PRELIMINARY ULTRASTRUCTURAL DESCRIPTION OF COELOMOCYTES OF THE LUMINESCENT OLIGOCHAETE, *Pontodrilus bermudensis* (ANNELIDA)

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INTRODUCTION

Pontodrilus bermudensis is a marine littoral member of the earthworm family Megascolecidae. On strong electrical, mechanical or chemical stimulation this species exudes a luminous fluid from the mouth and anus. The luminescence is whitish with peak intensity at the wavelength of 550nm. Emission of light by the exuded fluid is further stimulated by addition of hydrogen peroxide and/or *Diplocardia longa* luciferase, response to the enzyme suggesting that *Pontodrilus* luciferin is n-iso-valeryl-3-amino-propanal as in *D. longa* or a close analogue of this (1). The alimentary exudate of *Pontodrilus* has been shown to contain cells identical with coelomocytes despite the absence of dorsal pores through which coelomic fluid is exuded in *Diplocardia*. However, particle-bound or

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cellular luminescence has been denied for *Pontodrilus* (2; Lynch, personal communication). In *D. longa* luminescence has been directly observed to originate from discrete subcellular loci within large coelomic cells described as free chloragogen cells and in the sessile chloragogue (3). The source of luminescence in *Pontodrilus* requires re-examination and the present study is concerned with ultrastructural description of cells of the exudate and of the coelomic fluid preparatory to videomicroscope and other investigations of this source. Some reference will be made to cytochemical studies which are in progress.

Apart from the study on *Diplocardia* cells, no ultrastructural studies of megascolecid coelomocytes have been published. Constraints of space will not permit detailed comparison of *Pontodrilus* coelomocytes with those of species of other families but a review of the ultrastructural literature is given elsewhere (4).

II. MATERIAL AND METHODS

Worms collected from Peel Island, Queensland, were maintained for 16 hours in powdered filter paper moistened with filtered sea water to clear the gut. Coelomocytes were obtained either by placing individual worms in ice-cold glutaraldehyde (3%) in 0.1M phosphate buffer, when alimentary exudate was expelled directly into the fixative, or by dorsal incision of the worm in sea water, pipetting off the coelomic fluid and transferring this to glutaraldehyde. Alternatively exudate from electrically stimulated worms was transferred to the fixative. The samples were fixed in the glutaraldehyde for 2 hours, post-fixed in 1% osmium tetroxide in 0.1M buffer for 80 minutes, rinsing in buffer for 30 minutes before and after, and embedded in Spurr's medium after dehydration in a graded ethanol series (30 minutes in each of 20,40,75,90% and absolute). The temperature was maintained at 4°C from glutaraldehyde through 75% alcohol but at room temperature for later alcohols. At each stage samples were centrifuged for later alcohols. At each stage samples were centrifuged at 100-300g (100g was found preferable as higher speeds disrupted mucocytes) to allow removal and replacement of the supernatant liquid. Ultrathin sections were cut on an LKB Ultratome IV, stained with uranyl acetate and alkaline lead citrate, mounted on uncoated grids and photographed with an AEI Corinth electron microscope.

III. RESULTS

A. *Introduction*

At least nine morphological types are recognizable from their ultrastructure. Of the names applied to these, only two, mucocyte and linocyte, have previously been used. As the cells all resemble lymphocytes in occurring in body fluids outside the vascular system, they are here collectively referred to as lymphocytoids. A subset of these characterized by large numbers of secretory granules will be termed granulocytes, with various distinctive epithets. As in the terminology for human blood cells, granules may occur, though in lesser numbers, in cells not termed granulocytes. Mucocytes and linocytes will be regarded as a distinct subset of the lymphocytoid.

B. *Ultrastructurally Defined Coelomocyte Types*

1. Mucocyte (Figs. 3, 11). Mucocytes are the most abundant of the coelomocyte types; large clusters of them occupy much of the coelom of most segments in paraffin sections. The live cells, in alimentary exudate or released by dissection, rapidly disrupt, shedding their granules, in hyptonic media or on exposure to air if unfixed. They remain intact if expelled into glutaraldehyde or into sea water followed rapidly by fixation. The freed secretory granules become very numerous in untreated exudate and give rise by fusion to strands of moderately electron-dense material which is deduced to be the viscous mucus which characterizes the exudate. When the cells disrupt they also release GER vesicles and mitochondria; the residual free and contained membranes may form configurations resembling portions of the internal "threads" of linocytes (see below). The released granules and other organelles, or partial or whole mucocytes, are commonly phagocytosed by vesicular lymphocytoids in which they are observable in phagocytic vacuoles.

Uncentrifuged or gently centrifuged mucocytes are regular spheres. In resin sections the mean cell diameter is 8.3µm (S.D. 1.7, n 16) and the nuclear diameter 3.2 µm (range 2.8-3.7, n 8), with a cytoplasmic to nuclear ratio in diameters of 3.0 (range 2.5-3.5). Cells in paraffin sections (buffered formalin fixation) are larger, with mean diameter 9.9um (S.D. 1.4, n 68). Live, intact cells, uncompressed on slides, average 13.9µm (S.D. 1.4, n 35). The spherical to ovoid, or somewhat concavo-convex nucleus is invariably strongly

eccentric, only a thin layer of cytoplasm intervening between it and the adjacent plasma membrane. Chromatin lines the inner nuclear membrane as small clumps or broad irregular bands and forms scattered patches throughout the nucleus; the outer nuclear membrane, separated by a considerable cisterna, is ribosome-studded.

The mucocyte cytoplasm is packed with approximately equal numbers of large granules of mean diameter 0.6μm (range 0.34-1.02, n 18) and ribosome-studded vesicles (GER) of the same order of size to much smaller. The granules are irregularly or, more commonly, evenly subspherical and are membrane-bound but the thin membrane is observable only in occasional granules and then often for only part of the periphery. The granules vary from weakly to strongly electron-dense, becoming paler as they approach breakdown, and have a finely granular consistency. They are strongly PAS positive, indicating amino- or vic-glycols, and like the cytoplasm, stain strongly for β-glucuronidase. Wright's stain indicates that the cytoplasm is strongly acidophilic. These responses are not shown by other cell-types. The vesicles are negative for the former two tests. Several large, approximately isodiametric mitochondria with mostly short, irregularly disposed cristae are visible in a plane of section. In life and in resin sections several short pseudopodium-like projections are sometimes visible but a phagocytic role is not indicated.

A central Golgi complex has been demonstrated for some cells. The first cisterna, on the concave face, forms a few large interconnected dilatations with moderately electron-dense contents which have a granular structure resembling that of the large, mucous granules in the cytoplasm. These dilatations are here interpreted as prosecretory granules (vesicles) precursory to the cytoplasmic mucus-granules as seen in the mammalian parotid gland (5). A centriole is occasionally seen.

2. Linocyte (Figs. 4,12). Linocytes are large, ovoidal cells with a mean longer dimension of 10.9μm (range 8.3-18.2) and cytoplasmic to nuclear ratio of 3.3 (range 1.9-4.9) for a nuclear diameter of 3.4μm (range 2.2-4.9, n 9). The plasma-membrane is lined internally by a thin layer of cytoplasm which in places may be lacking or may be expanded sufficiently to include a rare mitochondrion or one or more small vacuoles. The nucleus, which is strongly eccentric, being almost in contact with the plasma-membrane, is irregularly ovoid or lobed and has dense masses of chromatin forming a wide, periodically constricted peripheral band or peripheral and scattered clumps; a degenerating nucleolus is sometimes

recognizable. In cells which seem least degenerate the large space internal to the cytoplasmic sheath is filled by a pleated double membrane which gives the appearance, in section, of a coiled thread wound on itself approximately one hundred times. In fact, however, the loops are linked with each other, mostly laterally, and with the cytoplasmic sheath so that they comprise a complex system of compressed vacuoles which are separated by double membranes which form the so-called thread. In some cells, however, in which the double membrane structure is still retained, the thread forms a single large whorl around a common centre. In other, probably more degenerate cells, the thread appears to consist of loose stacks of many transverse or longitudinal fibres lacking a clear double membrane condition, and may break up or become disorganized. Linocytes are often seen being engulfed or infiltrated by phagocytic lymphocytoids.

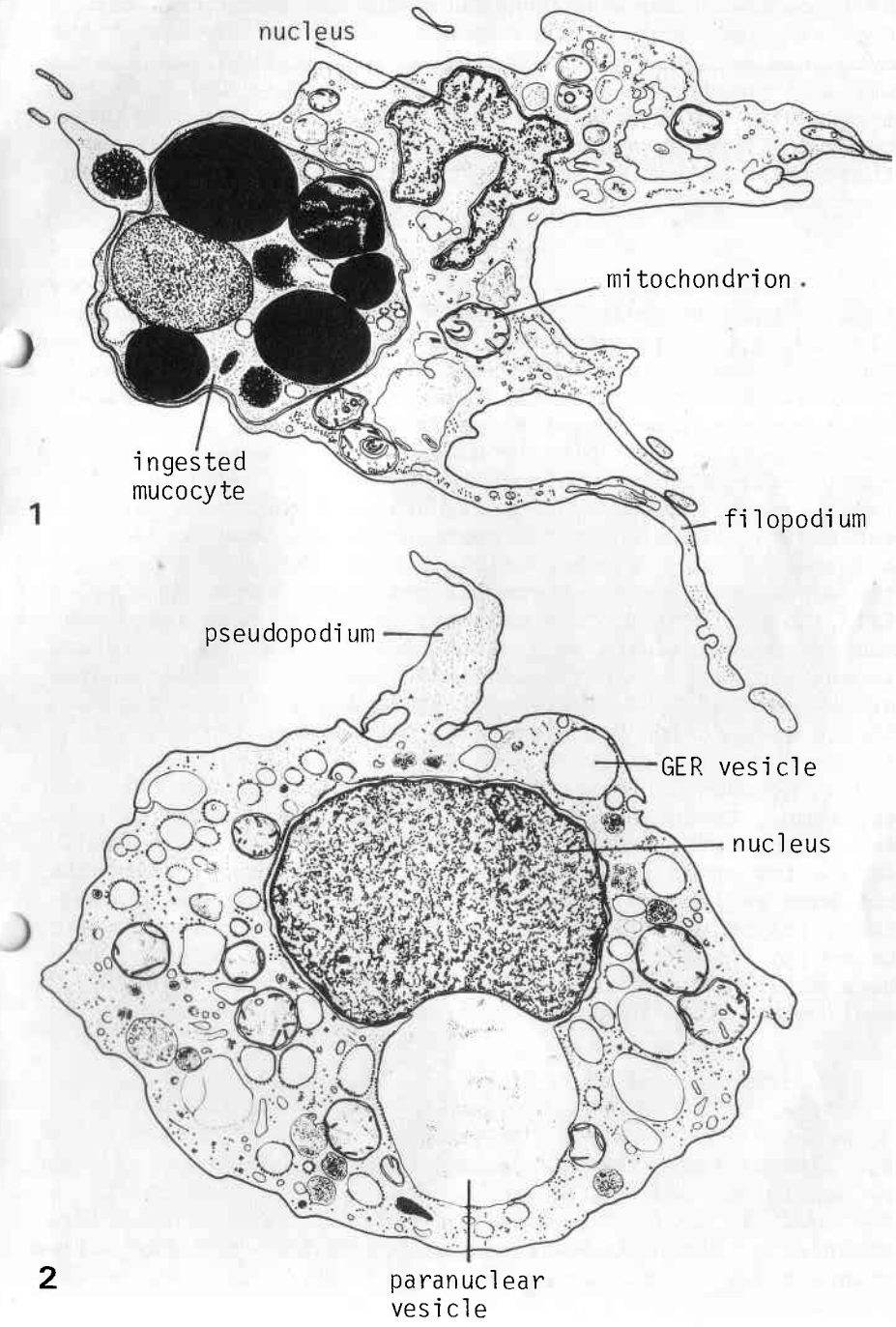
3. Vesicular Lymphocytoid (Fig. 2). This is a medium-sized cell, of mean diameter 9.1μm (range 7.1-11.2), cytoplasmic to nuclear ratio 2.9 (range 1.9-4.5) and nuclear diameter 3.5μm (range 1.9-5.7, n 10). It is possible that the vesicular lymphocytoid morph may be assumed by more than one of the other coelomocyte types but a typical form is recognizable which has the following characteristics. The nucleus is evenly or irregularly subcircular or somewhat kidney-shaped in outline. It is sometimes embayed by a large vesicle, here termed the paranuclear vesicle, which is a local enlargement of the perinuclear cisterna and like the latter is externally ribosome-studded. Chromatin is distributed evenly in the nucleus as small grains or may show few slight to conspicuous aggregations and a small but distinct granular nucleolus may be visible. Most distinctive are large numbers of moderate-sized to large subcircular to oval GER vesicles (sometimes small and ill-defined) which may or may not be accompanied by the paranuclear vesicle. Forms with small, indistinct GER vesicles have few, often incomplete mitochondria but whether the latter are forming or degenerating is not clear; the fact that these cells sometimes show a well developed Golgi apparatus suggests that they are young vesicular lymphocytoids. Also typical are several large mitochondria per section with round profiles, electron-pale matrices and mostly short, peripheral cristae. Some vacuoles have few or no ribosomes and may show their origin by endocytosis by a narrow, sometimes canalicular connection with the exterior. A few apparent phagosomes with electron-dense diffuse contents, and the occasional multivesicular body, are present.

Some variants, probably referable to this cell type, have large phagosomes containing ingested mucous granules released from mucocytes. A further variant, tentatively to be regarded as a vesicular lymphocytoid, has acquired considerable numbers of, probably intrinsic, electron-dense small oblate granules of the type seen in granulocytes and may show diffuse phagolysosomes with which oblate granules are fusing.

The outline of a typical cell is commonly very irregular, though often without definite pseudopodia, but one or more ragged filopods may be produced. Putative free ribosomes and polyribosomes are moderately numerous scattered throughout the cytoplasm. Larger, moderately abundant granules are probably glycogen.

4. Macrophagous Lymphocytoid (Fig. 1). These have mean maximum dimensions (excluding longer pseudopods) of 11.9µm (range 9.7-16.7), a mean nuclear diameter of 3.6µm (range 2.5-5.2) and a cytoplasmic to nuclear ratio of 3.8 (range 2.6-4.3, n 5). The cells are here termed macrophagous lymphocytoids because, in the large size and frequency of ingesta in their phagosomes, they are reminiscent of mammalian macrophages. They appear capable of ingesting intact mucocytes. The term macrophage may be applied for brevity with no implication of homology with vertebrate macrophages. Phagocytosis, although on a smaller scale, is widespread in other lymphocytoids, including granulocytes, but recognition of a distinct type for macrophagous cells is supported by additional morphological features which, in combination, provide a diagnosis for this type. A particularly distinctive feature is the form of the nucleus which has homogeneous, finely granular chromatin and is irregular in outline with abrupt, angular indentations. A nucleolus has been observed. Mitochondria are numerous and large, with pale matrices, and often appear to be in a state of dissolution, sometimes containing myelin figures. In view of the very active phagocytosis, the absence of secretory granules is surprising. This absence does not appear ascribable to utilization of granules which might have been present in a precursory phase as phagosomes containing obviously newly ingested, unchanged cell components are not accompanied by recognizable secretory granules. Some indistinct bodies of medium electron density may, nevertheless,

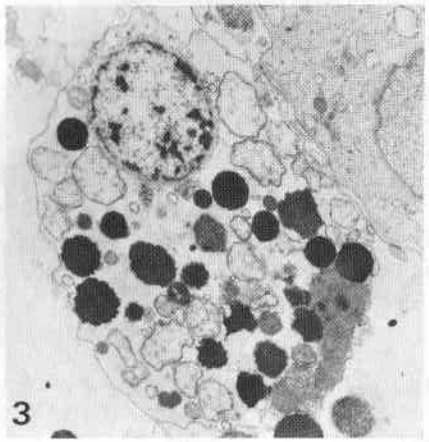
Figs. 1 and 2. Coelomocytes of Pontodrilus bermudensis. 1, macrophagous lymphocytoid with ingested mucocyte, X 12 100. 2, vesicular lymphocytoid with paranuclear vesicle, X 12 000.

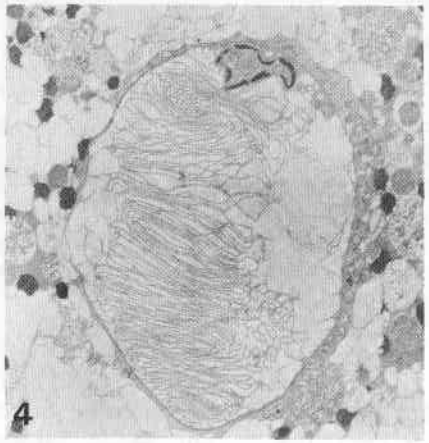


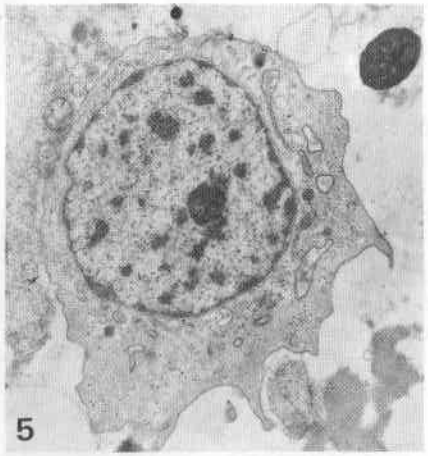
be lysosomes. Long tortuous filopodia are characteristic. Free ribosomes and polyribosomes are numerous throughout the cytoplasm but, unlike the vesicular lymphocytoid, association with the numerous vacuoles or vesicles is very low. One or more active Golgi complexes (with cisternae containing dense contents) from which small Golgi vesicles spread widely in the cytoplasm, and an adjacent centriole, have been seen in some macrophages.

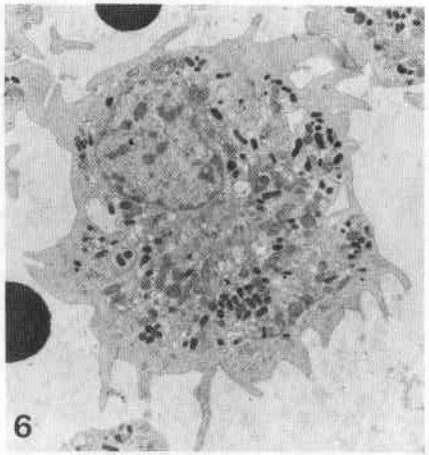
5. *Small Lymphocytoid (Figs. 5,13).* The small lymphocytoid is thus designated because of its morphological resemblance, in its agranular form, to the small lymphocyte of man The mean diameter is about 7.2µm (range 5.2-8.5) with small cytoplasmic to nuclear ratio, averaging 1.9 (range 1.7-2.6), for a nuclear diameter of 4.0µm (range 2.9-5.9, n 10). The nuclear outline is typically circular, though slightly to considerably indented but may be deeply cleft. Conspicuous large chromatin clumps are peripheral and scattered throughout the nucleus. The outer membrane is ribosome-studded and a large nucleolus may be visible. Very characteristic are the short profiles of narrow GER cisternae, often in single file, concentric with the nucleus. Putative free ribosomes and glycogen granules are scattered throughout the cytoplasm. In what appears to be the simplest (stem?) form mitochondria are absent and dark phagosome-like bodies are few and small though some fusion vacuoles, with nebulous contents, rarely in the form of canaliculi with terminal swellings, are present. Rounded GER vesicles and Golgi complexes are not apparent. Exceptionally a naked paranuclear vesicle is present. The cytoplasm of a typical cell tends to be rounded with a few broad ragged protrusions or broad short filopodia but some cells here classed as, and with the general characteristics of, small lymphocytoids, have numerous long, broad, branching filopods. These cells have acquired moderate numbers of elongate mitochondria with dark matrices and have small numbers of oblate to spherical very electron-dense

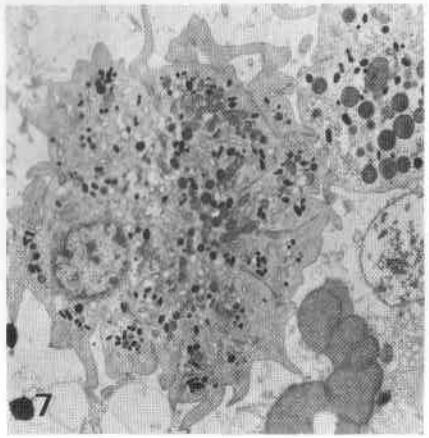
Figs. 3 to 8. Coelomocytes of Pontodrilus bermudensis. 3, mucocyte; note free mucus-granules in the medium, X 7 500. 4, linocyte invested by pseudopodia of a macrophage, X 3 200. 5, small lymphocytoid, X 8 000. 6, medium lymphocytoid, X 6 300. 7, filolobopodial granulocyte; released secretory granules of a mucocyte are coalescing to form mucus at bottom right, X 5 300. 8, lobopodial granulocyte with canaliculi, X 5 800.

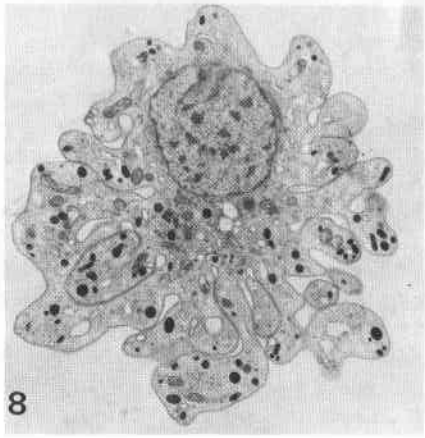








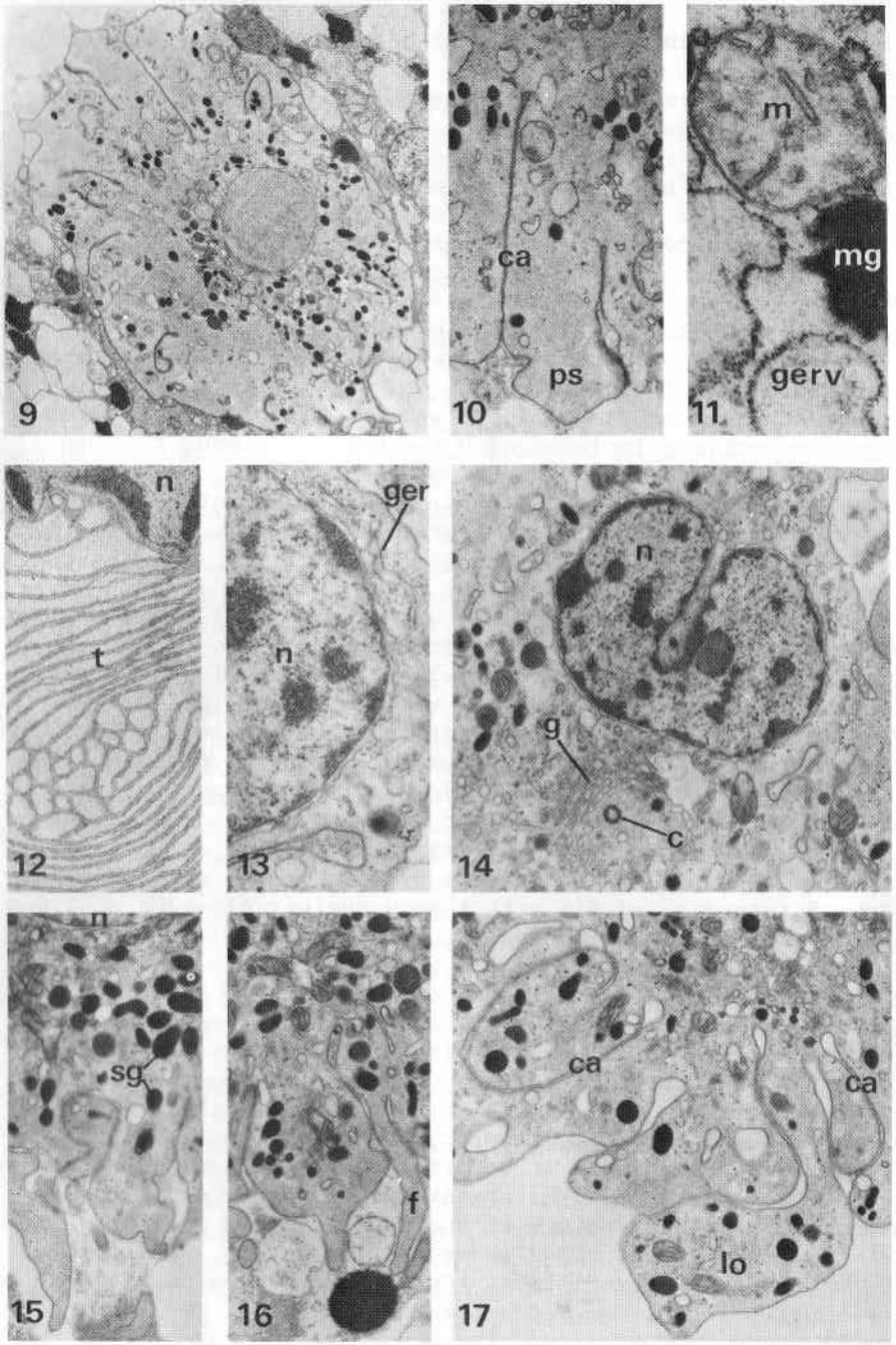




granules. The filopodia and a narrow band of peripheral cytoplasm are largely free of granules or inclusions, other than putative ribosomes and glycogen granules. The secretory granules, including larger spherical ones, do not exceed about 0.3μm. The actively filopodial cells often have large peripheral phagocytic vacuoles (residual bodies) containing minute, bubble like spherules of probably foreign origin. The cytoplasm fringing such vacuoles appears to have lost its bounding membrane in places; dense granules (lysosomes?) enter these vacuoles.

6. Medium Lymphocytoid (Figs. 6,14,15). The medium lymphocytoid is so designated because it shows ultrastructural similarities to the small lymphocytoid while being slightly larger. It shows no especially close similarity to the human medium lymphocyte. Origin from the small lymphocytoid is probable. Obvious distinctions from the latter are the slightly greater diameter, mean 8.5μm (range 8.1-9.2), which is due to the slightly increased ratio of the cytoplasmic to nuclear diameter, 2.5 (range 2.0-3.0), for a nuclear diameter of 3.4μm (range 2.8-4.3, n 7), and the large number of granules. Rod-to slightly dumbell-shaped dense granules resemble those of small lymphocytoids in appearance and size and it is not considered that larger, rounded granules reaching 0.4μm in diameter are a different type of inclusion. The nucleus again has patchy peripheral and general chromatin clumps, may exhibit a nucleolus, and has one or more slight to strong indentations, or may be deeply cleft, but tends to be more irregular,

Figs. 9 to 17 Coelomocytes of Pontodrilus bermudensis. 9, ectoplasmic granulocyte, X 5 100. 10 to 17, details of various types: 10, ectoplasmic granulocyte, X 10 800. 11, mucocyte, X 38 800. 12, linocyte, X 17 800. 13, small lymphocytoid, X 10 000. 14, immature medium lymphocytoid, X 13 400. 15, medium lymphocytoid with tendency to petaloid configuration of pseudopodia, X 23 500. 16, filolobopodial granulocyte, X 10 600. 17, lobopodial granulocyte, X 11 200. c, centriole; ca, canalicule; f, filopod interposed between pseudopodia which are lobopodia with filopodous extension; g, Golgi apparatus; ger, granular endoplasmic reticulum; gerv, granular endoplasmic vesicle; lo, lobopodium; m, mitochondrion; mg, mucus granule; n, nucleus; ps, broad pseudopodium bounded by canaliculi; sg, small, dense granule; t, "thread" of linocyte.



less circular in outline. Small phagosomes and multivesicular bodies are few. Clear, sperule-containing, residual bodies confluent with the peripheral cytoplasm resemble those of small lymphocytoids. GER cisternae are more dispersed and a few, scattered canaliculi may be present or, in some cells, canaliculi may be frequent between the bases of adjacent filopodia which have some tendency to a petaloid form. Mitochondria are moderately numerous. Putative free ribosomes and glycogen granules are scattered throughout the cytoplasm which again tends to lack inclusions or organelles in a narrow peripheral zone. GER vesicles are few and small.

Some cells with these features but fewer granules show a centriole with encircling Golgi complexes. These probably represent a young stage of the medium lymphocytoid. One cell-type tentatively included with the medium lymphocytoid has numerous large, dense apparent phagolysosomes, few small granules and has microfilament bundles extending into some at least of the filopods which thereby resemble axopods.

7. Filolobopodial Granulocyte (Figs. 7,16). The tendency to combine a petaloid configuration of pseudopodia with filopodia which was seen in some medium lymphocytoids is taken further in what is here termed the filolobopodial granulocyte. The obligatorily large number of granules warrants descriptive designation of this lymphocytoid as a granulocyte. It is not unreasonable to consider it a descendant or close collateral of the medium lymphocytoid. It is, however, significantly larger, mean diameter 12.1µm (range 10.0-15.7) an increase achieved by augmentation of the cytoplasmic to nuclear ratio to 3.5 (range 2.7-4.4), with a mean nuclear diameter of 3.6µm (range 2.4-4.6, n 3).

The filolobopodial granulocyte is readily identified by its peculiar pseudopodial structure; broad lobopodia, often bounded by narrow canaliculi which may wind deeply into the cytoplasm, bear or alternate with long filopodia. The appearance of the cell is thus somewhat intermediate between that of the foregoing medium lymphocytoid and the canalicular form of the lobopodial granulocyte described below. Like the former, but unlike the lobopodial cell, the secretory granules and organelles tend to be absent from a narrow peripheral zone. The ultrastructure of the cytoplasm and nucleus is too similar to that described above for the medium lymphocytoid to warrant full description. However, a nucleolus, though possibly present, has yet to be demonstrated. The number of small, rod- to dumbbell-shaped dense granules is greater and large numbers of spherical, moderately dense bodies are present. Whether the large bodies are true granules or are

phagolysosomes is not clear. Whereas canaliculi are well developed, comprising a definite lamellar-vacuolar system (*sensu* Droller) (6), GER-cisternae and especially -vesicles are scarce.

8. Lobopodial Granulocyte (Figs. 8,17). Granulocytes with small, rod- or dumbbell-shaped and with larger, spherical granules, the numerous pseudopodia of which are mostly large, round-ended lobopodia are here termed lobopodial granulocytes. Although common, they are about one sixth as abundant as mucocytes. Two forms are present, one with, the other without canaliculi but this is possibly a transitory distinction. Apart from the lobopodial form, they differ from small or medium lymphocytoids and from filolobopodial granulocytes in that the secretory granules and even the mitochondria, which in all these cells may enter the pseudopodia, are not excluded from the peripheral cytoplasm but frequently approach or abut the inner surface of the plasma-membrane.

Observed diameters vary from as small as the small lymphocytoids (5.7µm) to the largest known for a coelomocyte (18.6µm), mean 10.5µm, with a large cytoplasmic to nuclear ratio, mean 3.2 (range 1.9-4.5), for a mean nuclear diameter of 3.3µm (range 2.4-4.2, n 12). The nucleus is ovoid, variously notched or bi-partite in section. Chromatin clumps line the periphery and are scattered throughout it. A large nucleolus is sometimes visible. Secretory granules are numerous but, of those which are very electron-dense, small narrow oblate granules are relatively less common than in the preceding granulocyte-types whereas larger equally dense granules, circular in cross section and presumably spheroidal, are common. Nevertheless, these dense granules have mean maximum dimensions of only 0.3µm (S.D.0.06, n 55). In addition, however, a considerable proportion of cells have, often abundant, large, subspheroidal moderately electron-dense granules with flecked, granular contents. These range from 0.4-1.2µm, averaging 0.6µm in diameter (S.D.0.2, n 23). As such they are significantly smaller than chloragosomes measured in *Pontodrilus* chloragocytes and identity with these is therefore contraindicated.

Elongate mitochondria with dark matrices are moderately numerous. Golgi complexes, in a circular configuration, with or without associated centriole, are rarely observable. Several large vacuoles, commonly oval to dumbbell-shaped, are characteristic. These lack ribosomes and are here termed fusion vacuoles as, though usually empty, they appear to be the product of fusion of pseudopodia in an endocytotic process. The smaller fusion vacuoles may open by long narrow

canaliculi of the lamellar-vacuolar system (one per vacuole) to the exterior. Vacuoles in section commonly contain transected pseudopodia. Canaliculi often form closed circles, enclosing granule-containing cytoplasm. GER cisternae and vesicles, both small, are fairly common. Putative glycogen granules, polyribosomes and free ribosomes are scattered throughout the cytoplasm. Surprisingly, in view of the numbers of lysosome-like dense granules, phagosomes are questionably present and multivesicular bodies are uncommon. Scattered bundles of microfilaments are present and in some variant cells form axial elements of axopod-like, pointed pseudopodia.

9. Ectoplasmic Granulocyte (Figs. 9,10). The ectoplasmic granulocyte is an infrequent cell so-named because a broad peripheral band of cytoplasm, apart from containing scattered putative ribosomes and glycogen granules, may be virtually devoid of granules and other inclusions or there are at least large areas, some of which are peripheral, of such featureless cytoplasm. However, the chief characteristic of this type of granular lymphocytoid is the deep dissection of the cytoplasm by narrow canaliculi which extend inwards from the exterior and end internally in slight dilations. These canaliculi, comprising the lamellar-vacuolar system, separate broad, lobate pseudopodia which project very little beyond the cell periphery and are chiefly recognizable by virtue of their intervening canaliculi.

The cells are large, with mean diameter of 12.9μm (range 10.0-15.7, n 3). While the nuclear diameter remains in the order of 3μm, the cytoplasmic to nuclear ratio is high, at about 4.7. The nucleus is subcircular with ribosome-studded outer membrane and homogenously distributed, finely granular chromatin which lacks appreciable clumping. Its position varies from near central to strongly eccentric. Strongly electron-dense, membrane-bound, circular, oblate, or sometimes dumbbell-shaped granules with mean maximum dimension 0.24μm (S.D.0.06, n 18) are numerous. These granules do not appear to represent different types and therefore no dimorphism is recognized. No Golgi apparatus has been observed. Moderate numbers of mitochondria with circular to bilobed profiles, partial and complete transverse cristae, and matrices resembling the cytoplasm in their weak to moderate density are present. Small, subcircular to irregular GER vesicles, similar ones lacking ribosomes, and V-shaped, circular or tortuous canaliculi in addition to those at the cell margins are frequent. Wisps of microfibrillar material are ubiquitous. Phagosomes have not been seen but multivesicular bodies are present.

IV. DISCUSSION AND CONCLUSIONS

Of the nine types of coelomocytes in the coelomic fluid or alimentary exudate of *Pontodrilus bermudensis* only two, mucocytes and linocytes, have previously been described for any animal group. Mucocytes are acidophil cells the granules of which are PAS positive (suggesting presence of vic- and/or amino-glycols) and contain β-glucuronidase. Mucocytes have elsewhere been recognized ultrastructurally only in the Enchytraeidae (7 and Richards, personal communication) that described by Richards for *Enchytraeus albidus* being morphologically closest to the mucocyte of *Pontodrilus*. In enchytraeids the granules contain 1,2 glycols and varying amounts of acid mucopolysaccharide. The acidophil cell described ultrastructurally for *Lumbricus terrestris* (Lumbricidae) as the "inclusion-containg cell" (8) also resembles the mucocyte of *Pontodrilus* but differs notably in the small amount of endoplasmic reticulum.

The linocyte was first described in a megascoleid (9) for a cell which had the appearance by light microscopy of being filled by one to several coiled threads. Similar cells were termed nematocytes in *Nematogenia panamaensis* (Ocnerodrilidae) (10) and thread-containing cells in enchytraeids (11). Origin of linocytes from mucocytes after release of secretory granules (7) is here endorsed.

Of the seven *Pontodrilus* cell-types additional to mucocytes and linocytes, none has been recognized by previous workers and none can be identified with certainty with types described ultrastructurally for lumbricids (8,12,13), or enchytraeids (7). Whether homologues do occur or are to be expected in other families is debatable. Similarities with those of other families are apparent but have not been sufficient to justify an identical nomenclature. A brief consideration of genetic relationships of the cell-types within *Pontodrilus* is appropriate for recognition of interfamilial similarities. Origin of linocytes from mucocytes seems established but origin of the mucocytes is not known. ~~The lumin-escent cell of *Diplocardia longa* is not known~~. The luminescent cell of *Diplocardia longa* appeared to be a free chloragocyte (3) but ultrastructural investigation of the chloragocytes of *Pontodrilus bermudensis* in connection with the present study excludes such origin of mucocytes in *Pontodrilus*.

Lineages of lymphocytoids, including their subset the granulocyte, can be surmised only tentatively from this purely ultrastructural study. The small lymphocytoid, in its amitochondrial form, appears to be a stem cell which can,

however, on further differentiation become phagocytic and probably gives rise to the medium lymphocytoid. The filolobopodial granulocyte may well be a descendant of the medium lymphocytoid. The tendency to exclusion of organelles and granules from a very narrow zone of peripheral cytoplasm in these types and general characteristics such as granule type, and appearance in the filolobopodial form of a well developed lamellar-vacuolar system with a petaloid configuration of pseudopodia suggest that the ectoplasmic granulocyte is derived from or is a close collateral of the filolobopodial cell. The lobopodial granulocyte, with its tendency for organelles and granules to abut the plasma-membrane, is probably not derived from the small lymphocytoid-filolobopoidal lineage, a view supported by the existence of probable precursor cells (not described above) resembling the lobopodial cell but smaller with large cytoplasmic to nuclear ratio and with few or no pseudopodia. The lumbricid cell most closely resembling the lobopodial cell is the type II granulocyte (8) or "transportation amoebocyte" (14) but the type II cell is believed to be derived from the type II and this from the type I lymphocytic coelomocyte whereas the latter two cells approach the small and medium lymphocytoids of *Pontodrilus* in general ultrastructure though not identical with them. The ectoplasmic granulocyte superficially resembles the type I granulocyte of *Lumbricus* (8) which has a wide ectoplasmic band but definite canaliculi are not evident in the latter cell. The macrophagous lymphocytoid is conceivably derived from the vesicular lymphocytoid but the origins of this lineage remain to be elucidated.

Of all the nine cell types, the mucocyte, released granules of which form the viscous mucus of the alimentary exudate, is the most likely source of luminescence. Lobopodial granulocytes are also sufficiently abundant to be considered as possible sources of luminescence and the cooperation of two or more cell types in producing luminescence cannot yet be ruled out. As the luminescence is peroxidative it is curious that no *Pontodrilus* coelomocytes (nor those of lumbricids 8) are peroxidase positive. The possibility that minute inclusions such as the lysosome-like, 0.3μm, small dense granules of *Pontodrilus* granulocytes contain peroxidase but are too small or insufficiently numerous to give a visible positive reaction in smears under the light microscope warrants further investigation.

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