse sections. From the account of Vejdovský ('86, p. 404) it is at once evident that the œsophagus does not belong to the first type, and according to his description[1] and figures ('86, p. 404, Taf. XV. Fig. 35) it bears a

[1] Vejdovský says (p. 404): "Als Mundhöhle bezeichne ich das enge Kanälchen," etc. It is this portion of the alimentary canal which I regard as the morphological equivalent of the œsophagus of the second type.

strong resemblance to the second type. At any rate, I am unable to see the striking difference in this region on which Bürger lays great stress. As for the intestine proper, it is not of great importance whether the lumen be bounded by four or eight cells. There are evidently differences in the alimentary canal of the two forms; one of the most striking is the degeneration of the anterior portion in Gordius, and of the posterior part in Nectonema. This is, however, of minor importance on the question of general relationship.

At first sight nothing could appear more unlike than the reproductive systems in the two forms, and so far as external sexual organs are concerned there does exist a great difference. The papilla and terminal opening of the male Nectonema do not resemble in the least the forked tail and subterminal opening of Gordius. The female organs bear an external resemblance, but internally there is nothing in Nectonema parallel to the complicated structure of the system in Gordius. Too little is known of ovaries or testes in Nectonema to permit of a comparison, but the apparent absence of mesenteries and the probability that the organs are not paired in this case are certainly important differences. On the other hand, certain striking points of similarity must be noticed. The position of the sexual organs dorsal to the intestine is a peculiarity in Nectonoma which is shared only by Gordius among all the Nematodes at least. The same may be said of the fact that both male and female sexual organs possess terminal or subterminal openings. If my conjecture be correct that in Nectonema this is a cloacal opening, then this feature is also common to both. Moreover, of all Nematodes these two families are the only ones in which spicula are entirely wanting.

The body cavity in the two forms differs in that a lining epithelium is present in Gordius, but probably absent in Nectonema, except in the anterior chamber. The body cavity of both increases in size, however, by the cell masses which bound it taking part in the formation of sexual products or the nourishment of the body; but it is doubtful if this process goes so far in Nectonema as in Gordius, where it leaves only a thin row of cells, the peritoneal epithelium. This matter is, however, hardly cleared up for Gordius, even after the numerous investigations that have been made, and it cannot be regarded as more than formulated for Nectonema by the present study.

The lateral lines, as well as the contained excretory canals which are so characteristic of all other Nematodes, are wanting in both Nectonema and Gordius. In Gordius, moreover, no probable excretory system has

been shown to exist, and I have looked in vain for evidence of one in Nectonema. The only indication of a dorsal line in Gordius is the median dorsal interruption of the longitudinal muscles in the posterior portion of the body; in Nectonema, on the contrary, this organ is well developed. In both forms the ventral line is prominent, and in both it contains the ventral nerve cord.

Of the nervous system it may be said that the brain is more highly developed in Nectonema, the dorsal cells as well as the correlated anterior chamber being structures entirely without parallel in Gordius. But the ventral nerve cord in the two genera shows a similarity not only in position, but to a certain degree in structure, being made up in general of three portions, a median and two lateral. (Cf. Vejdovský, '86, Taf. XVI. Figs. 51, 63, *et al.*) Like many other Nematodes, both forms possess an anal ganglion.

Numerous lesser points of likeness may be mere coincidences. Such are the great numerical superiority of males over females, — which, among all Nematodes, is found only in these two groups, — the parasitic nature, and the mode of motion. The spermatozoa which I have described recall the unripe sperm of Gordius; yet such evidence is interesting rather than weighty.

In summing up this detailed consideration it may, I believe, fairly be said, that the points of difference between Gordius and Nectonema are more numerous than those of likeness, but that the latter are more general and important. This agreement in general characters is so striking that I cannot believe it is due to anything else than affinity. It will be noticed that the characters which separate the Gordiidæ from the other Nematodes are shared with Nectonema; thus the absence of lateral lines, the existence of *one* principal nerve cord (ventral), the dorsal position of the sexual organs, and the terminal openings of the same. Again, the points of difference between the two groups are largely those which separate the various families of Nematodes *s. str.* from one another; namely, the structure of the muscles and alimentary canal (?), and the character of the ducts and external sexual organs.

We do not know how much the change from salt to fresh water has modified Gordius, which is evidently the more degenerate form, as may be inferred, for example, from the greater reduction of the alimentary canal and of the nervous system. Certainly the rows of bristles in Nectonema are to be attributed to its free life and more active habits. With the latter also one would naturally look for a more highly developed nervous system. Further evidence, that to be gained from the

embryonic development as well as from the life history and structure of the larva, will make this matter clearer.

Nearly all writers agree in placing the Gordiidæ in an isolated position under the Nematodes. If, according to the proposal of some, this family be raised to the dignity of a separate order, then there is no doubt in my mind of the right of Nectonema to a position in that order as the representative of a new family, the Nectonemidæ. But whatever may be the final decision in regard to the rank of the Gordiidæ, this new family must take its position near that group. That the relationship is close enough to warrant the inclusion of the genus Nectonema in the family of the Gordiidæ is hardly possible, but a final opinion on this point can be given only in the light of more complete knowledge, especially on the points just enumerated. It is my intention to investigate the subject further, and to follow if possible the life history of this most interesting form.

CAMBRIDGE, March 25, 1892.

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## EXPLANATION OF FIGURES.

All figures were drawn with the aid of Zeiss lenses and an Abbé camera unless otherwise stated, and represent preparations of *Nectonema agile*, Verr. The method of staining, the lenses, and the magnification employed, are noted briefly for each figure.

## ABBREVIATIONS.

| | |
|---|---|
| *cd. n. v.* | ventral nerve cord. |
| *cl. coms.* | commissural cells of brain. |
| *cl. d.* | dorsal cell. |
| *cl. gn.* I, II., etc. | first, second, third, etc. large ganglion cell of brain. |
| *cl. in.* I.–IV. | intestinal cells I. to IV. |
| *cl. mu.* | muscle cell of body wall. |
| *cl. œ.* | œsophageal cell. |
| *cl. sns.* | sensory cell. |
| *coms. œ.* | dorsal (supra-œsophageal) commissure of brain. |
| *cp'* | corpuscle of body cavity fluid. |
| *cta.* | cuticula. |
| *e'th. pi't.* | peritoneal epithelium. |
| *fbr'. mu.* | contractile fibrillæ of muscle cell. |
| *fbr. n.* | nervous fibrillæ. |
| *gn. an.* | anal ganglion. |
| *h'drm.* | hypodermis. |
| *in.* | intestine. |
| *ln. d.* | dorsal line. |
| *ln. v.* | ventral line. |
| *mb. ba.* | basement membrane. |
| *n. a.* | nerve fibres anterior to brain. |
| *nl. d.* | nucleus of dorsal cell. |
| *nl. gn.* | nucleus of large ganglion cell. |
| *nl. gn.* I.–V. | nuclei of ganglion cells I. to V. of brain. |
| *n. l.* | lateral nerve of ventral nerve cord. |
| *nll.* | nucleolus. |
| *n. m.* | median nerve of ventral nerve cord. |
| *n. n.* | nervous nuclei. |
| *œ.* | œsophagus. |
| *pd. cl. d.* | stalk of dorsal cell. |
| *pr'c. gn.* | process of ganglion cell. |
| *sac. d.* | dorsal sac. |
| *sp'z.* | spermatozoa. |
| *st. pi'ph.* | peripheral layer of anal ganglion. |
| *te.* | testis. |
| *va. df.* | vas deferens. |

## PLATE I.

Fig. 1. Male Nectonema, natural size. The bristles are so minute that they could not be represented in proper proportions. Free hand drawing.

Fig. 1$^{a}$. Anterior end of the same to show torsion of the body. Somewhat diagrammatic.

Fig. 2. Dorsal view of anterior end of living animal. × 32.

Fig. 3. Lateral view of anterior portion of living animal. Drawn by A. Agassiz, August 5, 1871.

Fig. 4. Posterior portion of male. Drawn from specimen cleared in clove oil. Simple microscope. × 7.

Fig. 5. Posterior portion of female discharging eggs. Drawn from specimen cleared in clove oil. Simple microscope. × 7.

Fig. 6. Lateral aspect of central part of body. Drawn by A. Agassiz, August 2, 1877.

Fig. 7. Ventral aspect of body. Drawn from living animal, and afterwards reduced one half. 1. A. × $\frac{6}{2}^{2}$.

Fig. 8. Right half of anterior portion of the body drawn from a transparent object in clove oil, and represented as though the near (left) half of the body had been removed. 1. A. × 48.

Fig. 9. End of body of male viewed as a transparent object in clove oil. 2. a*. × 17.

Fig. 10. End of body of female viewed as a transparent object in clove oil. 2. A. × 50.

Fig. 11. Cross section from centre of body of male. Kleinenberg's hæmatoxylin. 2. A. × 98.

1. 1a. 4. 5. 8.

dd in.

ov.

in.d

11.

ir.

6.

ir.v.

10.

3.

9. sac d

in.

in.v

7.

2.

## PLATE II.

Fig. 12. Tangential section, including cuticula of dorsal line with bristles. 1. C. × 180.

Fig. 13. Cross section of cuticula with bristles attached, only the basal portions of which are represented. At the lower side lies a cross section of a bristle. 4. C. × 400.

Fig. 14. Cross section of cuticula with scales attached. Apochr. 4 mm. Compens. Oc. 4. × 360.

Fig. 15. Cross section of scale with cuticula and hypoderm. Apochr. 4 mm. Compens. Oc. 12. × 1,050.

Figs. 16, 17. Cross sections of scale on cuticula to show central canal. Apochr. 4 mm. Compens. Oc. 4. × 360.

Figs. 18, 19. Surface view of scales on cuticula. Apochr. 4 mm. Compens. Oc. 4. × 360.

Fig. 20. Cross section of cuticula and hypoderm. Ehrlich's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 8. × 720.

Fig. 21. Cross section of body wall just lateral to dorsal line to show structure of the muscles. Apochr. 4 mm. Compens. Oc. 8. × 720.

Fig. 22. Fragment of muscle after maceration in 60 % KOH, and subsequent treatment with potassic acetate. 3. C. × 320.

Figs. 23–26. Cross sections of body wall from different individuals to show the different widths of the contractile and protoplasmic zones in the muscular layer. The contractile zone is represented by a dark band, and the protoplasmic by a lighter tint. 1 A. × 52.

Fig. 27. Tailed nuclei from muscular layer. Mayer's HCl carmine. Apochr. 4 mm. Compens. Oc. 8. × 720.

Figs. 28–30. Portions from protoplasmic zone of muscular layer, showing the formation of corpuscles from the free ends of the muscle (?) cells. Gentian violet and eosin. Apochr. 4 mm. Compens. Oc. 4. × 375.

Fig. 28. Portion of cell nearly cut off at *x*.

Fig. 29. Portion of cell at *x*, which stains more deeply and resembles corpuscles (*cp'*.) of the body cavity.

Fig. 30. Corpuscles of different sizes completely separated from protoplasmic zone.

ctn.

hdrm.

21.

cl.mu.

28.

x.

29.

x.

cp'.

15.

d.

14.

x.

16.

30.

cp'.

18.

17.

19.

22.

fbr'mu.

23.

27.

te.

cd.n.v.

25.

26.

in.

24.

12.

13.

20.

hdrm.

cd.

H.B.W. del.

B.Mevel, lith. Boston.

## PLATE III.

Figs. 31–45. Transverse sections of the alimentary canal from a single series of sections. Mayer's HCl carmine. Apochr. 4 mm. Compens. Oc. 4. × 230.

Fig. 31. 7th section of the series.
" 32. 16th " " "
" 33. 25th " " "
" 34. 31st " " "
" 35. 32d " " "
" 36 38th " " "
" 37. 47th " " "
" 38. 48th " " "
" 39. 49th " " "
" 40. 50th " " "
" 41. 51st " " "
" 42. 52d " " "
" 43. 54th " " "
" 44. 55th " " "
" 45. 103d " " "

Figs. 46–48. Transverse sections of alimentary canal from the region where it is bounded by two cells alone to show diminution in size toward the posterior end. Apochr. 4 mm. Compens. Oc. 4. × 230.

Fig. 46. Kleinenberg's hæmatoxylin.

Figs. 47, 48. Ehrlich's hæmatoxylin.

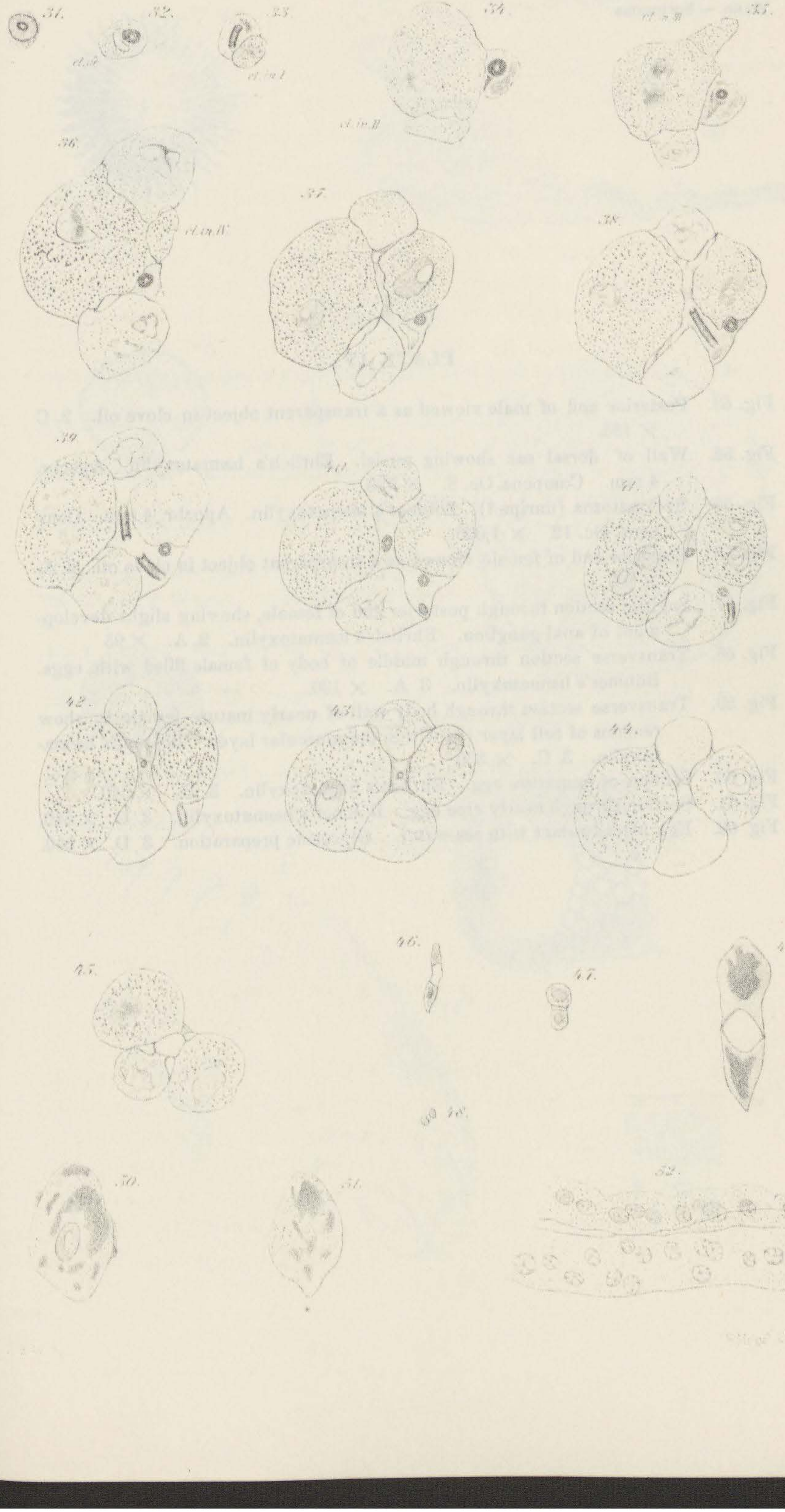
Fig. 49. Transverse section of alimentary canal to show the normal relation of the lumen to the cells. Kleinenberg's hæmatoxylin. 2. E. × 610.

Figs. 50, 51. Transverse sections of the œsophageal cell in the same series. Ehrlich's hæmatoxylin. 2. E. × 610.

Fig. 50. To show the relation of the nucleus to the chitinous tube.

Fig. 51. Two sections beyond the section in Fig. 50 to show disappearance of the chitinous tube.

Fig. 52. Lateral aspect of intestine. The two cells of the opposite side are not shown. Ehrlich's hæmatoxylin. 2. C. × 165.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## PLATE IV.

Fig. 53. Posterior end of male viewed as a transparent object in clove oil. 2. C × 165.

Fig. 54. Wall of dorsal sac showing nuclei. Ehrlich's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 8. × 725.

Fig. 55. Spermatozoa (unripe ?). Böhmer's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 12. × 1,000.

Fig. 56. Posterior end of female viewed as a transparent object in clove oil. 2. A. × 95.

Fig. 57. Sagittal section through posterior end of female, showing slight development of anal ganglion. Ehrlich's hæmatoxylin. 2. A. × 95.

Fig. 58. Transverse section through middle of body of female filled with eggs. Böhmer's hæmatoxylin. 3. A. × 130.

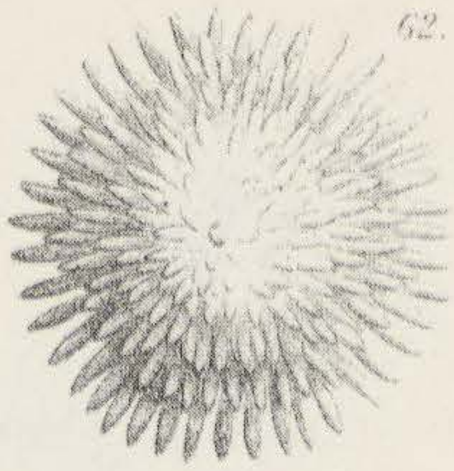
Fig. 59. Transverse section through body wall of nearly mature female to show remains of cell layer (*) within the muscular layer. Böhmer's hæmatoxylin. 3. C. × 330.

Fig. 60. Section of immature egg. Ehrlich's hæmatoxylin. 3. D. × 540.

Fig. 61. Section through nearly ripe egg. Böhmer's hæmatoxylin. 3. D. × 540.

Fig. 62. Egg after contact with sea-water. Glycerine preparation. 3. D. × 560.

62.



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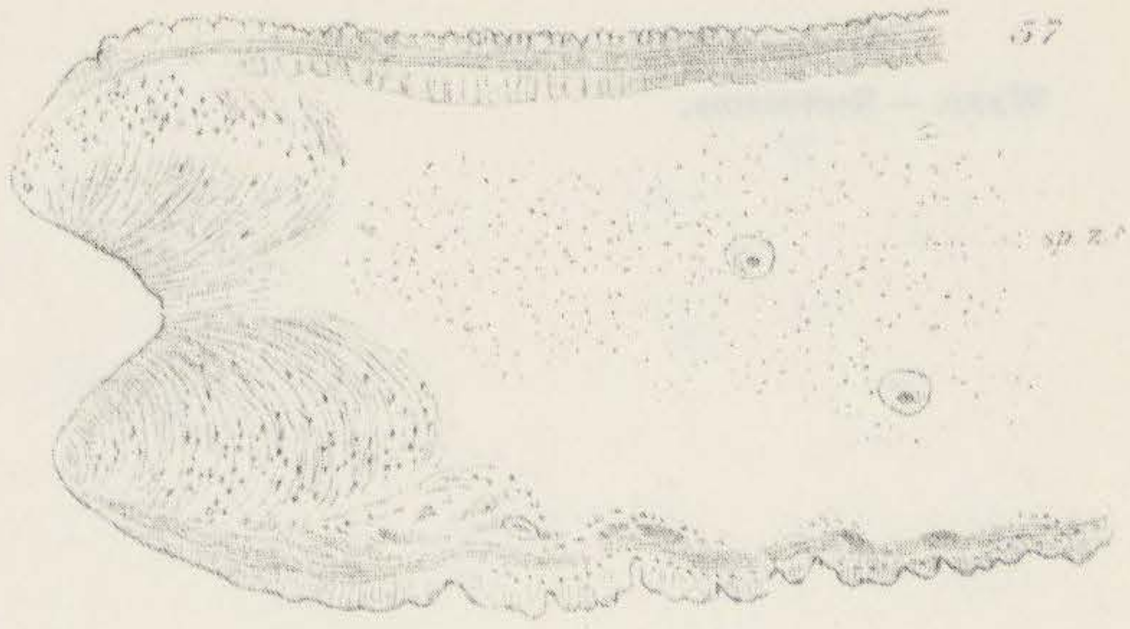

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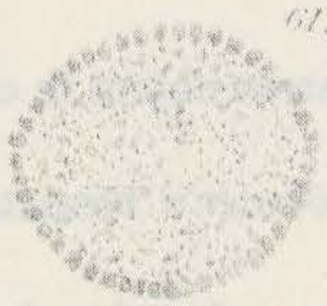

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sp. ?

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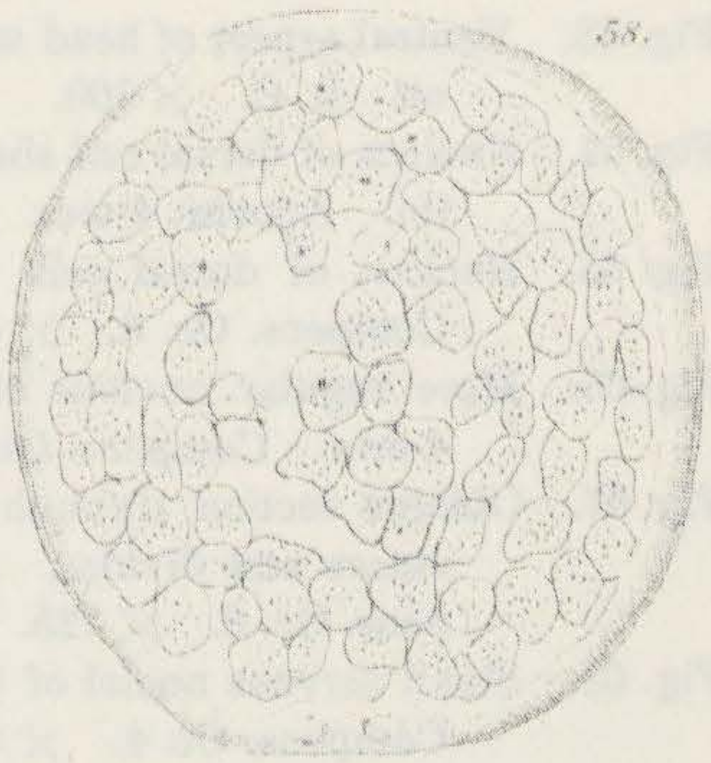

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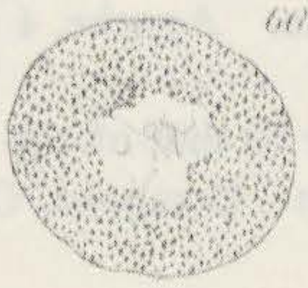

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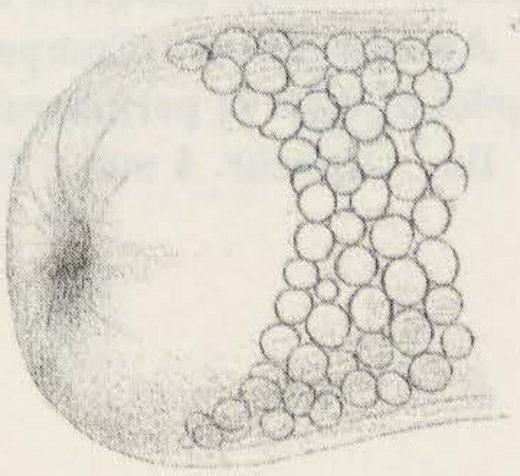

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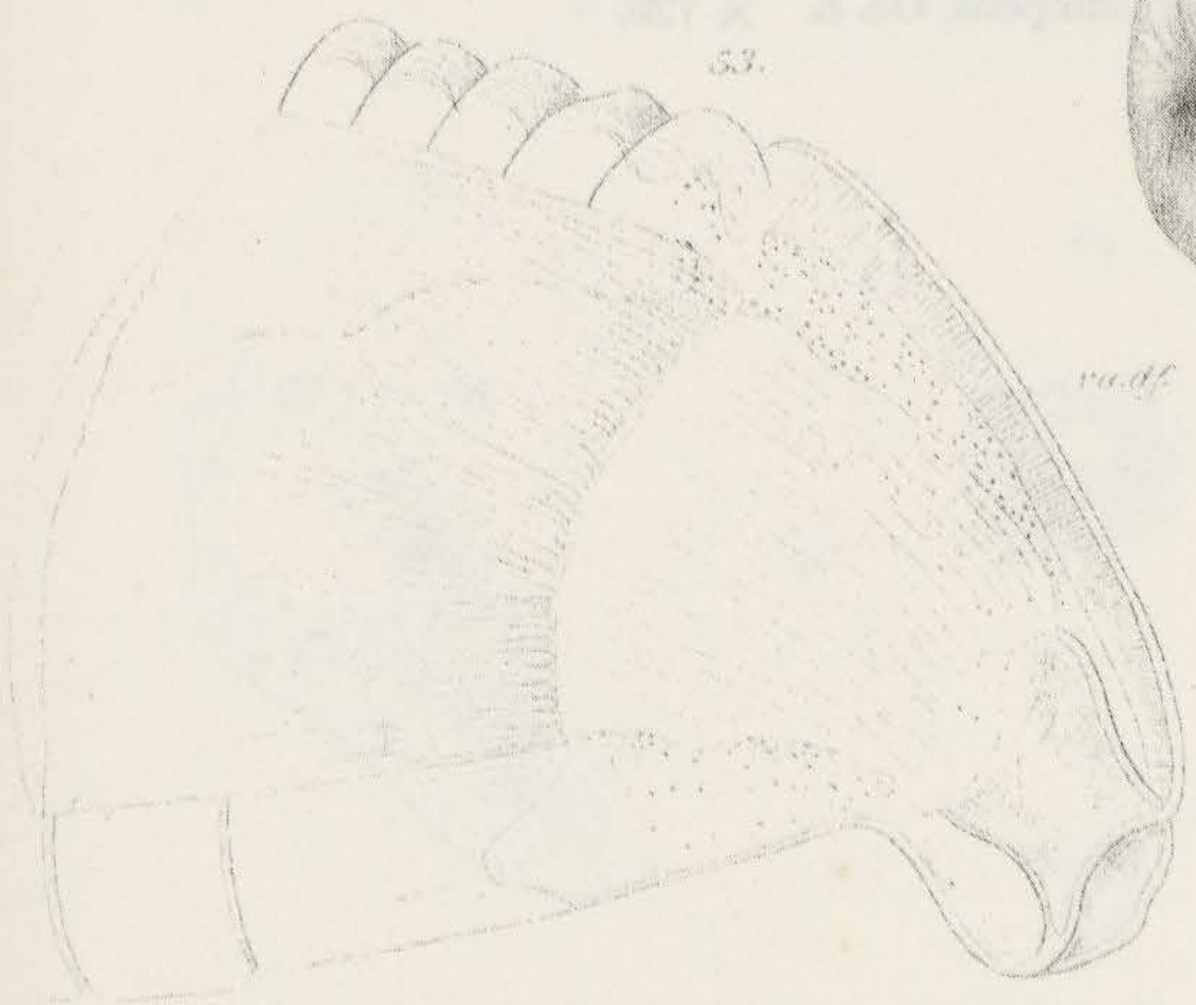


vu. sp.

59

el. mu.

## PLATE V.

Fig. 63. Ventral aspect of head with brain, viewed as a transparent object in clove oil. 2. C. × 160.

Fig. 64. Nucleus of dorsal cell showing enclosed body ($x$). Böhmer's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 8. × 680.

Fig. 65. Nucleus of dorsal cell. Kleinenberg's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 8. × 680.

Fig. 66. More regular nucleus of dorsal cell. Ehrlich's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 8. × 725.

Fig. 67. Oblique section through basal portion of dorsal cell where the process enters and divides. Böhmer's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 8. × 725.

Fig. 68. Small nervous nuclei of brain. Ehrlich's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 4. × 360.

Fig. 69. Nucleus of commissural cell showing enclosure similar to that of the dorsal cell. Böhmer's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 4. × 360.

Fig. 70. Nucleus of large ganglion cell in anal ganglion. Böhmer's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 8. × 725.

Fig. 71. Nuclei of cells in peripheral layer of anal ganglion. Ehrlich's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 8. × 725.

66.

67.

71.

63.

cl gn. I.

cl gn. III

cl gn. II.

pd. cl. d.

cl. gn. VI.

cl. gn. V

cl. gn. IV

64.

70.

68.

x

nli.

65.

69.

## PLATE VI.

Figs. 72–88. Successive transverse sections through the brain. The small nervous nuclei are represented by simple dots, the nuclei of the large cells as accurately as possible; the cell bodies are not represented.

Fig. 72 is the twelfth section from the apex of the head; each section is represented from its anterior face, and approximates 10 $\mu$ in thickness. Ehrlich's hæmatoxylin. 2. A. $\times$ 95.

Fig. 89. The posterior end of the male with anal ganglion and dorsal sac viewed as a transparent object in clove oil. Only a few of the numerous Gregarinida (*) in the body cavity are represented. 2. A. $\times$ 66.

72.

*ct.d.*

73.

*nt.gn.I.*

74.

75.

*nt.gn.II.*

76.

*pa.ct.d.*

*nt.d.*

77.

*nt.gn.III.*

78.

79.

80.

*comm.str.*

81.

82.

*nt.gn.IV*

83.

*nt.gn.V*

84.

85.

86.

87.

88.

*pr.c.gn.*

*sav.II*

89.

*vn.df.*

*gr.an.*

## PLATE VII.

Fig. 90. Sagittal section through posterior end of male, slightly lateral, showing large cells and processes in anal ganglion. Böhmer's hæmatoxylin. 1. C. × 180.

Fig. 91. Gregarinida from mass of spermatozoa. One in cross section is shown at *a*. Mayer's HCl carmine. 3. D. × 560.

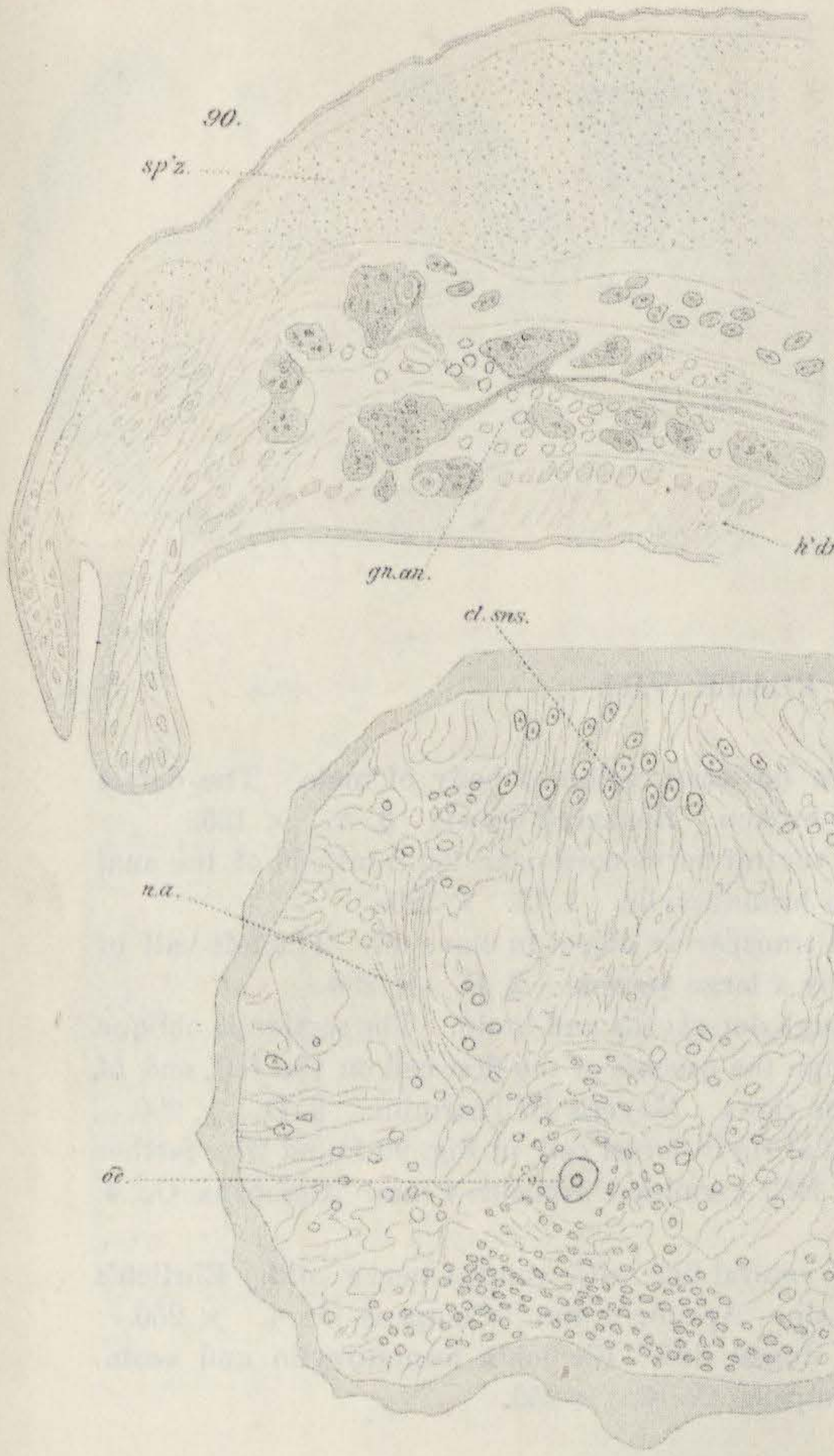
Fig. 92. Transverse section, slightly oblique, from near the tip of the head, showing sensory cells, and nerve fibres passing up towards them. Ehrlich's hæmatoxylin. 2. C. × 230.

Fig. 93. Isolated sensory cells from front end of head. Ehrlich's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 8. × 725.

Fig. 94. Diagrammatic representation of the relative size and position of the large ganglion cells in the brain. The extremes of variation in the position of the second pair (*cl. gn.* II.) are represented on the right and left of the figure. Dorsal aspect with the dorsal cells removed; the deep-lying cells appear fainter, cells which are nearer being more prominent. Drawing by A. G. Mayer.

Fig. 95. Oblique longitudinal section of posterior portion of the brain and the partition which cuts off the anterior chamber. The deepest and last portions of the processes of the dorsal cells are shown. Ehrlich's hæmatoxylin. Apochr. 4 mm. Compens. Oc. 8. × 725.

90.

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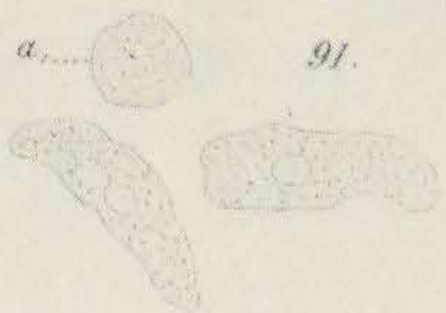


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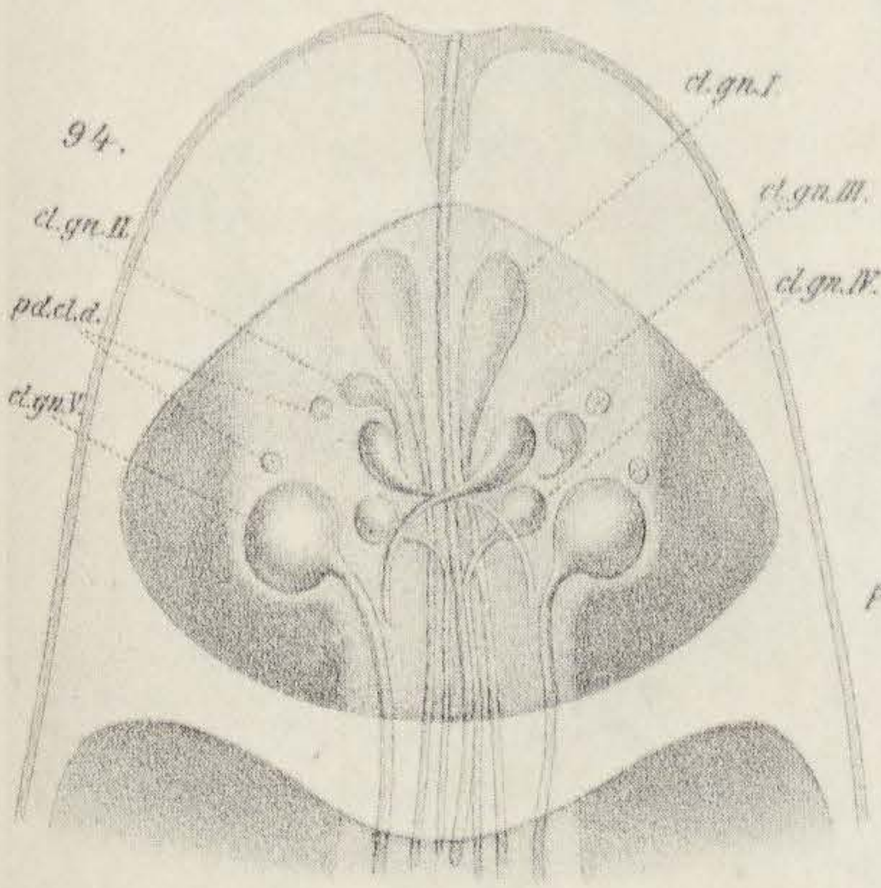


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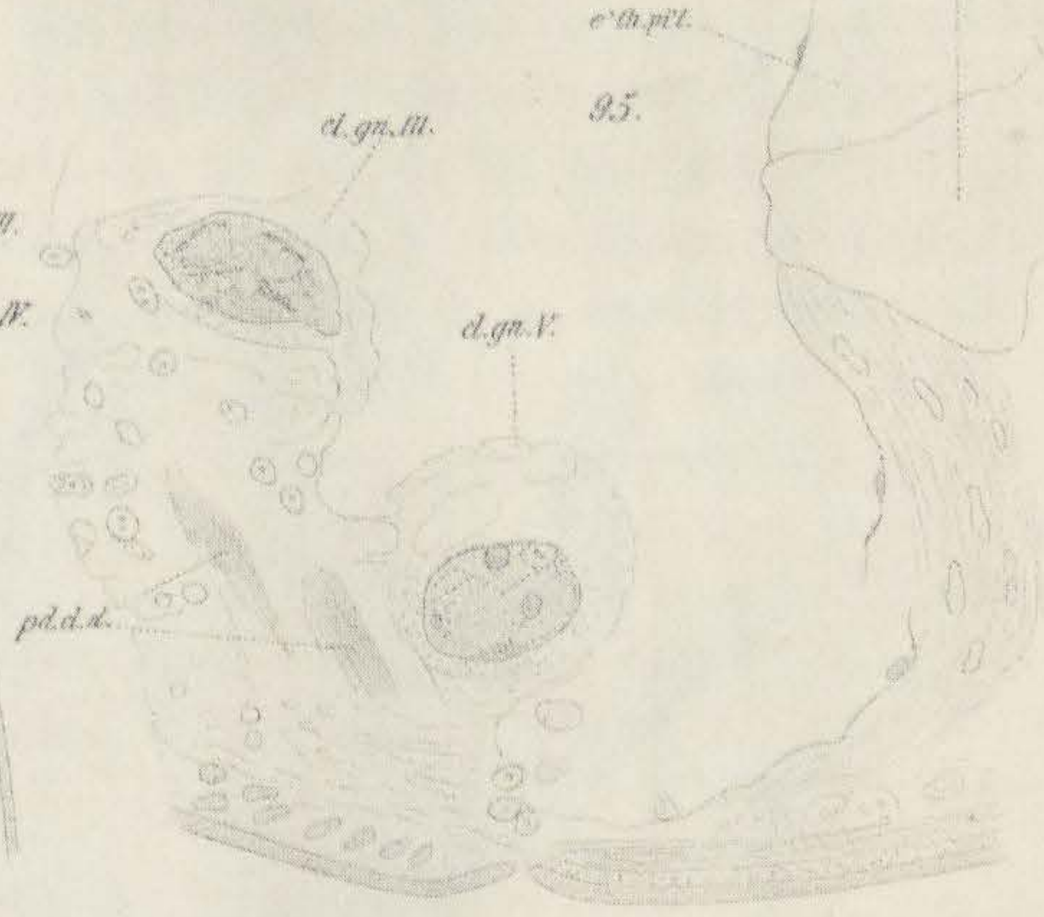



R.B.W. del.

B.Messel, lith. Boston.

## PLATE VIII.

Fig. 96. Transverse section from posterior region of body of male. The dorsal margin is somewhat broken. Bismarck brown. 3. A. × 125.

Fig. 97. Longitudinal section of ventral nerve cord near the front end of the anal ganglion. Böhmer's hæmatoxylin. 1. C. × 180.

Fig. 98. Dorsal cell viewed as a transparent object in clove oil. The left half of the cell is occupied by a large vacuole. 2. D. × 290.

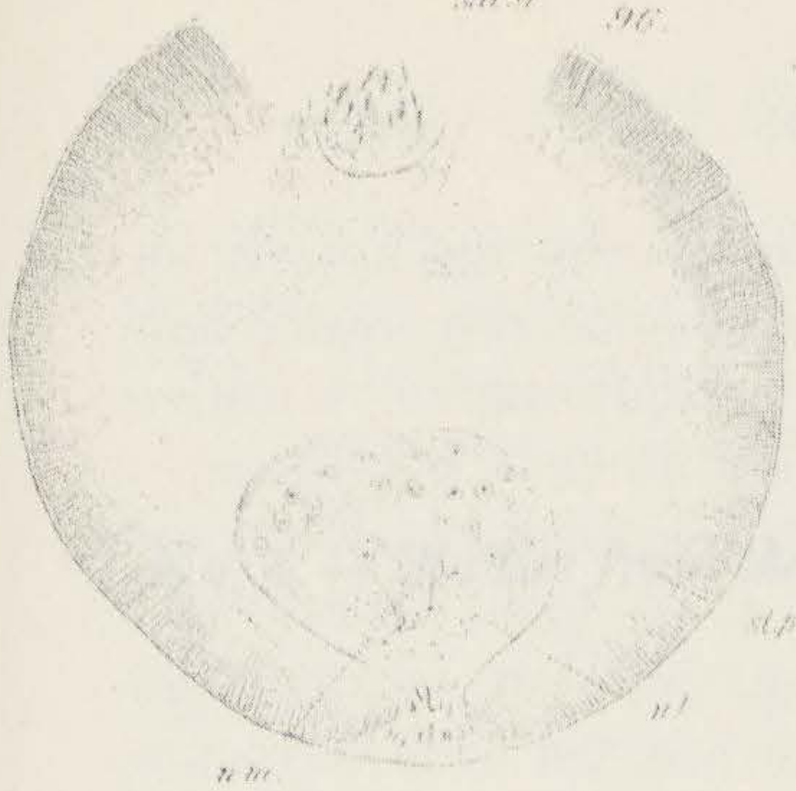
Fig. 99. Transverse section through dorsal cells and brain. The section is oblique enough to pass through the process of the first cell on the left, and of the second cell on the right. Mayer's HCl carmine. 2. D. × 290.

Fig. 100. Termination of process of right dorsal cell in Fig. 99 found 20 $\mu$ farther posteriad. Mayer's HCl carmine. Apochr. 4 mm. Compens. Oc. 4. × 360.

Fig. 101. Transverse section of ventral line with ventral nerve cord. Ehrlich's hæmatoxylin and eosin. Apochr. 4 mm. Compens. Oc. 4. × 250.

Fig. 102. Transverse section of dorsal line. Ehrlich's hæmatoxylin and eosin. Apochr. 4 mm. Compens. Oc. 4. × 250.

96.

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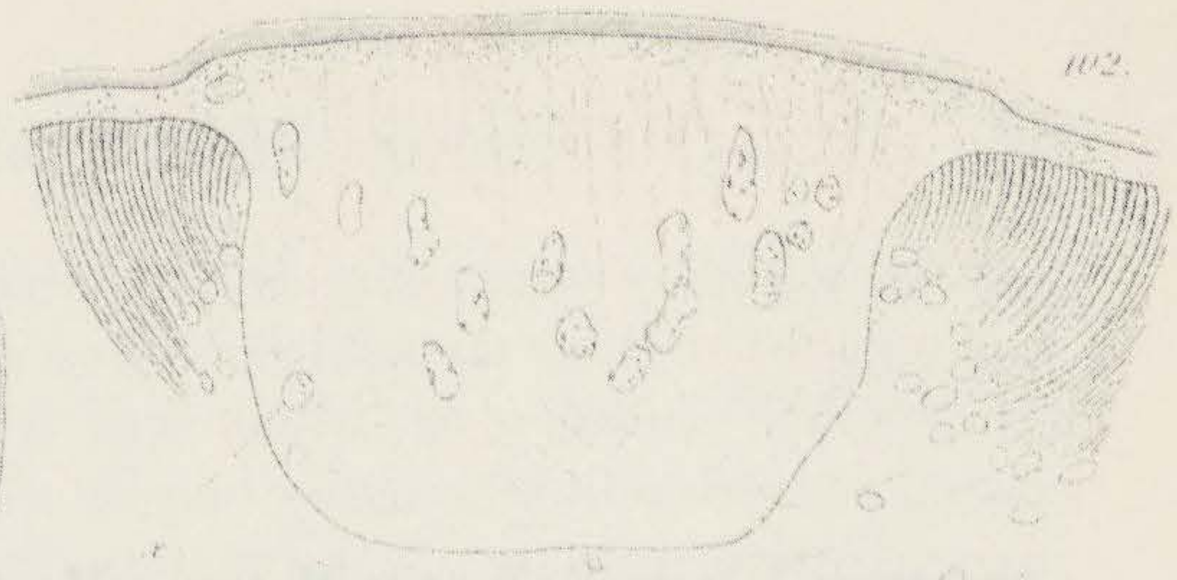


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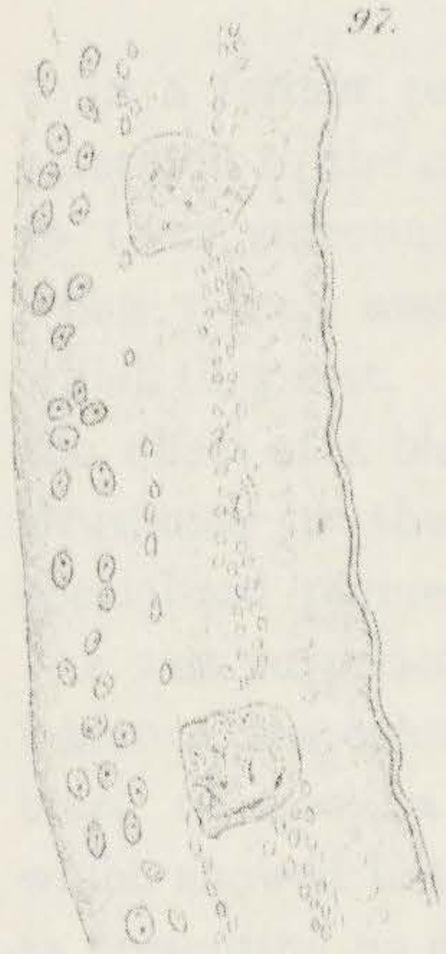

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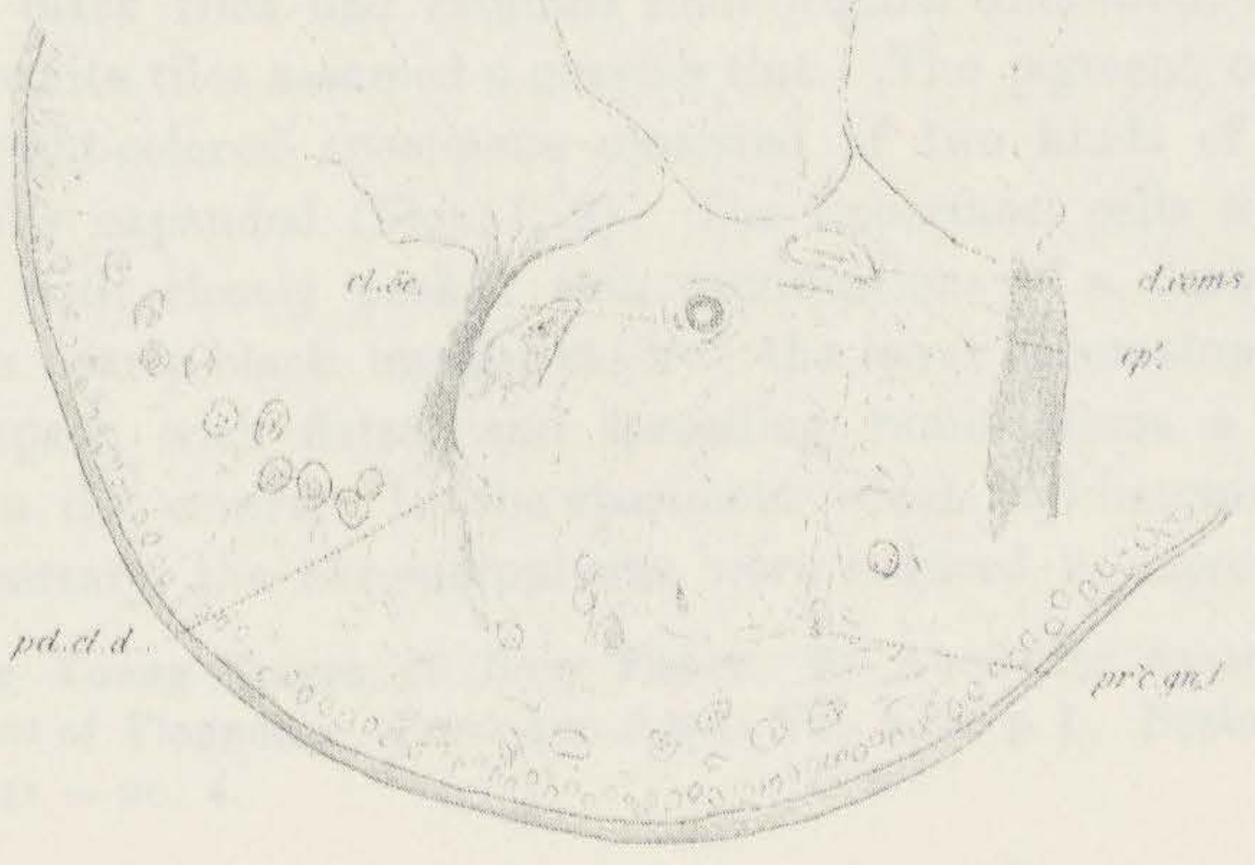


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