VIII. THE LYMPH GLANDS IN THE GENUS *PHERETIMA* WITH A NOTE ON THE COELOMIC ORGAN OF BEDDARD.

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(Plate VI.)

In the common Indian species of earthworms of the genus *Pheretima* there occur on either side of the dorsal vessel throughout the intestinal region a series of segmentally arranged whitish structures which constitute a prominent feature in the ordinary dissection of the animal. Since this genus is usually taken as a type for study in the Colleges of Northern India, and since but little has been published on these organs, I determined, at the suggestion of my Professor, Lieutenant-Colonel J. Stephenson, to investigate them in the three common species of *Pheretima* which occur in Lahore, *P. posthuma* (L. Vaill.), *P. heterochaeta* (Mchlsn.), and *P. hawayana* (Rosa). My grateful acknowledgments are due to Colonel Stephenson for the help and suggestions which I received from him in the course of my work.

Beddard, whose monograph sums up what was known on the Oligochaeta prior to 1895, speaks of these structures along with certain others in other worms as " Coelomic Organs of problematic nature " ; " in certain Perichaetidae there are a series of minute paired whitish bodies lying one on either side of the dorsal vessel in the middle region of the body, and springing from the septa (in *P. indica*), or from the dorsal vessel itself (*P. dyeri*). These bodies are quite solid, consisting of a mass of cells surrounding a few muscular fibres." *P. indica* is probably the species now known as *Pheretima heterochaeta*, and *P. dyeri* a synonym for *P. rodericensis*.

G. Schneider published (*Zeit. f. wiss. Zool.*, LXI, 1896) a paper entitled " Ueber phagocytäre Organe und Chloragogenzellen der Oligochäten " (I have not seen his preliminary account, published in Russian with a German abstract in *C. R. Soc. Natural. Pétersbourg* of the previous year). He also investigated *P. indica* and *P. dyeri*, and in addition *P. barbadensis* (a subspecies of *P. hawayana*). According to Schneider the dorsal vessel, at the place of origin of the glands in each segment, lies in a sheath, which is a funnel-shaped forwardly directed diverticulum of the septum ; the glands arise from this sheath. The sheath is deficient at a small opening on each side, and from the margins of this opening muscular fibres branch out into the gland ; the adjoining segments communicate with each other through this opening. The muscular fibres form the frame-work of the gland, which is not a solid mass, as Beddard states, but a tree-like branching structure, whose twigs in older examples lie so close that the whole gives the impression of a lobed

cell-mass penetrated by numerous canals and lacunae. The cells are thickened peritoneal epithelium ; their nuclei are similar to those of the peritoneal cells on the one hand, and those of the leucocytes on the other ; the outer cells of the gland form rounded projections into the peritoneal cavity. Foreign bodies and parasites are found in the glands, and also setae covered with a thick layer of leucocytes ; in addition to the leucocytes, which make up the bulk of the cells of the glands, there are also large clear cells, with small deeply staining and apparently shrivelled nucleus ; these are sometimes full of small round refractile granules, and mostly occur in small aggregates surrounded by leucocytes ; they are probably dead chloragogen cells. Fresh chloragogen cells are also present in the glands. The author obviously considers the gland-cells to be merely heaped around the supposed opening in the septum. He also describes similar organs in certain Lumbricidae, and performed a number of interesting physiological experiments in order to ascertain the function of the glands ; he comes to the conclusion that they are phagocytic organs.

Schneider introduced the name " lymph-glands " for these structures, which is quite appropriate.

Lloyd (*An Introduction to Biology for Students in India*, 1910) after describing the naked-eye characters of the organs, says " The function of these glands is unknown ; they consist of a mass of nucleated cells, which may be blood cells, or phagocytes in a state of development." He calls them " blood glands," an unsuitable term, which had better be dropped, especially as there are definite blood glands in some species of *Pheretima* (Lloyd's " oesophageal glands ").

METHODS.

The technique employed was the following :—The dissections made in order to describe the form and situation of the glands were made under the binocular dissecting microscope. The worms for sectioning were kept for a week and fed during this time on damp blotting paper renewed daily ; they were then narcotized, and fixed in 10 per cent. formalin for 24 hours, then washed and passed through graded alcohols ; some were cut into pieces and fixed in warm sublimate and acetic for an hour, then washed several times in distilled water and passed through graded alcohols.

The sections were first overstained with Delafield's haematoxylin and then differentiated in acidulated water (five drops HCl to 100 cc. distilled water ; I used acidulated water in preference to acid alcohol because in the latter case there is no graduated and regular transference of the sections from a watery to an alcoholic medium). After passing through graded alcohols up to 90 per cent. the sections were counter-stained in alcoholic eosin (1 per cent. eosin in 90 per cent. alcohol for one minute), then dehydrated and cleared in the usual way.

I also used Dobell's iron-haematein method (*Arch. Protistenkunde*, XXXIV, 1914). Films of the coelomic fluid, which I examined in the course of my work, were fixed in either sublimate or absolute alcohol, and stained in a similar manner to the sections.

THE GLANDS AS SEEN IN DISSECTION.

Pheretima hawayana.—The lymph glands are a double series of whitish bodies, situated on either side of the dorsal vessel, lobulated, segmentally arranged, beginning in segm. xxvi. In the anterior portion of their extent they occupy the posterior third of each segment, and extend from the dorsal vessel outwards about half way towards the lateral margin of the intestine. As we pass backwards they enlarge, until in the middle region they cover the greater part of the intestine in each segment (fig. 1). Still further back they diminish again, and ultimately they totally disappear in the last two or three segments. Each consists of a large number of very closely set small lobules.

The septa are pouched forwards where they cross the dorsal vessel so that the dorsal vessel is here enclosed in a tube-like sheath, the cavity of which is part of the cavity of the segment behind the septum. It is to the walls of this pouch that the glands are connected.

On some of the glands a number of small white bodies are to be seen, which on examination are found to be the cysts of the spores of a Gregarine,—probably of the *Monocystis* found in the seminal vesicles.

Pheretima heterochaeta.—The glands begin in segm. xvii. In the anterior part of their extent they appear attached by a short stalk; behind, the glands enlarge and a stalk is not to be distinguished; at the hinder end the glands of a pair meet and fuse over the dorsal vessel and below it, so that the vessel is enclosed by the glands. The glands are of simpler form than in *P. hawayana*,—not lobulated in the same way; though towards the hinder end a number of lobes, with a digitate arrangement, may be present (fig. 2).

Pheretima posthuma.—The glands begin, as in *P. hawayana*, in segm. xxvi; the lobulation and variations in size correspond to what was found in that species; some of the glands also show the spore cysts of Gregarines.

HISTOLOGY OF THE GLANDS.

A detailed description need only be given for one species; for this purpose I choose *P. hawayana.*

The lobules of the gland surround a central cavity, and this cavity opens into the cavity of the sheath round the dorsal vessel at this region; the interior of the gland is therefore morphologically in connection with the cavity of the segment behind that in which the gland itself lies. Fig. 3, actually drawn from *P. posthuma*, will illustrate this relation.

The boundary of the gland consists of an extremely fine membrane, in which nuclei appear at intervals as flattened swellings; these ovoid nuclei contain a deeply staining granule ("pseudonucleolus"), as well as fine irregularly distributed chromatin particles; the protoplasm surrounding the nucleus appears to be fibrillar in structure, and is continued into the membrane which forms the boundary of the gland.

Besides this bounding membrane, the interior of the gland is traversed by a reticulum, sometimes comparatively sparse, of the same character,—much flattened cells joined end to end,—and continuous with the limiting membrane, or capsule, as it may be called. In the centre of the gland this reticulum is almost or quite absent, so that there

is there an uninterrupted space, containing more or fewer of the cells to be described ; this space, as has been mentioned above, opens into the cavity of the sheath round the dorsal vessel.

From the margins of this opening, *i.e.*, from what may be called the mouth of the gland, a number of muscular fibres take origin, as has been described by Schneider ; these pass into the gland, and then branch and radiate ; they are perfectly distinct from the reticulum.

Within the gland are contained numerous cells, of irregular shape, with rounded nucleus containing a pseudonucleolus ; their processes may resemble pseudopodia, and the nucleus may be excentric. These are leucocytes, and as their characters are well known, they need not be further described.

These cells are more compactly aggregated at the periphery of the gland, where they form fairly solid masses corresponding to the lobulations seen on the surface ; each such lobule is surrounded by a corresponding outward bulging of the enveloping membrane or capsule. The cells are also contained in the meshes of the reticulum of the gland, but are here more loosely aggregated ; in the centre of the gland towards the opening into the sheath of the dorsal vessel they are still more scattered.

The cells are to be looked on as proliferated from the inner surface of the capsule within the peripheral lobulations ; thence they travel into the central part of the gland, and ultimately they reach the general body-cavity through the sheath around the dorsal vessel, which, as already explained, communicates with the cavity of the next posterior segment.

From what has been said, it will be seen that I regard the capsule as peritoneal in origin ; it is indeed, as fig. 3 shows, continuous with the septum, and may be looked on as in fact an irregular sac-like forward bulging of the septum, which has become extremely thin by the loss of all muscular elements,—which has been indeed reduced to a thin sheet of peritoneal cells only. No doubt this sheet is morphologically double, and results from the coalescence of the two layers of peritoneum covering the two faces of the septum, but its double character is not to be made out in the actual specimens.

I differ, therefore, from both Beddard and Schneider in the conception of the essential nature of these organs ; neither author seems to have recognised the capsule, or bulging of the septum within which the cells are contained. Beddard's idea is that the organ is a mass of cells surrounding a few muscular fibres ; while Schneider speaks of a definite opening in the sheath of the dorsal vessel, through which the cavity of one segment communicates with that of the next adjacent, and the gland is a tree-like branching structure originating from the margins of the opening.

I must guard myself from saying that the capsule is to be made out as a complete investment over the whole periphery of the gland in every section ; it seldom is so, in this species at any rate. At places the cells of the gland are closely adherent, so that the capsule does not stand off as a separate structure, and frequently the capsule is absolutely continuous with the cells. This of course necessarily follows from the

fact that the cells are budded off from the inner surface of the capsule. It is possible also that some cells are budded from the outer surface of the membrane ; or the cells which may be seen there may perhaps be leucocytes of the coelomic fluid which have become temporarily adherent.

In *Pheretima heterochaeta* the lobulation of the glands is less marked than in *P. hawayana*, and the outline of the glands in sections is comparatively smooth ; there is consequently not the same massing together of the newly formed and forming cells within the lobules, and the texture of the gland seems on the whole to be looser ; the capsule is as a rule more easily traced, and its connection with the reticulum within the gland is easily made out.

In *P. posthuma* the lobulation is similar to that of *P. hawayana*, and the relation of the capsule to the cells also is as described for that species.

OTHER CONTENTS OF THE GLANDS.

That the main mass of the cells of the glands are leucocytes with a phagocytic function has been established by the experiments of Schneider.

In addition I have seen the cells described by Schneider as containing small round refractile granules ; the cells may be partially or even entirely filled by the granules. Chloragogen cells are also to be seen, and may be met with in various stages of degeneration. Cysts and pseudonavicellae of *Monocystis*, which may be surrounded by an almost epithelial arrangement of leucocytes, are present. Setae and fragments of setae, similarly surrounded by leucocytes, are also found.

THE COELOMIC FLUID IN *PHERETIMA*.

I add a few notes on the coelomic fluid in this genus.

The fluid is of a yellowish colour, which varies according to the nature of its cellular contents. Its consistency also varies ; it is thick and gelatinous in specimens coming from a dry locality, thinner in those from places where there is abundant moisture. As is well known, it is coagulable by alcohol.

Its cellular constituents are of four chief kinds :—

(1) Leucocytes, granular and colourless, of various sizes ; the nucleus is usually spherical, and excentrically placed ; the chromatin is distributed as irregular granules, while in the middle of the nucleus is a larger aggregate, which may be called the pseudonucleolus. In normal salt solution these cells are seen to be actively putting out pseudopodia, fine filiform processes extending in various directions, which may anastomose with similar pseudopodia of other cells and lead to the production of plasmodia. The cells may sometimes become pear-shaped, with a fine filiform process which gives the appearance of a flagellated Protozoon till the movements are observed.

(2) Minute colourless nongranular cells, mostly spherical, but sometimes becoming elongated and pointed at the ends ; they are numerous, and may also form plasmodia. In stained preparations the clear protoplasm readily takes up the eosin stain ; the nucleus when present is

excentric, spherical, and contains a pseudonucleolus; there is a large clear vacuole in the middle of each.

(3) The cells described in the account of the lymphatic glands as containing a number of refractile granules or globules are also seen.

(4) Yellow cells,—the chloragogen cells,—in various stages of degeneration are found.

In addition, numerous rod-like bacteria are present; and also the sporozoite stage in the development of *Monocystis*.

THE COELOMIC ORGAN OF BEDDARD AND FEDARB.

Beddard and Fedarb have described ("On a new Coelomic Organ in an Earthworm," *Proc. Zool. Soc.*, 1902), in specimens of *Pheretima posthuma* sent from Calcutta by Mr. F. Finn when Deputy Superintendent of the Indian Museum, a number of pouches or tunnels on the inner surface of the body-wall. These, which were visible in the ordinary dissection of the worms, were found in a number of specimens,—it is not stated that they were absent in any. Their direction is transverse on the lateral and ventro-lateral body-wall; they occur on both sides, from segment xxii to the hinder end of the animal, being largest from about segment xl for about twenty segments onwards. Extending outwards and upwards from near the ventral nerve cord, they present the appearance of tunnels open at both ends, considerably constricted in the middle of their extent; or the two halves may be quite separate, *i.e.*, the constriction may be complete, resulting in the formation of two pouches on each side, those on the same side having their mouths facing in opposite directions, their narrow closed ends close together. The roof of the tunnels or pouches is thin and membranous,—merely an extension of the peritoneum. The structures are not equally marked in all specimens; but, as stated above, they are not said to have been absent in any of the specimens examined.

In a large number of dissections of *P. posthuma* I was unable to see these organs, even with the binocular dissecting microscope. I also prepared several series of sections for the same purpose, but the results were here also negative, except in one case, in a few segments taken from a little in front of the middle of the body. Here the tunnel was present, as described by the authors; while reaching to not very far from the ventral nerve cord below, they terminated above a little dorsal to the lateral line of the body.

The organs are therefore not found in all specimens of the species; in some localities, as at Lahore, they appear to be of rare occurrence.

A point not noticed by the previous authors is the modification of certain cells of the roof of the tunnel. A section across the tunnel,—such as is obtained in a longitudinal vertical series where it passes through the lowest part of the tunnel on the ventral body-wall,—shows the floor to be flat, and the roof a semicircular arch, just as in an ordinary railway tunnel (fig. 4). The floor is carpeted by ordinary peritoneal cells, clear and squarish; the sides of the arching roof consists of flattened cells joined at their edges, as in the case of the capsule of the lymph glands, previously described. The vertex of the roof is peculiar; it consists of cells which are much elongated vertically, joined together

at their bases, and projecting downwards into the tunnel, with their free ends, which are narrower than the bases, separate from each other, and so giving a ragged appearance to the lower side of the roof; the length of these downwardly projecting cells may be more than one-third of the height of the tunnel; they have a fibrillated structure, the fibrils running in the direction of the long axis of the cells.

In the tunnels numerous leucocytes are seen, and many nephridia, as mentioned by Beddard and Fedarb. I have no suggestions to offer as to the function of these organs.