XII.—On the Septal and Pharyngeal Glands of the Microdrili (Oligochæta). By J. Stephenson, M.B., D.Sc., Lecturer in Zoology, Edinburgh University. (With One Plate and One Figure in the Text.)

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INTRODUCTION.

In 1917 I published an account (16) of the so-called pharyngeal gland-cells of earthworms, unevenly but intensely staining cells (whence the name "chromophil" which I gave to them) which form lobular masses on the pharynx, and smaller aggregations in other places in the anterior segments. I came to the conclusion that the cells were not, as was usually held, epithelial in origin—were not cells which had fallen out of the epithelial layer while still retaining their connection with the pharyngeal cavity by means of a long neck functioning as a ductule. On the contrary, they were, I believed, mesoblastic in origin, and congeneric with the peritoneum; and were devoid of ducts leading into the pharynx.

Similar cells occur in the smaller Oligochæta also;—as paired masses on the septa of certain anterior segments, where they form the "septal glands" of the Enchytræidæ, of the genera *Naidium* and *Pristina* of the family Naididæ, and of certain Tubificidæ; sometimes on the pharynx, where they occupy a position similar to that of the pharyngeal glands of earthworms; and as smaller groups irregularly distributed in various other situations in the anterior part of the body of a number of Microdrili.

In my former paper I did not treat of these, but expressed my intention of returning to them later. The present communication gives the results of my investigation of the chromophil cells of the Microdrili—results which are largely similar to those I obtained in the case of earthworms, and which may be considered as confirmatory of a large part of my former work.

HISTORICAL.

VEJDOVSKY summarises in his monograph (19) the views of older authors concerning the septal glands of Enchytræids. HENLE had stated that they discharged into the hinder part of the œsophagus; but other investigators considered them to be parts of the reproductive apparatus, while RATZEL believed them to belong to a complicated pharyngeal nervous system. VEJDOVSKY himself maintains the opinion that they are glandular, and that they discharge into the pharynx; certain portions, however, form isolated masses unconnected with the ducts. He describes the glands in *Anachæta bohémica* (= *Achæta bohémica*), where there are two pairs; the hinder gland on each side is connected with the anterior by a strand in which a clear central canal could seldom be discovered (though such a canal is said to be obvious in other genera); the anterior gland is prolonged forward by a duct whose walls are

also composed of gland-cells; the lumen of the duct is most obvious at the anterior end where it discharges into the hinder end of the pharynx; there is also a cavity in the glands. Similar aggregates of cells, unconnected with the glands first described, are found on the posterior faces of septa 4/5 and 5/6, to which they are attached by a short stalk. Both groups have a peritoneal investment.

The septal glands are especially well seen in young examples of *Phreatothrix pragensis* (= *Trichodrilus pragensis*), a Lumbriculid. Here they may be present on both sides of the septa; there are four pairs, connected by strands as in the Enchytræids, and the duct discharges into the pharynx. In the adult it is difficult to make out the arrangement of the glands and ducts, since the ducts become surrounded by a proliferation of the cells, and the body-cavity is partly obliterated (the same happens, according to the author, in all Tubificidæ and Lumbricidæ). Certain Naididæ—*Naidium* and *Pristina*—are similar to the Enchytræidæ; but in *Æolosoma* (Æolosomatidæ) and *Chætogaster* (Naididæ) there is nothing corresponding to the septal glands.

MICHAELSEN in 1886 (7) described the septal glands in *Enchytræus möbii* (= *E. albidus*). After giving a general account of their shape and of the characters of the cells, he describes the strands which are prolonged forwards from the hindmost glands; these are said to be composed of bundles of parallel canals which convey the secretion from the interior of the glands. These strands pass through the two anterior pairs of glands, taking up their secretion also. Finally they split up; the individual canals separate and, joining the dorsal surface of the pharynx, discharge into the pharyngeal cavity.

HESSE in 1893 (3) also gave a description of the septal glands of Enchytræids. The glands are said to be hollow, and to have a peritoneal covering; a portion of the cells disintegrates during their functioning, and passes along the ducts with the secretion, presumably becoming dissolved, since further along the ducts the disintegrated matter ceases to be distinguishable as solid particles. The partly disintegrated cells may resemble ganglion cells in shape. The mode of discharge into the pharynx is seen in preparations stained with borax-carmin, which colours the more solid and granular parts of the secretion; this material is seen to become divided up into a number of small cords which penetrate between the elongated epithelial cells of the dorsal wall of the pharynx and reach the lumen of the alimentary tube through fine pores in the cuticular lining. The preparations are stated to be quite distinct. A figure gives an excellent idea of the cells, their shape and uneven staining, the fine interstices between them, the fine striation of the central portion of the gland and of the "duct"; there is no suggestion in the figure that the cells are continued forward as ductules.

HESSE returned to the subject in the following year (4). His conception of the septal glands had now undergone a change, in consequence, apparently, of his having investigated certain Naididæ. He states that in segment iii of *Stylaria lacustris* and of *Nais elinguis* numerous single-celled glands are present; the bodies of the cells stain darkly, like those of the septal glands; they discharge between the cells of the pharyngeal epithelium, and are themselves apparently long drawn-out epithelial cells. HESSE now states that a glance at his Enchytræid preparations immediately justifies a similar description of the cells of the septal glands; within each gland is a central mass consisting of ductules, and the preparations show in the most convincing manner that the strands continued forward from the glands are bundles of canalicules, the ductules of the gland-cells. Such a structure of the septal glands is established for *Pachydrius* (= *Lumbricillus*) *litoreus*, *Parenchytræus* (= *Enchytræus*) *litteratus*, *Fridericia ratzelii* and *galba*, *Tubifex*, *Psammoryctes*, *Limnodrilus*, and *Lumbriculus*. The author speaks of the cells growing back from the pharynx, and pushing the

peritoneal coat of the septa before them as they pierce through ; but this is rather an inference than the record of actual observations.

Again, in the same year (5), HESSE repeats this second conception of the glands. In connection with the anterior part of the gut of Oligochæta there exist a number of glandular appendages, consisting of long drawn-out cells, the narrow ends of which lie side by side and form their ducts ; these ducts separate on reaching the alimentary epithelium, and discharge between the epithelial cells. This is most plainly visible in the Naididæ, in *Psammoryctes*, and *Branchiobdella*, and is also easily to be observed in *Tubifex*. The cells which are most drawn-out are those of the septal glands of Enchytræids, where the condition is such that one can scarcely recognise the true nature of the glands without comparative observation ; thus their nature was missed by earlier authors, and, in spite of favourable preparations, by HESSE himself in his earlier paper ; but he now recognises that the above view is correct.

UDE (18) in describing *Bryodrilus ehlersi* takes HESSE's view of the constitution of the septal glands.

BEDDARD in his monograph (1) also adopts HESSE's view of the septal glands ; each cell is prolonged to form its own duct, and the ducts appear to enter the pharynx ; the structures seem to be simply epidermic glands which have been invaginated along with the stomodæum.

MICHAELSEN (8) finds septal glands in *Phreodrilus kerguelensis* which are strongly reminiscent of those of Enchytræids. The pointed ends of the cells pass into the ducts, which become aggregated into fairly thick strands, and discharge through the dorsal wall of the pharynx. Septal glands are also present in *Phreodrilus crozetensis* (8A).

The same author describes (9), under the name of salivary glands, cells of a similar kind in *Ilyodrilus hammoniensis* and *Tubifex filum* (Tubificidæ). The glands discharge through the pharyngeal wall. In the first species they are few, and extend back into segment v ; in the second, numerous glands extend backwards, much cut up, into segment vi.

I have described similar masses of cells in two Tubificids, *Branchiura sowerbyi* and *Limnodrilus socialis* (13). In the first of these worms they occur, surrounding the alimentary tube on all sides, in backward bulgings of the posterior septa of segments iii, iv, and v ; the cells are not compacted into definite lobes, and appeared to me to be aggregations of peritoneal cells ; there is no mention of any ductules. In *Limnodrilus socialis* there are paired masses of cells in segments vi and vii, and smaller masses in v and viii ; these masses are pear-shaped, with the small end below, near the ventral vessel ; a mass may unite round the ventral vessel with its fellow of the opposite side. Some of the cells in this species may contain chloragogen, or similar yellow granules, but the general characters of the cells are very different from those of chloragogen cells—they are more compact, less regular in size and shape, and stain more deeply ; they do not merge into the chloragogen cells, though they are in contact with them ; the masses have a distinct outline of their own. They occur in the anterior part of their segments, not in relation with the septa.

STIRRUP (17) finds that the septal glands of *Enchytræus pellucidus* have no central cavity ; they are solid blocks of deeply staining tissue, with a core which is continued forwards on each side to become continuous with a longitudinal strand. This strand, which thus connects together all the glands of a side, stains slightly, is solid, and appears quite incapable of carrying any liquid secretion ; each enters the substance of the large dorsal pharyngeal projection, first dividing into four or five branches ; but the author was never able to discover any opening into the lumen. The strands are not muscular, nor are they ducts ; they have a delicate fibrillar structure, and the author is inclined to view them as nervous.

NOMURA in 1913 (11) described the appearance in two species of *Limnodrilus* (Tubificidæ). "Septal sacs" and pharyngeal glands are distinguished; the sacs are described in detail as they appear in *L. gotoi* (= *L. socialis*), and the pharyngeal glands as they occur in *L. willeyi*. The septal sacs are attached in pairs to the posterior faces of septa 5/6–9/10, ventral to the intestine; each organ is composed of many club-shaped cells enclosed within a thin structureless membrane; the cells at the entrance of the sac are "undoubtedly chloragogues," while those at the bottom of the sac are smaller and have a dense cytoplasm; between these two forms there are cells of intermediate characters. The septal sacs have an intimate relation to the blood-vessels running through them. The pharyngeal gland-cells are arranged in six longitudinal rows on the dorsal side of the pharynx; the outermost row on each side extends back to segment v, the other rows to the middle of segment iii, "but all open into the pharynx in segment iii." The cytoplasm of the gland-cells stains slightly, but contains numerous secretory granules which take up Delafield's hæmatoxylin well; the proximal end of the cell forms the duct, which opens into the pharyngeal cavity. One of NOMURA's figures shows indications of a few cells insinuating their narrow ends between the epithelial cells of the dorsal wall of the pharynx; but from other figures, where the cells and their ducts are shown on a larger scale, it appears that there may be four cells on a single stalk, or there may be a series of several cells on each side of a central axis; a large cluster of such ducts is shown penetrating the pharyngeal epithelium.

MICHAELSEN in 1914 (10) recorded large-celled salivary glands in segments iii–v of *Limnodriloides winckelmanni* (Tubificidæ); these cover the dorsum of the pharynx in a thick layer in segment iii, and in iv and v appear as masses with numerous small lobes which take up a large part of the body-cavity. Nothing is said as to the manner of their discharge.

NOMURA in 1915 (12) described the condition in *Monopylephorus limosus* (Tubificidæ). There are here no septal glands; but glandular cell-masses, always richly supplied with fine blood-capillaries, occur attached to the peritoneum in segments iv and v, often in iii also. These appear to be differentiated from the peritoneum, since cells are seen with characters intermediate between the glandular and the peritoneal cells (the author thinks it possible, however, that these might be glandular cells which had just discharged their contents). They occur, projecting into the cœlom, mostly on the ventral side of the line of the dorsal setæ, where they are conspicuous by their staining reactions; they contain many secretory granules which stain deeply with hæmatoxylin. In the same worm there are four rows of pharyngeal glands longitudinally arranged on the dorsum of the pharynx. The glands consist of pear-shaped cells enveloped by a thin connective-tissue layer containing very fine capillaries. The cytoplasm is somewhat granular; the proximal end of each cell is prolonged into a duct, which either unites with others coming from neighbouring cells or opens directly into the pharyngeal cavity through the interspace between the endodermal (?) cells.

Miss DIXON, in her study of *Tubifex* (1915) (2), finds septal glands in the œsophageal segments, and to a less extent in those occupied by the pharynx. The glands are attached primarily to the septa, but may extend in to the walls of the œsophagus, and in a large degree lie freely in the body-cavity. Each gland is a mass of pear-shaped cells, the narrower part of each being prolonged into a duct which opens into the œsophageal region of the alimentary tube.

In 1915 I described, in *Hæmonais laurentii* (Naididæ) (14), gland-cells in groups on the side of the pharynx, the groups being separated from each other by muscular strands passing

outwards from the pharyngeal wall. The individual cells were pear-shaped or fusiform, and were continued by means of a narrower neck into the epithelial layer of the pharynx, "to which," I stated, "they thus properly belong." But I added, "It is very possible, however, that some of the cells in this situation are of peritoneal origin and have no direct connection with the pharyngeal epithelium."

Lastly, I found similar cells in 1917 in *Monopylephorus parvus* (15). Here the pharyngeal gland-cells are arranged in four longitudinal rows, dorsally and dorso-laterally on the pharynx, as in *M. limosus* according to NOMURA. There are also numerous similar cells on the body-wall, where they form considerable masses at the level of the hinder part of these rows, as well as for some distance behind this, as far as segment vi; there are a number also on each side of the nerve cord in the œsophageal region. I made no statement about their manner of discharge; but those on the body-wall at any rate could hardly discharge into the pharynx or œsophagus.

CRITICAL.

Of the writers mentioned above, those who have treated this subject at greatest length are VEJDOVSKY and HESSE; the accounts of other authors mostly occur in the course of general anatomical descriptions of the various worms, and it is quite possible that there are other similar accounts scattered in the literature, which I have missed.

The majority of authors state that the gland-cells discharge into the alimentary tube, generally into the pharynx; while, however, the earlier writers seem to have conceived the ducts, in the case of the septal glands of Enchytræids, as patent tubes of some size, HESSE was the first to describe the cells as drawn out into long processes—extremely long in the case of the Enchytræids—which function as ductules, and along which the secretion of the cells passes. Subsequent investigators have usually confirmed HESSE; STIRRUP, however, is an exception; NOMURA finds that the cells of the pharyngeal glands of *Limnodrilus* are of the type described by HESSE, while those of the septal sacs in the same genus, and those of certain granular masses of *Monopylephorus*, have no such form; while I believed that the pharyngeal glands of *Hæmonais* communicated with the pharynx in the way described, but that the chromophil cells of Tubificids had no such connection.

It is, however, difficult to get the evidence of figures in favour of the discharge through a canaliculus prolonged from the cells. HESSE's figure (in 4) is frankly diagrammatic, and is meant only to represent the author's view of the constitution of the glands and their ducts. NOMURA's figures also seem to be highly schematic, and again illustrate the author's interpretation of the appearances rather than the appearances themselves. I cannot help feeling this, for example, in regard to the figure showing four cells on a single stalk, and that showing a series of cells attached at regular intervals on each side of a central axis. Miss DIXON's figure is on rather a small scale; and though the text states that the narrower part of each cell is prolonged into a duct which opens into the œsophagus, in the figure the cells do not come into any sort of relation with the œsophageal epithelium, and are separated from it by a membrane and a space; it seems possible, however, that the authoress, in the text, is meaning rather to quote the experience of others than her own findings.

The only well-executed lithograph, on an adequate scale, that I have seen is that in the plate which accompanies HESSE's earlier paper (3), before he adopted the view of the prolongation of each cell to form a ductule; there is here no suggestion whatever of such a mode of discharge as the author later came to believe in.

I may perhaps make one or two remarks on the wording of HESSE's descriptions. In the first (3), where there is no question in the author's mind of the prolongation of the cell into a long drawn-out canaliculus, his preparations are stated to be quite distinct in the sense in which he interprets them. In the following year (4), "a *glance* at my preparations *immediately* allows me to affirm" (italics mine) ("doch ein Blick auf meine Präparate liess mich diese Frage sofort bejahen") that such prolongations exist. Again, in the second paper of the same year (5), HESSE states that the cells are most drawn-out in the septal glands of *Enchytræids*—so much so, that without comparative observation ("ohne vergleichende Betrachtung") one can scarcely recognise the true nature of the glands; thus their true nature was not recognised by the earlier observers, nor, *in spite of favourable preparations* (italics mine) ("trotz meiner günstigen Präparate"), by the author himself in his earlier account. One might almost paraphrase these last sentences as meaning that anyone examining even favourable preparations with an unbiassed mind would not suppose that the cells were prolonged to form their own ducts.

I can, however, scarcely criticise HESSE's change of attitude—unless it is allowable to animadvert on the insistence with which he first assures us that his preparations are unequivocal in the one sense, and then that they are so in the opposite one—since I have myself been in two minds in the matter; whereas I formerly thought that in the *Naididæ* the pharyngeal cells were prolonged as far as the lumen, I no longer believe this to be the case. It hardly seems too much to say that even good preparations may suggest either one thing or the other, and that something, at least, depends on the ideas with which the observer comes to the examination.

If, then, the ordinary methods of histological investigation are liable to give equivocal results, it will be well to inquire if it is possible to supplement these in any way. The following procedures have suggested themselves to me:—

(1) An examination of developmental stages; for example, if, as HESSE supposes, the cells of the septal glands grow back from the pharynx, and push the peritoneal covering of the septa before them as they pierce through, some stages of this process ought to be discoverable.

(2) An investigation of the production of the gland-cells in asexual reproduction and regeneration; *e.g.* in the new formation of the head, including the pharyngeal region, which takes place in fission, the usual mode of reproduction in the *Naididæ*; and in the regeneration of the head end in *Lumbriculus variegatus*, where the fragmentation of the body and regeneration of missing parts is so frequent as almost to constitute a regular mode of reproduction.

(3) An examination of the exact sites of the occurrence of the cells; in certain cases it may happen that from their position and their close association with other structures a connection with the alimentary lumen is practically excluded.

Without exhausting the subject, I have endeavoured to add to the information to be gathered from ordinary morphological investigation something of that which may be obtained in the ways just suggested.

MATERIAL AND METHODS.

Most of the material used in the present investigation was obtained at Ascog on the island of Bute during the summer of 1920—some of it on the shore, some in ponds near at

hand on the island. For the hospitality of the Bute Laboratory I have to thank Prof. L. P. W. RENOUF, at that time Director.

The specimens of *Pristina æquiseta* which furnished the material for the description of the formation of the septal glands during asexual reproduction were taken by Professor W. N. WOODLAND at Allahabad, and were sent by him to the Indian Museum, whence I had them for identification; the method of fixation was not stated. I also owe the specimens of *Limnodrilus* sp. to the Indian Museum. I have also worked over again some of the material used in the papers of my own already referred to.

My Ascog specimens were fixed in either hot formalin (10 per cent.) or hot sublimate and acetic. The solutions were used hot (not boiling) in order to kill the worms before they had time to curl up so much as to make section-cutting difficult, as well as to ensure more rapid and effective penetration.

For staining, Delafield's hæmatoxylin with eosin as a counter-stain was found to be most generally useful. I also used Dobell's iron-hæmatein, iron-brazilin, picro-indigo-carmin, muchæmatein, and methylen blue—the last two in order to distinguish any mucin in the secretion of the gland-cells.

THE CELLS IN THE TUBIFICIDÆ.

(1) *Tubifex benedeni* Udek.

The chromophil cells are present in segments iii, iv, and v; they occur in masses which are applied by an extensive flat base to the anterior face of the septa; they are seen in numbers on the blood-vessels, especially in segment iv—the vessels sometimes have a fairly thick covering of the cells (fig. 1); they are found on the muscular fibres passing outwards from the pharynx in segment iii; and once a group of pear-shaped cells was seen attached by their narrow ends to the posterior face of one of the septa.

In shape the cells are oval, angular, pear-shaped, or irregular; the cell body, 30μ or more or less in long diameter, stains deeply and unevenly, and is granular and spongy in appearance, with the margins frayed out and disintegrating. The nuclei are 5μ in diameter, with central or excentric nucleolus, and chromatin granules peripherally arranged; a clear space is left round the nucleolus (fig. 2, and *cf.* fig. 3 of *Limnodrilus* sp.).

In some cases the granules in the interior of the cell have the appearance of arranging themselves in rows, so that the cell-body begins to take on a fibrillar structure; and in some cells the more deeply staining portion forms definite long threads, which may extend for some distance and constitute connections with other cells (fig. 2).

If a reference is made to my previous paper on the chromophil cells of earthworms (16), the great similarity of these cells to those there described will immediately be apparent. The cells of earthworms are large, the cell-bodies stain deeply but unevenly with nuclear stains, and disintegrate at their periphery; the vesicular nuclei are characterised by a large nucleolus and peripheral chromatin. The chromophil cells of the Tubificidæ are indeed more like those of earthworms than are those of any other family of Microdrili.

In the case of the cells on the posterior face of the septum there was no membrane of any kind passing over the free ends of the cells, which appear to be modified peritoneal cells. So, too, in the masses on the anterior face of the septa the cells take the place of the usual flattened peritoneal nuclei seen on the posterior face (*cf.* fig. 4); and on the blood-vessels also they appear to be modified peritoneal cells.

(2) *Tubifex insularis* Steph.

In a species of *Tubifex* closely related to the above, which I found at Ascog, and which I am describing elsewhere under the name of *T. insularis*, the chromophil cells again have the same very typical characters.

They are found in segments iii, iv, and v: on the anterior face of septum $3/4$ as a flattened mass, on the blood-vessels and muscular strands in segment iv, enveloping the blood-vessels in segment v, and as masses attached to the anterior face of septum $4/5$ by non-staining strands of tissue.

In one specimen a strand of tissue was seen, continued from one segment to another through the opening in the septum which transmitted the ventral vessel and œsophagus; this strand was continuous with chromophil cells in both segments iv and v (compare the septal glands of Enchytræids, and their connections from segment to segment by means of strands).

(3) *Clitellio arenarius* (Müll.).

The cells are numerous, and their characters well marked. There are occasional indications of fibrillar change.

They are present in segments iii, iv, and v. Here again they show a marked association with the blood-vessels, which they may in places entirely surround. They are also frequently attached in lobular masses or narrow fingerlike lobes to the septa; the attachment is often effected in a curious and characteristic manner—by narrow strands or stalks of connective tissue. These strands do not stain, or only slightly; they are nucleated, and are continuous with the bodies of their associated chromophil cells.

In segment vi, in one specimen examined, is a mass of the cells, intensely stained, closely associated with the ventral blood-vessel, and by its other surface closely apposed over a considerable distance to the surface of the high, clear, peritoneal cells that here clothe the gut. If the chromophil cells had connections with the gut lumen, these must have been obvious as deeply stained processes passing through the non-staining peritoneal layer towards the alimentary canal. Such processes are, however, absent.

In none of the specimens of Tubificidæ was anything seen which could suggest the possibility of a relation of the chromophil cells with the gut lumen; the conditions in the section which has just been described are indeed so particularly favourable for observation as to allow of its being used as a test-case.

(4) *Limnodrilus* sp.

In a not fully mature and therefore indeterminable species of *Limnodrilus*, sent to me by the Indian Museum, from Sona Sar, a small lake in Kashmir, at an elevation of 12,500 feet, chromophil cells occur in segments iv–vi; in places they form a continuous layer ventrally from side to side below the alimentary canal, and have their origin apparently from the floor, and perhaps also from the lateral walls, of their segments; they are present on the blood-vessels, and on septa $4/5$ and $5/6$ (on both sides of the latter). There are some interesting points in connection with the cells and their distribution, and the appearances seem worthy of detailed description (figs. 3, 4).

(a) *The Cells*.—These are large, but various in size; a small one measures 20 by 12μ , a large one 35 by 20μ . Their shapes are also various and irregular; they do not fit closely together. The nucleus of a large cell may measure 7 by 5.5μ ; the nucleolus is large, nearly

2 μ , homogeneous and not very deeply staining; round the nucleolus is a clear space, and the chromatin granules are peripherally situated. The cytoplasm stains deeply but unevenly; the darker staining portions are here amorphous or granular in constitution, not fibrillar or reticular. The boundary of the cell is sometimes indefinite, its periphery dissolving away into a granular faintly staining more or less scattered material which has no precise outline. A band of rather granular-looking structure may connect two neighbouring cells together.

(b) *Relation to the Body-wall.*—In a number of sections cells are seen on the parietes, and none elsewhere (fig. 3). They rest on the longitudinal muscular layer, though there seems to be usually a thin membrane or a few smaller (peritoneal or connective-tissue) nuclei intervening. A length of a blood-vessel is sometimes seen lying on the inner surface of the body-wall: such a vessel may be covered over by chromophil cells which lie directly on it. Some of the chromophil cells appear to be directly derived from the cells of the lateral line, and to be actually continuous with them (the cells of the lateral line are seen in a transverse section between the upper and lower halves of the longitudinal muscular coat, cf. fig. 13, *Lumbriculus variegatus*); so too in other specimens there appears to be continuity of the cell-body with the connective-tissue or peritoneal cells which lie on or between the muscle fibres of the longitudinal coat.

In many cases membrane-like expansions connect neighbouring cells, and bridge over the interval between them. There is no other covering membrane than these expansions, which stretch from cell to cell, and which are specialisations of the cells themselves—no separate peritoneal layer on the coelomic side of the cells.

Here and there a focus of proliferation of the chromophil cells may be discovered on the body-wall. A mass of nuclei is seen, like those of the developed chromophil cells except that the peripheral chromatin takes up a rather greater space; there are no cell-limits in the mass, the nuclei being crowded together with the intervention of only a small amount of lightly staining cytoplasm. Traced towards the coelom, cell-limits become established in the mass, the amorphous deeply staining substance appears, and the cells become typical chromophil cells.

(c) *Relation to the Ventral Vessel.*—The cells are also seen in transverse sections as winglike laterally situated masses on the ventral vessel. Each aggregate is roughly pear-shaped, the narrow end being attached to the vessel by the intermediation of one or two elongated cells, which are on the one hand continuous with the substance of the vessel-wall, and at their other ends, directly or by means of a band, with the chromophil cells.

(d) *Relation to the Alimentary Canal.*—Some cells appear to originate from the dorsal side of the ventral vessel, between the vessel and the alimentary canal. Cells of the winglike expansions just described may come into relation with the alimentary canal; other blood-vessels in the body-cavity may be the seat of masses of chromophil cells, and these masses also may impinge on the alimentary canal. In one place the appearances suggest that certain peritoneal cells surrounding the alimentary tube have been modified to form chromophil cells, in the same way as on the body-wall and on the blood-vessels. But there is no evidence whatever of any connection of the chromophil cells with the alimentary epithelium.

(e) *Relation to the Septa.*—In a series of longitudinal sections masses of the cells occur on septa 4/5 and 5/6 (fig. 4). Those next the septum are sessile, often by a broad base, on the muscular fibres of the septum, and thus take the place of the peritoneal layer which may be seen elsewhere, e.g. on the opposite side of the septum (fig. 4). Peritoneal elements are ordinarily absent over the surface of the cell-masses, though one or two oval or flattened nuclei, with the characters of those of the peritoneum, may be identified here and there on the coelomic face of the aggregates.

In these aggregates the chromophil cells may be mixed with others. Some of these are large, round cells, containing eosin-staining granules of some size, the nucleus with a small nucleolus scarcely distinguishable from the numerous chromatin granules; these seem to be of the nature of coelomic corpuscles. Also contained in the masses of cells are nuclei with the characters of peritoneal nuclei.

THE SEPTAL GLANDS IN THE ENCHYTRÆIDÆ.

In this family the chromophil cells occur exclusively in the form of septal glands—

Diagram illustrating position and relations of septal glands in the Enchytræidæ. *c.g.*, cerebral ganglion; *dors. gld.*, dorsal lobes of the three septal glands; *ph.*, pharynx (the dorsal projection of high columnar cells is indicated); *retr.*, retractor muscles of the pharynx; *sept.*, septum; *str.*, the strand which puts the glands in connection with the dorsal wall of the pharynx; *vent. gld.*, the ventral lobes of the septal glands, connected together by the posterior part of the strand; *v.n.c.*, ventral nerve cord.

cell aggregates on certain of the anterior septa, usually three pairs, connected to the alimentary tube by means of strands of tissue which begin behind within the hindmost gland of each side, and pass through the more anterior glands to become attached to the dorsal surface of the pharynx (*cf.* text-fig.).

I have, for the purposes of the present paper, examined the glands in *Enchytræus albidus*, *Marionina semifusca*, *Lumbricillus subterraneus*, *L. viridis*, and *L. pagenstecheri*, but it will not be necessary to describe them separately in all these species. Though descriptions already exist (*cf.* Historical section, VEJDOVSKY, MICHAELSEN, HESSE, STIRRUP), I give the following account, in order, as I think, to correct certain points and to emphasise

others which seem to me to be of importance, but which have not been noted by previous inquirers:—

(1) *Lumbricillus viridis* Steph.

The glands occur as large lobed masses in segments iv, v, and vi, a pair in each segment, one on each side of the oesophagus. They rest behind on the septum, which they cause to bulge backwards, so that they lie in a depression. Vertically, this posterior portion of the gland occupies the greater part of the height of the segment. In each segment the ventral part of the gland is prolonged forwards as a large rounded lobe, which in the case of the two posterior pairs reaches as far as the anterior septum of the segment in which it lies.

The cells (fig. 5) which compose these masses are of various shapes, determined by mutual accommodation—rectangular, oval, spindle-shaped, pear-shaped, triangular; they are 48–64 μ long, possibly in cases even longer, and of very various breadth. On the whole they fit closely together, sometimes showing linear interstices between individual cells.

They stain very unevenly, since the cytoplasm contains small patches or areas where granules of deeply-staining material are closely aggregated together. The nuclei are 5–6 μ in diameter; they show a large circular nucleolus, 2.5–3 μ in diameter, staining lightly and evenly, and of a somewhat pinkish tinge (eosin); around the nucleolus is a large clear space; the chromatin, if present, must be peripheral, but it is difficult to distinguish it from the granules of the surrounding cytoplasm, and in some cases there seems to be no peripheral chromatin. Some of the cells are continuous with the matter of the strand which leads off from or passes through the gland.

A peritoneal investment covers in the anterior face of the glandular mass. Posteriorly the masses rest against the septa, which in this species are (in the anterior region of the body) notably thick and muscular.

The strands, to which allusion has already been made, are stout cords .035–.05 mm. in thickness. They begin in the interior of the hindmost gland on each side, and pass forward through the ventral portion and anterior lobular extensions of the other glands (*cf.* text-fig.). Except where it is enveloped in the glands, each strand has a thin peritoneal investment. Each consists of a faintly-staining basis, which contains (*a*) a certain amount of more darkly-staining granular matter; (*b*) nuclei, oval in shape, 4–5 μ in long diameter, evenly staining; (*c*) remains of cells, which melt away at their periphery into the basal substance of the strands, or become continuous with this at their pointed ends. The whole strand has a faint, indefinite, longitudinal striation, due to the arrangement of the contained granular matter. Some of the cells around the strand melt into and are continuous with the strand tissue (fig. 5).

If the cell-remains included within the strands are still of some size, they possess the characteristic nuclei. But the cell-bodies are apparently undergoing disintegration, and as they do so the nucleus becomes homogeneous, and persists in this condition after the whole of the cell-body has disappeared. The first change in the nucleus is that the nucleolus becomes more refractile; the clear space round the nucleolus then comes to take up the stain, but evenly and to a moderate degree only; lastly, the nucleolus disappears; the cell-body has meanwhile disintegrated.

The pharynx of all Enchytræids presents a projection from the dorsal wall into the lumen—a raised plate consisting of a very high columnar epithelium. The nuclei, situated in a thick layer at approximately half the height of the cells, are also much elongated; the free surface is covered with a layer of cuticle; the bases of the cells form a well-marked even line, but my preparations do not show a definite basement membrane.

The strands leading forwards from the septal glands can be followed forwards in sections towards the dorsal surface of the pharynx (*cf.* text-fig.); there they split up amongst the numerous muscular bands which are inserted into the pharyngeal wall; and tracts composed of the material of the strands, staining only lightly, with the contained granules arranged in indefinite striæ, can be followed as far as the bases of the epithelial cells. Here the granules are seen to penetrate between the bases of the cells, and to scatter themselves in the basal region of the epithelium (fig. 6).

Another feature of the epithelium is a very regular layer of spindle-shaped bodies, not very deeply stained, about 3 μ in length and 1 μ thick, situated at a little distance from the free border; some of them (or most, in certain sections or specimens) are traceable upwards for some distance as fine lines, towards the level of the nuclei of the cells. The bodies are apparently aggregations of the granular matter of the strands, about to escape on the free surface of the pharyngeal epithelium. I have not myself seen any canalicules in the cuticle, and therefore think it possible that the granular matter can only escape on the surface when the cuticle is detached and renewed (as seems to be happening in fig. 6). The ovoid aggregates would represent the granular matter dammed up and waiting an opportunity to escape.

(2) *Marionina semifusca* (Clap.).

The posterior portions of the glands are enclosed within the septa, the anterior layer of the peritoneum passing in front of the cell-mass, and the rest of the septum—muscular and

posterior peritoneal layers—behind. The anterior lobe of the gland in each segment has (or may have—it is not obvious in all cases) a fine investment of peritoneum with nuclei.

The cells correspond to the description given for *Lumbricillus viridis*. They are continuous in many cases with the peritoneal capsule, a corner of the base of a triangular cell, or the narrow end of a pear-shaped cell, being drawn out into a process which joins the investing membrane. There is a distinct layer of peripheral chromatin granules in the nucleus.

Here again some of the cells merge into the slightly staining mass of the strands where these originate in or pass through the glands, in such a way that it is impossible to define the limits of the cell or to delimit it from the strand. In other cases the cells have rounded internal ends, and do not shade off into the strand. The constitution of the strand is as before.

In two specimens (out of six), parts of the cells, and the granules lying in the strands, have stained with eosin. Here the strands with their granules can easily be seen to reach the base of the pharyngeal epithelium; the granules penetrate in all directions within the epithelium, and are, as before, aggregated in small ovoid masses in a layer near the free surface. The recognition of the condition (described already for *L. viridis*) happens here to be rendered easier by the exceptional staining.

(3) *Young Enchytræids.*

Batches of young worms are sometimes met with, of small size and quite immature; I obtained a number of these, some not more than 2.5 or 3 mm. long, which I sectioned, in the hope of obtaining earlier stages in the history of the septal glands. In the absence of the sexual organs it is not possible to say to what species the worms belonged; but from their associations I believe they were young *Lumbricillus viridis*.

I obtained no very early stages in the development of the glands; but in the smallest specimens there are one or two features of interest.

(a) To the best of my observation, some of the glandular masses are not continued forwards by means of strands. This happens most frequently in the case of the most posterior glands, which are not joined to those in front, and hence gain no connection of any kind with the alimentary tube; I have never found the most anterior glands disconnected from the pharynx. It is naturally difficult, in longitudinal and still more in transverse sections, to be certain that there is no continuation forward of a few thin fibres, which might put one gland in connection with the next. But, however it may be in regard to actual connection, there can be no doubt that in a number of these young specimens the connecting strands, if present at all, are at least extremely tenuous, and present a marked contrast to the stout cords, half as thick as the anterior lobe of the glands, seen in the adults.

Between the anterior gland on each side and the pharynx the strands appear wrapped round by muscle fibres; many nuclei are embedded in the strands—indeed, the strands appear, in this part of their course, to be almost cellular cords.

(b) If the hindmost glands be examined in a series of transverse sections, beginning from their posterior ends, the first appearance of strand tissue is found near the anterior end of the gland; in other words, there is no such core of non-staining strand tissue extending into the centre of the gland as is found in adult specimens.

The conclusion from this observation and the last is that the strand tissue is a secondary formation, and makes its appearance later than the gland cells.

(c) In one specimen there appears to be a connection of one of the glands with the parietal peritoneum on one side. The appearances are consistent with (I will not say more in regard to this specimen) the supposition that the glandular cells are arising from the peritoneal lining (I describe below a similar condition in the developing head of *Pristina*; cf. also above, *Limnodrilus* sp., (b) "Relation to the Body-wall"). In another case the glands are apposed to (though not united with) the parietes over a considerable area—half or one-third of the periphery of the gland. I do not recall having seen this relation in any adult example. The inference would be that the glands originate from the peritoneal lining of the body-wall, and move to a more internal situation in the adult.

THE SEPTAL GLANDS IN THE GENUS PRISTINA (NAIDIDÆ).

Septal glands resembling in many ways those of the Enchytræidæ are found in two genera of Naididæ, *Pristina* and *Naidium*. I have examined them in two species of the former genus.

(1) *Pristina æquiseta* A. G. Bourne.

The masses of cells are not wholly "septal"; they are applied to the inner surface of the body-wall, from whence they extend on to the septa. As in the Tubificidæ, there seems to be no connection—I would say no possible connection—with the alimentary tract.

The specimens, stained with Dobell's iron-hæmatoxylin, show very well the cytological characters of the cells.

The glands, as seen in longitudinal section (fig. 7), are roughly hemispherical or lens-shaped. The chromophil cells measure up to 20μ ; their shapes are various—elongated and bipolar or pear-shaped, of the shape of an elongated triangle with curved sides, or massive and irregular. They are separated from each other by clefts, the clefts being filled up to some extent with fragments of the amorphous material into which some of the cells seem to fade away.

The cytoplasm stains deeply on the whole, but the depth of staining varies in different, even in contiguous, cells. The staining of any given cell is also unequal. The cell-margins may dissolve into grains or small blocks of amorphous matter, and the cell-outline may thus not be clearly definable.

The nuclei are subspherical, 2.5 – 3.5μ in diameter, the nuclear membrane being distinct. The nucleolus is large, more than 1μ in diameter, homogeneous, staining only slightly, somewhat excentric; the chromatin granules are peripherally situated.

The cell-masses are seated on the anterior faces of septa $4/5$, $5/6$, and $6/7$. With regard to their position and relations, the following observations are of interest:—

(a) In some cases there is actual continuity between the septum and the amorphous granular material at the periphery of the cell.

(b) There is no separate membrane enclosing the cellular mass in front; the cells themselves may join at their margins by membrane-like expansions, and form the limiting surface of the mass. Again, the septum against which the cells rest, and which in a number of cases passes down behind the cells as a thin membrane with a few slightly flattened nuclei, is in other cases not traceable over the whole of the posterior surface of the mass; where it is wanting, the hinder (as well as the anterior) surface of the gland is merely the surface of the cells of which it is composed, which here also may be united by expansions from their neighbouring edges.

(c) Sometimes two neighbouring cells at the surface of the mass appear not to be connected; but a connection may sometimes be discovered on focussing a little up or down. The surface of the mass would thus appear to be fenestrated, allowing coelomic fluid to percolate in the intercellular spaces.

(d) The cell-masses are ventrally and laterally sessile on the parietes near the ventral nerve cord, and lie in contact with the longitudinal muscular fibres of the body-wall.

(e) Pear-shaped cells with all the characters of chromophil cells are in places seen hanging from the parietes into the coelom, attached by narrow ends.

(2) *Pristina longiseta* Ehrbg.

The glands and the individual cells have exactly the characters described in the preceding species.

It should be mentioned that the chief point of difference between the septal glands of the Enchytræidæ and of the genus *Pristina* (and *Naidium*) is that in these latter there are no strands connecting the glands of the same side together and to the pharynx. There are indeed no connections of any kind between the cells and the alimentary tract; cell-processes passing independently across the coelom can be excluded from consideration.

(3) *The Septal Glands in the developing Head.*

The above species of *Pristina* reproduce (as do all the Naididæ) principally by fission, the anterior segments of the posterior of the two animals into which the parent divides being newly produced in a "zone of budding."

In *Pristina æquiseta*, sections through the zone of budding, in a case where the hinder of the two animals already has a proboscis of some length, and is therefore almost ready for separation, show the following features:—

Numerous cells are seen in the region of the glands (fig. 8). Their length is about 7–9 μ , with a maximum of 10 μ ; the nuclei are 3–3.5 μ in long diameter, are shortly ovoid, and constitute by far the clearest part of the cell. The nuclei have the characters previously described, but are much larger relatively to the cell-body; the nucleoli are possibly slightly larger. The cells as a whole, however, are considerably smaller than in the adult head, there is not the same difference between the light and dark parts of the cell, and the periphery is more definitely limited. The cell-body is not, however, homogeneous, and as a whole is noticeably darkly staining. The cells may be in places contiguous to the chloragogen cells of the gut-wall; but no signs of a transition between the two were seen, nor any extension of the cells towards the alimentary lumen.

In another specimen the attachment of the cells to and apparent origin from the parietes were well seen, a number of the cells being applied by broadish bases to the longitudinal muscular coat ventro-laterally in the segment. The same was the case in still another series of sections through a not fully differentiated head (fig. 9). The cells were seen in various situations, but were not aggregated into definite glands. A layer of the cells one cell thick is seen on the body-wall; most of them have blocks of deeply staining matter, and their other characters also mark them out as septal gland cells. They are totally separated from the gut.

In a specimen of *P. longiseta* which appears to have separated recently, the head having apparently not yet attained its full development, masses of the cells are seen on the body-wall; the cells seem to be arising from the peritoneal layer at this spot. There seems to be no possible connection with the alimentary tract.

THE PHARYNGEAL CELLS IN THE NAIDIDÆ.

(1) *Nais simplex* Piguet.

The pharyngeal cells of this species are squarish or rectangular, pear-shaped, or irregular in shape, and are of varying sizes; the largest are about 18 by 7 μ , a small one would be 9 by 7 μ , and the average size is about 11 by 9 μ . They are at once recognisable by their deep staining; the staining is somewhat uneven. The cell outlines are sometimes irregular, the margins appearing to be disintegrating. The nuclei are oval, 4.5 μ in long diameter, the chromatin being distributed as scattered grains. In general appearance the cells are similar to those of *N. communis*, represented in fig. 10.

Round the pharynx and the anterior part of the œsophagus the cells lie singly, isolated from each other, amongst the muscular fibres which pass backwards and outwards from this portion of the alimentary tube; and they may be seen at some distance from the wall of the canal, without appearing to be directly connected therewith. Further back, in segments v and vi, the cells form considerable lobed masses on the sides of the œsophagus, and are more closely compacted and more closely apposed to the alimentary wall by their flat surfaces, though there often is a narrow chink between the two.

A small pear-shaped lobe of a few similar cells may be seen sometimes, attached by its narrow end to a septum in the angle of its junction with the œsophagus.

I could never see a prolongation of the cell as a duct towards the alimentary lumen; the side of the cell which lies towards the tube is often flat, and there is often a slight interval between the cell and the alimentary wall—indeed, other structures may insert themselves between the two, *e.g.* muscular fibres, a flattened peritoneal nucleus, or some non-nucleated chloragogen fragments.

There is no peritoneal covering over the lobular masses in segments v and vi.

(2) *Nais communis* Piguet.

The cells (fig. 10) resemble generally those of *N. simplex*, and are seated by, for the most part, flattened surfaces on the basement membrane of the alimentary epithelium. The cell-body is composed of closely-aggregated deeply-staining granular matter, and there are no visible processes penetrating the epithelial layer.

Towards the hinder limit of their extent the cells, as represented in the figure, form compact lobular masses; in these the cells are closely applied to each other and to the alimentary wall. There are no peritoneal or chloragogen cells over the masses.

(3) *Stylaria lacustris* (L.).

The pharynx, as seen in transverse section, is prolonged upwards into a dorsal diverticulum, the lining of which, and of the dorsal wall of the pharynx, is a well-marked palisade epithelium. Over the pharynx, and extending back on to the œsophagus, the pharyngeal cells appear to be arranged in small pear-shaped or spherical masses; nine to twelve such lobular masses are seen in a section, and in each lobule are half a dozen or more cells. The lobules are 12–24 μ in height, the greatest diameter of the cells is 12–18 μ , and of the nuclei 3.5–4 μ . The cytoplasm stains sometimes a little unequally, but not very deeply, with hæmatoxylin; the nuclei are not obscured; there are indications here and there of a slight loosening of the margins of the cells. The nuclei show fairly

large grains of scattered chromatin, but a number have a distinct central particle with a small clear space round it; the central particle and surrounding space are much less distinct than the corresponding features in the cells of the septal glands of the Naididæ, or of the chromophil cells of the Tubificidæ.

The appearance of lobules seen in transverse section is explained by tangential sections (fig. 12). The cells are disposed along the alimentary tube in longitudinal rows, or double rows, which anastomose in places, and between which muscle fibres emerge; it is the sections of these rows that appear as lobules.

In some cases muscular fibres intervene between the base of the lobule and the epithelium of the pharynx or œsophagus. In other cases the base of the lobule is in contact with, and even appears to be continuous with, the epithelium, without demarcation; but I have never seen any duct-like prolongation penetrating between the epithelial cells of the lining of the canal. A spherical cell was seen to lie separate from the rest, attached to a muscular fibril, without any indication of a prolongation towards the pharynx; the nucleus of this cell, those of the lobules, and the peritoneal nuclei on the radiating muscular fibres and on the parietes are similar to one another, and differ from those of the pharyngeal and œsophageal epithelium, which are more elongated.

Behind the dorsal diverticulum the appearance of lobulation in transverse section becomes less regular, and ultimately ceases, while the cells, 12 by 8.5μ in size, are less closely applied to the alimentary wall, and are isolated or nearly so. Some cells can now be seen on the ventral surface of the tube. Just before the cells disappear, they are represented by a few ovoid or spherical cells lying on the muscular coat. Any connection with the lumen of the canal in this hinder region seems quite excluded.

(4) *The Cells in newly-forming Heads.*

On the differentiating pharynx of a posterior zooid of *Nais communis* there is, outside the pharyngeal epithelium, a thickish layer of cells apparently peritoneal in origin (fig. 11). These are closely apposed to the pharyngeal epithelial layer, but with a definite line of demarcation. The layer fades away in front and behind, and has the appearance of being a thickening of the peritoneal coat.

The cells are closely packed together, and cell outlines are not always distinctly visible. The nuclei are large relatively to the size of the cells, and are roundly ovoid, with the long axis of the nuclei parallel to the pharyngeal wall; while the long axes of the nuclei of the very crowded pharyngeal epithelial cells are at right angles to the pharyngeal wall. The differential staining is beginning in a number of the cells.

In a transverse section through the developing head of a posterior zooid of *Nais simplex*, at a younger stage than the above, a layer, mostly one cell thick, of apparently newly-proliferated small cells is present over the differentiating pharynx. These seem to be of the same character as those on the body-wall (where masses of embryonic cells form a small-celled mesoblastic proliferation which furnishes the material for much of the new intercalated segments), except that they are mostly larger than these latter, which are little else than nuclei. Their cytoplasm stains deeply, but so does that—the little there is—of the other newly-formed elements in the section. I would not call the appearances in this specimen decisive, though, taken in conjunction with those just described and with those in the adult, they are suggestive of the origin of the pharyngeal cells from proliferating mesoblastic elements.

THE CELLS IN THE LUMBRICULIDÆ.

Lumbriculus variegatus (Müll.).

In a series of transverse sections the cells make their appearance, few at first, a little way behind the mouth. They are situated in the loose texture of muscle fibres and blood-vessels which intervenes between the œsophagus and parietes (fig. 13). They are of various and irregular shapes, and here fit together without any interstices. The contour of the cells is on the whole definite, though traces of the loose granular margins so often seen elsewhere are indicated in places. The cytoplasm stains deeply and slightly unevenly.

Further back the cells become more numerous, and may in their total bulk in the section equal, or even exceed considerably all other structures (vessels and muscle fibres) in the body-cavity. Linear interstices are present between the cells, which now have looser margins with an appearance of disintegration, and a granular and often unequally staining cell-body. Their size varies—19–29 by 13–21 μ ; the nucleus is 7.5 μ or somewhat less in long diameter, and the chromatin is in scattered grains.

The groups of cells are often quite naked; sometimes a flattened nucleus may be seen lying on a mass of several chromophil cells. Sometimes the cells are in close association with blood-vessels, e.g. the dorsal vessel, and may partly wrap them round. A vessel may have on it here and there scattered ovoid peritoneal nuclei, and at one place a cluster of chromophil cells. Other groups of the cells cluster round and in between muscle fibres. None ever have the faintest sign of connection with the alimentary canal.

In a series of longitudinal sections an additional feature comes out. Masses of the cells are applied by a broad base to the anterior face of the anterior septa, and here take the place of the peritoneal coat, the nuclei of which are seen, as normally, on the posterior face of the septum. The appearances are similar to those seen in *Limnodrilus* sp. (fig. 4).

In a specimen in which the head end had been recently regenerated (evidenced by the absence of pigment), the cells are more various in size, but throughout are smaller than in the above examples, while their nuclei are relatively larger. The cells occur scattered amongst the peritoneal nuclei on the vessels and muscular fibres. Here and there are intermediate forms—nuclei with a small investment of deeply-staining protoplasm. Throughout they are at some distance from the alimentary canal: indeed the cells are neither in contact with the alimentary tube, nor is there any sign of a connection. The association of the cells is rather, in both this and the adult examples, with the vessels and muscle fibres.

DISCUSSION AND SUMMARY.

In certain of the anterior segments, most commonly iv, v, and vi, of the four families of Microdrili investigated (Tubificidæ, Enchytræidæ, Naididæ, and Lumbriculidæ) there are thus regularly present in considerable numbers large cells, the bodies of which stain deeply with nuclear dyes (chromophil cells). Some, at least, of the cells, and usually by far the greater number, have an indefinite outline, owing to the disintegration of the periphery of the cell and its transformation into loosely granular, amorphous, or sometimes fibrillar material.

While the above characters are common to all the cells described in the present paper, a distinction may be made between two groups according to the features of the nucleus. In the one group the nucleus has a large, lightly-staining nucleolus, around which is a clear space, and the granules of chromatin are peripherally situated. In the other no nucleolus is to be seen, and the chromatin granules are scattered throughout the nucleus. To the first group belong the cells of the Tubificidæ and Enchytræidæ, and those of the septal glands of

the Naididæ (*Pristina*, and doubtless *Naidium*); these are identical in cytological characters with the corresponding cells of earthworms; to the second, the cells of the Lumbriculidæ and the pharyngeal cells of the Naididæ. The pharyngeal cells of *Stylaria* (Naididæ) are to some extent intermediate between the two groups; some of these have a small nucleolus with a small free space round it (indicated in some of the cells in fig. 12).

The questions of chief morphological interest in respect of these cells are two: (1) Do they connect with and discharge a secretion into the lumen of the alimentary canal? (2) If so, are they cells of the alimentary epithelium which have left their place, or what is their origin? (3) If not, what is their function?

(1) *The Connection with the Alimentary Canal.*

A reference to the body of the paper will show that I have never found a connection (compare what I have described as a "test-case" in *Clitellio arenarius*), and consider it to be out of the question, in the Tubificidæ and Lumbriculidæ; for the sake of illustration, I may point to fig. 3 (*Limnodrilus* sp.) and fig. 13 (*Lumbriculus variegatus*). Cell processes do not span the coelom to join the alimentary epithelium; such processes would, of course, be continually in danger owing to the mobility of all the parts concerned. And there is no evidence that such cells as those shown on the muscular fibres in fig. 13 send processes along these fibres to the body-wall, thence along the body-wall to the septa, then along the face of the septa to the alimentary tube, which is the alternative. I come to the same conclusion from the histological evidence in the case of the septal glands of *Pristina* (Naididæ) also.

The matter is not quite so easy in regard to the pharyngeal cells of the Naididæ. Here the association with the alimentary canal is much closer (figs. 10, 12), and there is not the same improbability of the occurrence of connecting processes as in the previous cases. I can only say that I have never seen processes of the cells dipping between the pharyngeal epithelial elements; in fig. 12, for example, a basement membrane, or a thin fibrous layer, intervenes.

It is less easy still in regard to the septal glands of the Enchytræidæ. There is a stout connection between the glands and the dorsal wall of the pharynx in the form of the "strand" on each side, to which frequent reference has been made. Around the origin of the strands there are disintegrating gland-cells, and in the strands, in a non-staining basal substance, there are disintegrating cells in all stages, nuclei—the last remnants of the cells, and granular matter resulting from their disintegration (fig. 5). But the cells of the gland do not, so far as I can discover, give off processes into the strand; no such threadlike prolongations are discoverable in the strands—much less are the strands wholly composed of such prolongations.

The matter resulting from the breaking up of the cells is possibly liquid (the granular appearance being perhaps due to coagulation by fixatives), and able to percolate down in the substance of the strand. It reaches the epithelium, between the cells of which it penetrates, forms the "pockets" illustrated in fig. 6, and discharges into the lumen, possibly at such times as the cuticular covering of the cells is shed and renewed. The process is similar in many respects to what has been shown by KEILIN (6) to take place in earthworms (compare, however, what follows, and the concluding section of the present paper).

But this is a very different thing from saying that the cells are prolonged as canalised processes—each cell forming its own duct by means of an immensely lengthened neck. For (1), as just said, I have found no indication of such cell processes in the strand. (2) The cells, where they abut on the core, or beginning of the strand within the gland, show no evidence

of giving off such processes—rather is their outline indefinite, merging into the strand tissue. (3) Where the strand discharges its matter into the pharyngeal epithelium, the matter is seen in fixed preparations as free granules (fig. 6) and is not contained in tubular canals, however fine. (4) The septal glands, and the cells of which they are composed, are in *Pristina* similar in all respects to those of the Enchytræids; but here there are no strands. The same is the case with the septal aggregates of similar cells, usually less definite than those of the former groups (though also called septal glands by authors) of the Tubificidæ. And in the Enchytræidæ themselves in the youngest specimens which I have been able to examine, I have found the strands passing forwards from the posterior glands either absent, or at any rate of extreme tenuity.

As to what the basal substance of the strand is, I am not certain. The only hints I can discover are derived from the observation in *Tubifex insularis* of a small strand passing through the ventral aperture of a septum and connecting two groups of chromophil cells, one in each segment; and, in *Clitellio arenarius*, of small non-staining strands attaching groups of cells to the septa, nucleated, and in continuity with the bodies of the cells themselves. These, and the immensely more developed strands of the Enchytræidæ, may originate in the transformation of peritoneal cells; thus in very young Enchytræids the nuclei in the anterior part of the strand, near the pharynx, are very numerous, so that at this time the strand is more cellular than later.

(2) *The Origin of the Cells.*

In the genera which I have investigated, the cells are associated (except in the case of the pharyngeal cells of the Naididæ) not so much with the alimentary canal as with the septa (with which their substance may be continuous, *e.g.* in *Pristina æquiseta*), the muscle strands, and the blood-vessels of the anterior part of the body (*e.g.* figs. 1, 13). This association with the blood-vessels comes out especially in the Tubificidæ—in *Tubifex*, markedly in *Clitellio* and in *Limnodrilus* (here especially with the ventral vessel); I had previously found it to be the case in *Limnodrilus socialis*.

The cells are also found on the body-wall (*e.g.* figs. 3, 9), where, even in the adults, they have the appearance of originating; in *Limnodrilus* sp., foci of proliferation were found on the parietes. In the developing head of *Pristina* (two species) this is also the case. In the adult *Pristina æquiseta* some of the cells hang from the body-wall into the cœlom, attached by their narrow end. In very young Enchytræids the septal glands may rest on the body-wall by one of their surfaces, while in the adult they are entirely suspended in the cœlom; this points to an origin of the gland cells from the parietes.

There are numerous indications, in both adult and developing specimens, that the cells belong to the peritoneal layer—are differentiated from it, or, it may be, take its place. The relations of the cells on the blood-vessels (compare what is said under *Limnodrilus* sp., and *Lumbriculus variegatus*), of those on the body-wall (figs. 3, 9), and especially those on the septa, seem to show this. On the septa (figs. 4, 7, 8) they take the place of peritoneal cells over the area which they cover; they correspond on the one side of the septum to the peritoneal cells on the other (fig. 4, and note the solitary chromophil cell on the posterior side of the septum; cf. *Lumbriculus variegatus* also). In the regenerating head of *Lumbriculus variegatus* the developing chromophil cells are scattered amongst the peritoneal nuclei, and intermediate forms between peritoneal and chromophil cells occur. Fig. 12 shows how the pharyngeal cells of *Stylaria* take the place of the peritoneal layer on the dorsum of the pharynx; fig. 11 shows how, in the developing head of *Nais*, the cells arise as a modification

of this layer. An interesting condition is that of the septal glands in *Pristina*, where the septum may actually be absent both in front of and behind the mass of septal cells; these thus take the place of the septal membrane—in other words, they constitute the septum at these places. It may be recalled that NOMURA believes the cells in *Monopylephorus* to be differentiated from the peritoneum, and finds intermediate forms. And in the “septal sacs” of *Limnodrilus gotoi* (where again there is an intimate relation to the blood-vessels) chloragogen cells occupy the entrance to the sac, smaller cells with a dense cytoplasm (which I take to be chromophil cells) the bottom of the sac, and between the two are cells with intermediate characters.

(3) *The Function of the Cells.*

The chromophil cells of the Microdrili may form very considerable masses; in some of the Enchytræidæ they are the bulkiest and most conspicuous organs in the body; and it seems safe to say that their function must be one of some importance to the animal.

KEILIN (6) finds that the corresponding cells in earthworms secrete mucin. He quotes WILLEM and MINNE, who extracted from the pharynx of earthworms a substance which digested fibrin in alkaline media and produced peptone; these authors concluded that this substance was produced by the pharyngeal cells (i.e. the chromophil cells), since these were the only ones whose structure was compatible with a glandular function.

This does not, perhaps, help us very much in deciding the function in the Microdrili, where (except in the Enchytræidæ) there is no discharge of secretion into the alimentary tube. The secretion is an “internal secretion,” and is given off into the coelom.

It is to be remembered that the coelom, with its contained coelomic fluid, is of far more importance in the economy of the Annelids than in that of Vertebrates. In the latter, the cavity is merely virtual; and the whole of the coelomic fluid—pericardial, pleural, and abdominal—does not in man exceed a few drachms. The functions of the Annelid coelom are nutritive and circulatory, phagocytic and excretory, and reproductive. There seems to be no evidence of a phagocytic and excretory function of the chromophil cells. In earthworms and Enchytræids, however, the cells are brought into relation with the alimentary canal and discharge into it; and it is perhaps allowable to conclude from this that the function of the cells in the Microdrili in general, where they discharge into the coelom, comes under the first of the above heads—nutritive and circulatory.

The body-cavity in these worms is often crowded with coelomic corpuscles. These must live their life, die, and disintegrate. While the excretory products they contain will then be dealt with by other organs, there must be a good deal of matter set free which is of potential value to the organism and capable of being worked up again into assimilable substances. May it be that the secretion of the chromophil cells acts on the broken-down corpuscles, digesting and preparing for absorption such portions as may be capable of reassimilation?

I conclude therefore that—

(1) There are in the anterior segments of the Microdrili numerous cells with distinctive and easily recognised cytological characters, of which the deep colouration of the cell-bodies with nuclear stains is the most obvious (chromophil cells).

(2) These cells are associated with the blood-vessels, muscular strands, septa (“septal glands”), body-wall, and pharynx and oesophagus (“pharyngeal glands”).

(3) In most cases the cells have no relation to the alimentary tube; but in the pharyngeal glands the cells rest on the pharyngeal or oesophageal wall; while the septal glands of Enchytræids (but not of other families) are connected to the dorsum of the pharynx by a strand of non-staining tissue on each side.

(4) Only in the Enchytræidæ do the cells discharge into the lumen of the alimentary tube. Their products—largely disintegration products—here percolate down the strands and penetrate between the cells of the dorsal wall of the pharynx.

(5) In none of the species examined, not even in the Naididæ, where the pharyngeal cells rest directly on the pharynx, do cell processes penetrate the alimentary wall to take their places in the epithelial lining of the tube. The cells therefore (except in the case of the Enchytræidæ) constitute “ductless glands,” and their secretion (largely disintegration products) is an “internal secretion,” which, in most cases, mixes with the coelomic fluid.

(6) The cells themselves arise, not as cells of the alimentary epithelium which have migrated inwards, but from the peritoneal lining of the coelom.

A NOTE ON THE PHARYNGEAL CELLS OF EARTHWORMS.

My paper of 1917 (16), to which I have already referred, was followed in 1920 by that of KEILIN (6), who criticises my results, and comes to conclusions in some respects opposed to mine.

In my paper I showed that these cells had usually been considered “as gland-cells belonging to the epithelial layer of the alimentary tube, and . . . supposed to pour their secretion into the lumen of the tube *by means of long fine ductules prolonged from the cell-body.*” According to my observations the cells were not of epithelial origin, and had no connection with the pharyngeal epithelium; nor did I see any structures that could be interpreted as ductules; the cells originated, I thought, from the peritoneal, not the epithelial layer, and were therefore mesoblastic. I rejected the usual supposition that the cells poured a secretion into the alimentary canal.

In this last point I was mistaken; KEILIN has amply demonstrated that a secretion which contains mucin is produced by the cells, and reaches the pharyngeal lumen.

With regard to the ducts, KEILIN, speaking (p. 45) of the “gradual passage of the glandular salivary cells into the salivary or mucin ducts,” might be supposed to agree with those authors who described “long, fine ductules prolonged from the cell-body.” This is, however, not so; the conducting channels are “large mucin ducts,” which “subdivide and pass gradually into smaller ducts”; finally “numerous small ductules . . . penetrate between the epithelial cells.” The ordinary arrangement of ducts and ductules is thus reversed; instead of small ductules joining to form larger ducts and these again combining, the large ducts are supposed to split up into progressively smaller canals.

But are the channels in which the secretion flows ducts or ductules at all? My own impression, on reading KEILIN’s paper, was that they were channels amongst the thick felt of muscular fibres which pass upwards and backwards from the dorsum of the pharynx, to which, perhaps, the name “ducts” might loosely be applied, but that they were not tubes with definite walls—not, in short, definite structures at all; in other words, that the channels were functionally, though not structurally, ducts. There is not much in the way of description of the ducts in the text (p. 45); but Dr KEILIN was kind enough to answer my enquiry by saying that “the salivary secretion which is constantly formed in the glandular cells does not flow in the mere intermuscular spaces, but in the real ducts. The walls of these ducts are very thin, reduced almost to a protoplasmic layer with its nuclei. The latter are shown in all of my figures.”

I must, however, confess to being still unconvinced that there are any ducts in the usual sense, *i.e.* tubes with their proper walls. (a) There is in KEILIN’s paper no indication

of how they begin—of their relation to the cells at their origin; the “gradual passage of the glandular salivary cells into the salivary or mucin ducts” does not seem to me to be satisfactory—it would serve as a description of the origin of a fine canalicular cell process, but does not apply to the wide spaces shown as ducts in, *e.g.*, KEILIN’s fig. 6. (*b*) The “ducts,” according to KEILIN’s description, differ from glandular ducts in splitting up into successively smaller ductules; the reverse is the case with the canals usually described as glandular ducts—smaller unite into successively larger. (*c*) There are no indications of the “walls” mentioned in Dr KEILIN’s letter (but not, I think, in his paper) in any of his excellent figures. Nuclei, of course, there are in this region. I have treated of them at length (16, pp. 264, 265, 266, etc.); but these appear to me to be connective-tissue nuclei, derived, in part at least, from the disintegrated chromophil cells themselves. I have re-examined my own preparations again without finding any sign of ducts with proper walls of their own.

Dr KEILIN’s figures (*e.g.* figs. 3, 5, 6, 7) appear to me to show exactly what the “ducts” are. There is no question of the existence of ductules as fine canalised processes—immensely elongated necks, in fact—of cells which retain in this way their original connection with the epithelial layer of the pharynx—of such a connection with the pharynx and of such ductules as I particularly had in mind in writing my former paper.

With regard to the origin of the cells, KEILIN says: “STEPHENSON’s statement that they are derived from the peritoneal layer appears to me to be doubtful. His description, and especially his figures, do not give the slightest support to this opinion.” This is a little overwhelming. I would hope, however, that it is not quite as bad as that; and that the pages (pp. 274–281) in which I trace the cells in successively younger specimens of earthworms back to embryo taken from the cocoon may still be allowed some value. But as Dr KEILIN does not descend to detail, I must be content to leave the pages as they are, and trust myself to the judgment of those who read them.

The results arrived at in the present communication are in many respects similar to those of my former paper. In the following ways, I think, they confirm them:—

The chromophil cells in the Microdrili, as in the earthworms, have no direct connection by means of long drawn-out necks with the alimentary epithelium. In the case of the Microdrili, the distribution of the cells in many cases makes the decision on this point easy; and where at first sight there seems a possibility of such a connection, closer observation fails to substantiate it.

The condition of the septal glands of Enchytræids is—although the gland cells are there much further removed from the pharynx—closely comparable with that in earthworms. I believe that in both, while the secretion certainly reaches the pharyngeal lumen, it does so, not by definite ducts, but by percolation along the strands in the one case, and through the felt of muscular fibres in the other.

In the Microdrili the cells arise, as far as I have been able to observe, from the peritoneal lining of the coelomic cavity; and this, I think, helps to confirm my previously expressed opinion as to the origin of the cells in earthworms, that they are mesoblastic, and not cells of the alimentary epithelium which have retreated inwards.

REFERENCES TO LITERATURE.

- (1) BEDDARD, F. E., *A Monograph of the Order of Oligochaeta*, Oxford, 1895.
- (2) DIXON, G. C., "Tubifex," *L.M.B.C. Memoir*, No. xxiii, 1915.
- (3) HESSE, R., "Beiträge zur Kenntnis des Baues der Enchytraeiden," *Zeit. f. wiss. Zool.*, vol. lvii, 1893.
- (4) — "Über die Septaldrüsen der Oligochaeten," *Zool. Anz.*, vol. xvii, 1894.
- (5) — "Zur vergleichenden Anatomie der Oligochaeten," *Zeit. f. wiss. Zool.*, vol. lviii, 1894.
- (6) KEILIN, D., "On the Pharyngeal or Salivary Gland of the Earthworm," *Quart. Journ. Mic. Sci.*, vol. lxxv, 1920.
- (7) MICHAELSEN, W., *Untersuchungen über Enchytraeus möbii Mich., und andere Enchytraeiden*, Kiel, 1886.
- (8) — "Die Oligochäten der deutschen Tiefsee-Expedition," in *Wissenschaftliche Ergebnisse der deutschen Tiefsee-Expedition*, Jena, 1902.
- (8A) MICHAELSEN, W., "Die Oligochäten der deutschen Südpolar-Expedition," *Ergebnisse*, iii, 1905.
- (9) — "Hamburgische Elb-Untersuchung: IV. Oligochaeten," *Mitt. Mus. Hamburg*, vol. xix, 1903.
- (10) — "Oligochaeta," in *Beiträge zur Kenntnis der Land- und Süßwasserfauna Deutsch-Südwestafrikas*, Hamburg, 1914.
- (11) NOMURA, E., "On Two Species of Aquatic Oligochaeta," *Journ. Coll. Sci. Tokio*, vol. xxxv, 1913.
- (12) — "On the Aquatic Oligochaete *Monopylephorus limosus* (Hatai)," *Journ. Coll. Sci. Tokio*, vol. xxxv, 1915.
- (13) STEPHENSON, J., "On *Branchiura sowerbyi* Beddard, and on a new species of *Limnodrilus* with distinctive characters," *Trans. Roy. Soc. Edin.*, vol. xlviii, 1912.
- (14) — "On *Hæmonais laurentii*, n. sp., a representative of a little-known genus of Naididae," *Trans. Roy. Soc. Edin.*, vol. l, 1915.
- (15) — "Fauna of the Chilka Lake: Oligochaeta. Supplementary Report," *Mem. Ind. Mus.*, vol. v, 1917.
- (16) — "On the so-called Pharyngeal Gland-cells of Earthworms," *Quart. Journ. Mic. Sci.*, vol. lxii, 1917.
- (17) STIRRUP, H. H., "A Descriptive Study of an Oligochaete Worm of the Family Enchytraeidae; with an Appendix on certain Commensal Protozoa," *Proc. Zool. Soc.*, i, 1913.
- (18) UDE, H., "Beiträge zur Kenntnis der Enchytraeiden und Lumbriciden," *Zeit. f. wiss. Zool.*, vol. lxi, 1895.
- (19) VEJDovsky, F., *System und Morphologie der Oligochaeten*, Prag, 1884.

EXPLANATION OF FIGURES.

Chrom., chromophil cells; *circ.*, circular muscular layer of body-wall; *corp.*, coelomic corpuscles; *cut.*, cuticle of pharynx, apparently disintegrating and being cast off; *d.v.*, dorsal vessel; *ep.*, surface epithelium; *ll.*, lateral line; *long.*, longitudinal muscular layer of body-wall; *m.* muscular fibres attached to dorsum of pharynx; *æs.*, oesophagus; *pal.*, palisade margin of pharyngeal epithelium; *per.*, peritoneal cells or nuclei; *ph. ep.*, pharyngeal epithelium; *secr.*, secretion of septal glands penetrating between epithelial cells of dorsal projection of pharynx; *sept.*, septum; *set.*, seta in setal sac; *str.*, "core" of strand tissue; *v.n.c.*, ventral nerve cord; *v.v.*, ventral vessel; *x.*, minute aggregations of secretion near the free surface of pharyngeal epithelium.

Blood-vessels tinted red throughout.

Fig. 1. Longitudinal section through segment iv. of *Tubifex benedeni*; on the surface are seen the small papillæ characteristic of the subgenus (*Pelosclex*) to which this species belongs. The section passes at some distance from the middle line, hence the apparent thickness of the longitudinal muscular coat. $\times 240$.

Fig. 2. A group of chromophil cells of *Tubifex benedeni*, in which the deeply staining matter is arranged largely in the form of fibrils. These cells were near the ventral nerve cord. $\times 620$.

Fig. 3. *Limnodrilus* sp., part of a transverse section through an anterior segment, showing the body-wall with chromophil cells resting on and apparently originating from it. $\times 400$.

Fig. 4. *Limnodrilus* sp., a group of chromophil cells on septum 5/6, in longitudinal section. $\times 385$.

Fig. 5. Part of a septal gland of *Lumbricillus viridis*, in longitudinal section; there is a "core" of strand tissue, the cells around the "core" fade off into it; in the strand tissue are disintegrating cells, nuclei of cells which have disappeared, and granules of stainable substance. The nuclei of the gland-cells are largely obscured by the deep staining of the cell-bodies. $\times ca. 350$.

Fig. 6. Part of the dorsal pharyngeal projection of *Lumbricillus viridis* in longitudinal section, showing the matter which has descended the strand from the septal glands making its way between the epithelial cells, and accumulating in minute "pockets" near the free surface. $\times 250$.

Fig. 7. *Pristina æquiseta*, longitudinal section through the septal gland of segment vi. (Dobell's iron-hæmatoxylin.) $\times 500$.

Fig. 8. *Pristina æquiseta*, longitudinal section through a young aggregate of chromophil cells in the not fully formed head of a zooid about to separate. (Dobell's iron-hæmatoxylin.) $\times 1000$.

Fig. 9. *Pristina æquiseta*, transverse section through the body-wall in the dorso-lateral region, in the not fully differentiated head of a zooid preparing to separate. There are no aggregates on the septa. (Dobell's iron-hæmatoxylin.) $\times 1000$.

Fig. 10. *Nais communis*, transverse section through œsophagus with chromophil cells attached as lobular masses. $\times 580$.

Fig. 11. Longitudinal section through the differentiating head of a zooid of *Nais communis* which is about to separate. The figure shows the dorsal wall of the pharynx, with the first appearance of chromophil cells on it. The pharyngeal epithelium is apparently being renewed, and a number of the old and degenerating nuclei are seen near the free border, while the new and deeply staining nuclei are being formed near the base of the cells. $\times 680$.

Fig. 12. Somewhat oblique but approximately longitudinal section through part of the dorsal wall of the pharynx of *Stylaria lacustris*. The pharynx is passing at the right-hand end of the figure into the œsophagus. The palisade margin ("Stäbchen") of the epithelium is seen. $\times 620$.

Fig. 13. The half of a transverse section through an anterior segment of *Lumbriculus variegatus*, showing the association of the chromophil cells with the blood-vessels and muscular fibres. $\times 230$.

Where the mode of staining is not stated, Delafield's hæmatoxylin and eosin have been used.

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J. S.

J. STEPHENSON: SEPTAL AND PHARYNGEAL GLANDS OF THE MICRODRILL.