ULTRASTRUCTURE AND FUNCTION OF THE GUT OF A MARINE BRYOZOAN

by

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Résumé

L'ultrastructure du tube digestif du Bryozoaire marin *Cryptosula pallasiana* est décrite. L'existence, admise par de précédents auteurs, de types cellulaires acidophiles et basophiles est sans doute en rapport avec la colorabilité de cellules fixées sélectivement. Chez *Cryptosula,* ces deux types de grandes cellules ne sont pas décelables au microscope électronique, bien que ces cellules possèdent des corps de Golgi différents. Des inclusions orangé-brun de la paroi stomacale sont des lysosomes secondaires et des corps résiduels qui se colorent comme la lipofuscine. Des cellules coecales ressemblent de manière frappante aux cellules de certains Mollusques. Des terminaisons d'apparence neuro-sécrétrice se rencontrent au niveau des cellules ciliées de l'estomac.

Introduction

Bryozoa have a U-shaped digestive tract, a feature found in sedentary animals other than Lophophorates e.g. Entoprocts, Sipunculoids, Pterobranchs and Ascidians, groups which, at one time or another, have been compared structurally with Lophophorates. With the exception of Sipunculoids, these groups are ciliary suspension feeders which share a number of features in their gut morphology (Morton, 1960). These features are related to the common needs involved in the transport and digestion of small particles, joint functions that are also found in the molluscan digestive gland. Morton's observation that bryozoan stomach cells greatly resemble the absorptive-digestive cells of molluscan digestive glands is one that can be verified ultrastructurally in this study, now that there are a number of recent publications on the fine-structure of Molluscs.

Anatomical studies (Calvet, 1900; Ries, 1936; Braem, 1940; Bobin et Prenant, 1952; Brien, 1960) have shown that the bryozoan gut is uniformly one cell thick, though differentiated throughout its length (Silén, 1944). Form and function of the various regions have been fairly well characterized although opinions have varied as to the number of cell types found in the stomach (Calvet, 1900; Silbermann, 1906; Rey, 1927; Bronstein, 1939). It has generally been regarded that there are two cell types, one basophilic,

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the other acidophilic—in Gymnolaemates (Calvet, 1900; Silbermann, 1906; Rey, 1927; Soule, 1954; Brien, 1960), in Stenolaemates (Borg, 1926) and in Phylactolaemates (Miiller, 1914; Becker, 1938). Basophilic cells were said to be glandular and secretory, and acidophilic cells vacuolated and absorptive with inclusions. Calvet (1900: 224) did, however, observe that cell boundaries were difficult to distinguish and that the glandular cells were not all of the same height or stainability, commenting that the degree of uptake of the stain depended on the degree of vesiculation of the cell. Bronstein (1939), commenting on Rey's (1927) and Borg's (1926) observations, stated that the differences were not clear-cut. It was a purpose of the present study to determine how many cell types there are.

Intracellular digestion is known to take place in the stomach but the manner in which food is incorporated into the cells is not known nor is it very clear what the relationship is between absorption and the occurrence of orange-brown inclusions in the stomach wall. These inclusions led earlier workers to suspect a functional similarity with the digestive or hepatopancreatic organs of other invertebrates. In an early zoological text we read, in a chapter about Polypi, "Brown follicles cover the external wall of the stomach and seem to represent the liver" (Van der Hoeven, 1856). Farre (1837) also attributed the brown colour of the stomach to a secretion from "hepatic follicles". Joliet (1877) refers to the "hepatic cells" of the stomach, and Hincks (1880: 21) describes the colour of the stomach as "being due to the presence of numerous glands on its inner or lining membrane which secrete a brown fluid and probably discharge the functions of a liver" and (on p. 26) "The biliary glands pour in their secretion and the food takes on its rich brown colour".

On the other hand, Van Beneden (1845) stated that the colour of tha stomach depended on the nature of the food ingested as Bronstein (1939) and Jebram (1968) later observed, and Harmer (1931), using methylene blue staining techniques, created blue stomachs whose colour faded after a period of starvation. Rey (1927) suggested that the stomach produced melanin during the course of digestion. Bronstein (1939) felt that incomplete elimination of undigested material from cells in which intracellular digestion takes place led to accumulation of the material. In addition, then, to characterising general structure and function from pharynx to rectum, at the ultrastructural level, it is the aim of this account to consider specifically the points mentioned above, especially the nature and origin of the orange-brown inclusions that accumulate and which are believed to contribute to polypide regression.

Materials and methods

Observations were made on *Cryptosula pallasiana* (Moll), a monotypic, cosmopolitan, marine-fouling Gymnolaemate (Ryland, 1965), from Grave's Island, Nova Scotia. Freshly collected colonies were removed from rocks or algae and fixed immediately in preparation for the following treatments.

a. *Transmission electron-microscopy.*

The most favourable fixing solution was ice-cold 6 p. 100 glutaraldehyde in seawater with 1 p. 100 sucrose for 1-3 hours, although Millonig's and 0,1 M. cacodylate buffers at pH 7.4 in lieu of sea water were fairly satisfactory. After washing in buffer or seawater for some hours specimens were post-fixed in 1 p. 100 buffered osmium tetroxide (1 hour) and subsequently dehydrated in a graded acetone series. Polypides were either dissected from zooecia prior to epon embedment or colonies were decalcified in 10 p. 100 EDTA after glutaraldehyde fixation and subsequently embedded undissected. Thin (c. 60 μm*)* sections were cut with glass knives or a Dupont diamond knife, mounted on formvar-coated 200 mesh copper grids or on uncoated 300 mesh grids and stained in aqueous 4 p. 100 uranyl acetate (or saturated in 70 p. 100 methanol) for 10 min and in Reynold's (1963) lead citrate for 5 min. Micrographs were taken on a Zeiss EM 9 operated at 60 kv.

b. *Histochemistry and fluorescence microscopy.*

Stains were used on whole mounts and on paraffin and epon sections. Interpretation of electron micrographs was aided by 1 µm thick epon sections stained for light microscopy in toluidine blue according to the method of Trump et al. (1961). Characterization of the orange-brown inclusions in the stomach wall was carried out through vital staining of live whole mounts with brilliant cresyl blue and Nile blue sulphate, and staining of paraffin sections of Bouin-fixed material with Nile blue sulphate. Further characterization was carried out on a Zeiss Large Fluorescence Microscope on live stained (acridine orange) and unstained polypides using exciter filter UG 5/3 which transmits almost exclusively UV light. Photographs were taken with Kodachrome X (64 ASA) as it is fairly resistant to reciprocity failure (Pearse, 1972: 1203). Some stained sections were photographed on a Zeiss Photomicroscope II employing Nomarski Interference microscopy and Panatomic X film.

RESULTS

General morphology

The gross morphology of the bryozoan gut was the subject of a study by Silén (1944) who attempted to stabilize the terminology of the various regions. Broadly speaking, these regions are as follows in *Cryptosula* (Fig. 1). Immediately below the mouth is a muscular unciliated pharynx (oesophagus according to Silén). This is separated by a valvular constriction from the next section, a long tube leading to the stomach, designated the cardia by Silén. The stomach proper is a bipartite sac, the lower half of which is called the caecum. The upper half tapers into a dome-shaped pylorus which connects by a sphincter to the remaining section the rectum, terminating at the anus on the tentacle sheath. Lacourt (1949), apparently unaware of Silén's (1944) paper, proposed a terminology that, on the basis of his illustrations, is less concise than Silén's and involves new names for parts of the gut that are better known by more familiar ones e.g. ventriculus for central stomach, fundus ventriculi for caecum, etc. Subsequent authors and reviewers have adhered to Silén's terminology which will be used in this paper and commented on where appropriate. The various sections of the alimentary tract of *Cryptosula* will be described systematically in the following account.

Pharynx.

The Cheilostome pharynx is a vacuolated myoepithelium. It has been adequately described by Bullivant a Bils (1968) and Matricon (1973). The pharynx of *Cryptosula* does not differ except in a few minor points. The sarcomeres in *Cryptosula* are 2-3 µm long. In the smaller pharynx of *Zoobotryon* they are only 1-2 µm (Bullivant and Bils, 1968). Surrounding the pharynx in *Cryptosula* is a thin basal lamina in which the collagen fibrils are aligned in two layers at right angles to each other as in the tentacles. Outside the lamina is a sheet of circular muscle, serially continuous with the circular muscle of the

mouth. No longitudinal muscle was seen. Each sarcomere of this striated muscle is 3.5-5.0 µm long and there are fifteen sarcomeres per cell. At many of the Z bands is an indentation of the cell surface, presumably for the passage of ions.

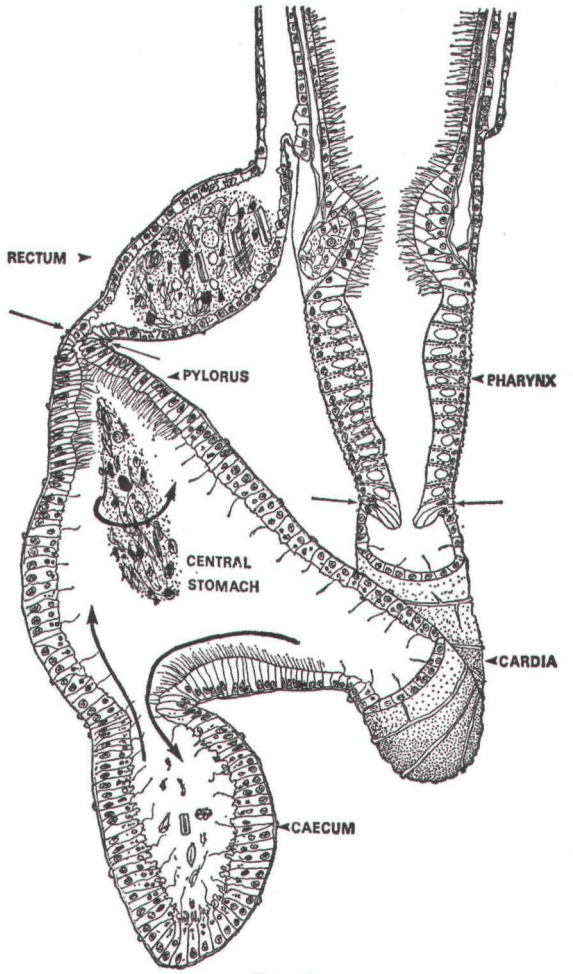

FIG. 1
Cryptosula pallasiana
Diagrammatic representation of the alimentary tract.
Pairs of thin arrows indicate sphincters. Arrows in the stomach indicate passage of material in and out of the caecum and the rotation of the ergatula in the central stomach.

Stomach.

According to Silén (1944) the next part of the gut, a long tube leading from the pharynx to the central stomach in *Cryptosula,* is better designated cardia rather than oesophagus. His view is acceptable as this section is histologically related to the rest of the stomach and is separated from the pharynx by one of the two major sphincters in the alimentary tract (excluding the mouth and anus). The stomach,

then, is tripartite (cardia, stomach sac and pylorus) or quadripartite if we consider the stomach sac to be divided into the upper central stomach and lower caecum.

a. *Cardia*

The cardiac stomach of *Cryptosula* comprises columnar cells **12-15 µ**m tall from base to apex (q.v. Plate 2 B). The cell surface is a brush border of slender microvilli c. 3 µm long. Cilia occur but are rare. They have a short rootlet with a basal centriole. Cells are united near their apices by gap junctions. Between the microvillar shafts and occupying the cardiac lumen is a fairly dense homogeneous mass of fine fibrous material of extrinsic origin which occupies canaliculi and vesicles at the cell apex.

Judging from the amount of rough endoplasmic reticulum and the well developed Golgi bodies secretion is of some importance. Golgi bodies give rise to vesicles 50-75 µm diam containing a rather dense secretion. At the perimeter of the Golgi field, the vesicles are mostly 75 µm diam whereas those closer to the saccules are 50 µm and the former have a dense halo with a clear core. Varying with the age of the polypide, the cardiac cells may also contain secondary lysosomes which tend to accumulate in the distal half of each cell, and small myelin figures which are often found in the basal half. Secondary lysosomes occur early in the life of the polypide, soon after feeding, as they do elsewhere in the stomach, and contain dense granules, clear areas and occasional membranous elements. The lysosomes appear as orange-brown inclusions in life, which stain intensely with brilliant cresyl blue applied in *vivo.* This reaction occurs throughout the whole stomach region. Brilliant cresyl blue is said to be a lysosome marker indicating acid phosphatase at the staining sites (Gahan, 1967), although this claim is disputed (Dr Michael Locke, University of Western Ontario, *in litt.* 1973).

The pharyngeocardiac junction is marked by a valve of cardiac cells which prevents backflow of ingested material as the pharynx dilates. The basal lamina of the cardia is not as structurally organized as that of the pharynx. Circular muscle is no longer a sheet of adjacent bands and there are now longitudinal strands of muscle. These two features (the lamina and the muscle) are related to the different activities of the pharynx and the cardia. The pharynx is continuously active and exhibits peristalsis. The cardia, on the other hand, shows slow dilation and contraction, in conjunction with the rest of the stomach.

b. Central stomach and caecum.

Two strongly ciliated areas occur in the stomach. One of these is the pylorus, the other is the floor of the cardia at the point where it enters the caecum, which allows any material from the cardia to enter the caecum directly. The upper part of the bipartite stomach, which Silén calls the central stomach, accommodates a rotating cord of food material projecting from the pylorus, about which more will be said later.

The cells of the central stomach are similar to those of the cardia but **are** more ciliated. The caecum is the site in the digestive tract

long known to be primarily responsible for intracellular digestion, and is better developed for this purpose than the rest of the stomach. There is no abrupt differentiation of cell types in the stomach although there is a gradient in the degree to which cytoplasmic systems are developed, correlated with a change from increasing ingestive and secretory capacity towards the caecum. In addition, there are fewer cilia and more microvilli towards the caecum and *vice versa* in the opposite direction.

Caecal cells are tall and columnar (Fig. 2), flattening upon dilation of the caecum. The apical half of the cell is concerned with secretion and absorption and contains Golgi bodies and digestive and endocytotic vacuoles. The cell apex is complexly canalized and vesiculated, the

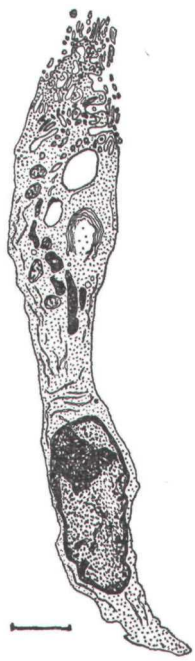

FIG. 2
Cryptosula pallasiana
A caecal cell.

Zonation of cell activity is very plain. Endocytic invaginations occupy the top third, digestive activity occurs in the cell centre and the nucleus occupies the cell base. *Scale* 2 µ*m.*

vesicles becoming larger by fusion deeper in the cell (Plate 2, A). They are lined on their internal face by a fine coating of the matrix material occurring between the microvilli, and on the cytoplasmic face by a fuzzy coat. During ingestion of particulate material, including whole diatoms if they are sufficiently small, cell apices do not fuse into a syncytium, as reported for *Zoobotryon* by Ries (1936).

There are two types of Golgi body, which have not been seen to occur in the same cell. One type is responsible for the production of macrovesicles of zymogen-like material (Plate 2, D) which appears to leave the cell, as well as smaller vesicles 50-75 (µm diameter with a fuzzy membrane and clear center. This Golgi body occurs throughout the stomach. A second type of Golgi body, saucer or cup-shaped, produces two sizes of vesicles as well (Plate 2, C). Macrovesicles are budded from any point on the Golgi body—periphery, convex, or concave face, and contain fibrous elements. In addition, small coated

vesicles 60-100 µm diameter are customarily budded from the periphery. These sometimes appear in chain-like form (Gordon, 1973) although this may be due to irregularities in the surface of the cisterns. The purpose of the small vesicles is not known. The contents of the macrovesicles are somehow deposited intercellularly and were not seen to be secreted at the cell apex. This second type of Golgi body occurs mostly in the central stomach and upper caecum. It is found also in digestive gland cells of the Bivalves *Cardium* and *Nucula* (Owen, 1970, 1973). Apart from these, I am aware of the occurrence of this type of Golgi secretion in only two other organisms, viz. the Chelicerate *Limulus* in the hepatopancreas (Herman & Preus, 1972) and possibly in the digestive gland of the Opisthobranch *Trinchesia* (Schmekel & Wechsler, 1968: fig. 8). Owen found this type of Golgi secretion (i.e. with fibrous elements) to be a characteristic feature of all Bivalve digestive glands that he studied, but he was not able to ascribe a function to it. In *Cardium,* two types of vesicle are also produced from this Golgi body. Herman & Preus suspect that the macrovesicles in *Limulus* may be primary lysosomes but neither they nor Owen observed fusion of the vesicles with food vacuoles and, in *Nucula,* they were found to be AcPase negative (Owen, 1973). Rarely these intercellular deposits have been seen in the lophophore base and tentacle epithelium of *Cryptosula* although a Golgi body to produce them was not seen. The small vesicles of this Golgi body were characterized by a fuzzy coating. Such bristle-coated vesicles are usually associated with micropinocytosis at the plasma membrane, but they are also known from Golgi bodies and their function is not altogether clear (Beams & Kessel, 1968: 227).

Beyond the caecum in the central stomach, cilia occur somewhat more frequently and the cell apex is somewhat less microvillous, otherwise the cells are like those of the caecum and possess the same kinds of Golgi bodies. Stomach cells are united by occasional septate desmosomes and more commonly gap junctions. The stomach is provided with a thin basal lamina overlain with bands of circular muscle with thin longitudinal strands. At intervals where these cross they are seen to be derived from the same cell. A single layer of peritoneal cells lies outside the muscle over the whole stomach. This is a diffuse covering which tends to slough off during specimen preparation.

c. *Pylorus.*

The pyloric cells share the same cytoplasmic features as other cells of the stomach but are densely ciliated and lack the large secondary lysosomes (Plate 3, B). The pylorus is a dome-shaped structure and its prime function seems to be to condense food material and skeletal fragments rejected from the caecum into a compact structure before passing it on to the rectum. The beating cilia cause the mass of particles, bacteria and fragments (collectively termed an ergatula by Morton, 1960) to revolve with its free end projecting into the central stomach (Plate 3, A).

One other feature is worth mentioning. In recta of polypides of *Cryptosula* which had not yet fed, I saw a semitransparent structure (Plate 1, A). This is apparently the same structure that Silén (1944)

saw in the pylorus of *Membranipora membranacea* which he later saw moved into the rectum. Afer the first defaecation of the polypide in both *Cryptosula* and *Membranipora,* it is no longer seen. It is believed to be meconium (Harmer, 1891).

d. Orange-brown inclusions.

A conspicuous feature of the stomach is the relatively large inclusion bodies (secondary lysosomes and residual bodies) which appear in the light microscope as orange-brown granules and which give the stomach its distinctive brownish colour. They are a well-known feature of Bryozoans but are lacking in newly-formed polypides. They cause a gradual darkening of the stomach wall during the life of the polypide. They stain in *Cryptosula* intensely with brilliant cresyl blue and Nile blue sulphate and exhibit a yellowish-green auto-fluorescence (Plate 1, B) when excited by UV light, suggesting that the granules are lipofuscin. Kœnig (1963) found that AcPase-containing granules fluoresce when irradiated with UV light but it is not clear whether fluorescence is associated with the membrane or content of the lysosome (Gahan, 1967).

In stomachs of older individuals the granules are more numerous and generally larger. They are derived from ingested food material and grow by addition of smaller vesicles, but the membranous elements within them (Gordon, 1973) are somewhat puzzling. Either they are elaborated from the ingested food or derived from autophagy of cell organelles during their normal turn-over, or from Golgi vesicles which carry digestives enzymes to these sites. It seems most likely that the membranes are derived from the cell itself. Other inclusions are amorphous material and osmiophilic droplets. Clear spaces may be due to the leaching out of some compounds like lipids during dehydration prior to specimen embedment.

Rectum.

The rectum is a short but distinctive thin-walled structure separated from the rest of the gut by the pyloric sphincter. Depending on the state of tension of the enveloping muscle bands during peristalsis it can taper for some distance towards the anus but it normally appears as a short swollen sac containing the condensed bundle of material passed on from the pylorus. The most notable feature of rectal cells is a brush border of microvilli (Plate 3, C) up to 2.5 µm long, below which is an extensive system of endocytotic channels. These channels fuse into inclusion bodies resembling digestive vacuoles in the caecum. A feature of some of the inclusions is their para-crystalline contents. At the level of light microscopy, differences in the nature of the inclusions of the rectum and caecum are quite apparent. The orange-brown inclusions in the caecal cells are not seen in the rectal cells and in UV light the caecal inclusions fluoresce yellowish-green whereas rectal inclusions fluoresce an orange-yellow colour.

RER is not well developed and Golgi bodies are not as prominent as in stomach cells, indicating less secretion by rectal cells. Compared

to the stomach, lateral cell membranes are much more complexly folded, resembling the condition in absorptive cells of the insect midgut (Berridge & Oschman, 1972). In its combination of characters, the rectum of *Cryptosula* would appear to be primarily absorptive in function with associated intracellular digestion on a smaller scale.

Some septate desmosomes unite adjacent cells. Nearer the anus the microvilli become shorter and fewer in number and the surface becomes covered with a fine fibrous material such as occurs on the surface of the cuticle where the anus opens onto the tentacle sheath.

Innervation of the alimentary tract.

The nerve supply to the gut is not extensive. One major nerve descends the dorsal side of the pharynx with a minor nerve either side which may have split from it (Plate 4, A). In *Electra pilosa,* Lutaud (1969) pictures three nerves descending the pharynx. In *Cryptosula,* there are a number of vesicle types, viz. small clear vesicles 40-80 nm diameter, resembling synaptic vesicles; small dense vesicles 50-80 nm diameter; large clear vesicles 115-150 nm diameter; and large, cored vesicles 100-160 nm diameter, resembling neurosecretory vesicles. These nerves innervate the myoepithelial cells of the pharynx and/or the circular muscles. No myoneural junctions were seen but some of the pharyngeal cells contained small, cored vesicles.

Other nerve branches were encountered in sections of the stomach, rectum and anus. Two types of nerve endings were found. In Plate 4, B, an actual synapse is not seen although the nerve is in close apposition to a muscle fibril (of a cardiac cell). The small vesicles have the appearance of synaptic vesicles.

The second type of nerve ending occurs at ciliated areas of the stomach from nerves running under the basal lamina. The endings contain only two types of vesicles—large dense-cored neurosecretory-type vesicles 100-160 nm diameter and small clear vesicles 50 nm diameter (Plate 4, C). Terminals of this kind containing two such vesicle sizes are typical of neurosecretory terminals which have been investigated by a number of workers, notably Smith (1970), Nagasawa et al. (1970), and Douglas et al. (1971). These workers have demonstrated exocytosis of the large vesicles and the micropinocytotic origin of the small vesicles. The large dense vesicles are not known to originate by endocytosis. Since it is most unlikely that a nerve terminal will take up a dense secretion from a stomach cell (which has not been observed to produce these vesicles) it is certain that exocytosis of the dense vesicles from the nerve terminals in *Cryptosula* takes place. Membrane-bounded dense vesicles identical to those in the terminals occur in the adjacent stomach cells. Assuming, then exocytosis from the terminals, there must be a mechanism by which stomach cells capture the released secretion.

DISCUSSION

The differing opinions by earlier authors as to the number of cell types in the Bryozoan stomach was based mainly on the types of staining reactions obtained. Results obtained from *Cryptosula* indicates that stainability of stomach cells can also depend heavily upon fixation. In my experience, fixation of adjacent cells can be vastly different, leaving one intact and a neighbour with some precipitation of cytoplasm (Plate 2, B) when using even the relatively critical methods of fixation employed for electron microscopy. Thick sections of EM-prepared material stained in toluidine blue for light microscopy give identical results, causing the cells with dense cytoplasm to appear more basophilic than their poorly preserved neighbours. Older methods of fixation are likely to enhance artifacts of this kind giving the appearance of cells with different staining characteristics. Also, the number and size of inclusions in the stomach wall, while intracellular digestion is occurring, will cause variations in staining characteristics. Therefore, while earlier reports of different cell types based on staining characteristics may be partly true, great care must be taken in ensuring that differential fixation does not give false results. There is no free mucus in the alimentary tract of *Cryptosula,* although the intermicrovillar matrix is mucopolysaccharide. Although two types of Golgi body occur in different cells or in the same cells if they replace each other, these are not detectable by light microscopy.

Observations on *Cryptosula* at the EM level show that Bronstein's (1939) conclusions concerning the nature of the pigmented inclusions in the stomach wall are correct, viz. that incomplete elimination of undigested material from the cells in which intracellular digestion takes place leads to accumulation of this material. In addition, there is probably the contribution of autophagy. The occurrence of membranous elements in the vacuoles indicates a local origin of these rather than their elaboration from digested material.

Digestion in Bryozoans is recognized to be both extra-cellular and intracellular, with the caecum being the primary site of digestion (Calvet, 1900; Bronstein, 1939; Silén, 1944; Brien, 1960). Intracellular digestion is common in a number of Invertebrates and structural similarities in the cells of different types should be expected. In fact, similarities between *Cryptosula* stomach cells, especially those of the caecum and other non-ciliated areas, and Molluscan digestive cells are striking. In the digestive gland of the freshwater Pulmonate *Biomphalaria,* digestive cells exhibit the following features. The basal region of the cell contains the nucleus and RER; the cell apex is microvillous with extensive endocytosis; in older cells, absorptive vacuoles contain yellowish-brown inclusions (secondary lysosomes and residual bodies) and these vacuoles respond positively in staining reactions for acid phosphatase and lipofuscin (Meuleman, 1972). Meuleman's identification of the yellowish-brown inclusions as lipofuscin complements results for *Cryptosula* using Nile blue sulphate, brilliant cresyl blue

and fluorescence microscopy (autofluorescence and acridine orange). Nile blue sulphate has been used to locate lipofuscin (Ortolani & Patricolo, 1972). Brilliant cresyl blue is said to be a lysosome marker (Mulnard, 1961) and lysosome autofluorescence and orange fluorescence after acridine staining is attributed to lipofuscin (Allinson & Young, 1969; Kœnig, 1963; Weglicki et al., 1968). Furthermore, lipofuscin is viewed as originating in the manner of residual bodies (Daems et al., 1969).

Meuleman (1972) has also observed that in *Biomphalaria,* animals fed carmine-stained food acquire red residual bodies 24 hours later, a situation comparable to that observed by earlier workers on Bryozoans (e.g. Harmer, 1931). Her conclusions about the absorptive capacity in this Mollusc are particularly relevant to *Cryptosula* as the processes appear to be completely identical. Vesicles formed from the cell apex are heterophagosomes (after De Duve's and Wattiaux' lysosome theory, 1966). "By fusion of heterophagosomes with others and with primary lysosomes, secondary lysosomes are formed". "When digestion is finished the secondary lysosomes develop into residual bodies... these residual bodies fuse to form the gradually enlarging yellow granules" (Meuleman, 1972:398). In *Biomphalaria,* the whole cell containing the residual bodies is thought to be released into the lumen of the digestive gland. Unfortunately, Meuleman was unable to determine the life span of a digestive cell but as these features occur in *Cryptosula,* this may shed light on the longevity of these cells in Molluscs.

In *Cryptosula,* a polypide survives for a period of 15-72 days during which time accumulation of residual bodies in the stomach cells reaches a point beyond which no more digestion can take place presumably and the whole polypide regresses. Because no mitoses were observed, it seems that there is no cell replacement in the stomach of *Cryptosula,* whereas there is in *Biomphalaria.*

The yellowish inclusions in certain cells of starved *Limulus* hepatopancreas (Herman & Preus, 1972) seem to be comparable to the orange-brown granules of *Cryptosula* and the yellowish-brown granules of *Biomphalaria.* Resorptive cells of Crayfish hepatopancreas have been observed to accumulate residual material also (Loizzi, 1971). Atkins (1933) observed the accumulation of yellowish spherules and small granules in rectal cells of *Loxosoma* (Entoprocta) whose vacuoles took up neutral red (a lysosome marker (Mulnard, 1961; Byrne, 1964). While Hincks (1880) was mistaken in assuming the brown colour of the stomach to be due to a "bilious brown fluid", it seems that he, Farre (1837), Van der Hoeven (1856) and Joliet (1877) were not far astray in ascribing a hepatopancreatic function to the bryozoan stomach, something that Morton (1960) also recognized when he stated that bryozoan stomach cells "resemble much in appearance the absorptive-digestive cell of the molluscan digestive gland".

The pylorus, while possessing a minor absorptive function, is concerned with creating an eddy in the stomach which concentrates particles leaving the caecum into a revolving rod. Silén (1944) does not regard compaction for defaecation as the prime function of the rotation mechanism, but as a means of further reducing organic material by enzymic digestion and perhaps also mechanical dissolution.

The whirling rod in the bryozoan stomach is nown in the Gymnolaemata and Stenolaemata but not in the Phylactolaemata, which lack a ciliated pylorus (Nitsche, 1868; Kraepelin, 1887). Silén realized that a whirling rod of food or secreted material occurs in Entoprocts and many Molluscs and concluded that its common occurrence and functional similarity must be related to the nature of the food ingested which, in these types, is particulate.

Morton (1960) discussed this relationship at length in a number of ciliary feeders which have the common problem of transport and digestion of small particles. In many of these groups, peristalsis, assumed to be inefficient at moving small particles, is reduced, and a section of the gut is designed to rotate its contents, generally mixed with mucus, the effect of which is transmitted anteriorly, such that particles are gradually wound in on a mucus cord at a controlled speed. This theme is variously modified in different animal groups, but where it occurs all possess the one common feature, whirling material in some part of the gut, termed an ergatula by Morton, and found in many Archaeogastropods, Mesogastropods, Thecosomes and Bivalves (as a crystalline style or protostyle), Entoprocts, all Lophophorates, Enteropneusts, Tunicates, Cephalochordates (Morton, 1960) and *Rhabdopleura* (Stebbing, 1972). That the effect of the whirling cord is felt anteriorly in *Cryptosula* is doubtful, however. There is no mucus secretion which would assist this, and food leaving the cardia passes rapidly into the caecum rather than becoming caught up in the motion of the ergatula.

The muscle fibrils of the stomach, which consist of striated circular muscles from which longitudinal fibrils branch, are like those of Crayfish hepatopancreas (Loizzi, 1972). Here, the diverticula expand and contract by simultaneous contraction of both circular and longitudinal fibrils, internal fluid pressure being assumed to effect expansion. Such a mechanism would explain Silén's (1944: 34) observation that peristalsis does not occur in the bryozoan stomach and caecum while dilation and contraction do. In *Cryptosula,* a rhythmic pumping-like action of the caecum occurs, at rates seemingly dependent on degree of fullness, counteracted by a similar motion of the cardia and central stomach. Loizzi also observed, associated with the hepatopancreas, nerve terminals with neurosecretory-like vesicles and suggested that the muscles may be influenced by these. In *Cryptosula,* however, presumed neurosecretory vesicles enter the actual stomach cells, comparable to *Phoronis* perhaps, where Vandermeulen (1970) observed chromaffin-like granules in proventricular and intestinal epithelial cells. Meuleman (1972) likewise observed presumed neurosecretory terminals near secretory cells of the digestive gland of *Biomphalaria.*

The gut of *Cryptosula,* a sessile suspension feeder, is thus seen to be a mosaic of structural and functional features found in a wide variety of phyla.

Summary

1. The ultrastructure of the alimentary tract is described **for *Cryptosula pallasiana.***

2. It was found that, contrary to earlier literature, there is no clear distinction between cell types in the stomach which can **be** based **on** staining characteristics at the level **of** light microscopy. Light and dark-staining cells which have been previously regarded as acidophilic and basophilic are almost certainly the result **of** differential fixation of adjacent cells. There are obvious differences in the types **of** cell apex found in the caecum and central stomach and the ciliated tracts but, where these areas merge, there is a gradation from one to the other. Two cell types can be distinguished on the basis of different Golgi secretions but even here the Golgi bodies are the sole distinguishing characteristic.

3. Caecal cell apices do not fuse into a syncytium. Ingestion is by endocytosis.

4. The orange-brown inclusions are secondary lysosomes and residual bodies, which respond to certain stains and UV light in the manner of lipofuscin. Cells of the central stomach and caecum containing these inclusions are strikingly similar to digestive gland cells of some Molluscs, confirming at the EM level Morton's (1960) statement of this similarity at the level of light microscopy.

5. The function of the pylorus appears to be that of compaction of food residue before entering the rectum, which in turn, seems to be essentially absorptive in function.

6. Muscle fibrils of the stomach are striated circular muscles from which thin longitudinal fibrils branch.

7. Neurosecretory-like terminals occur on ciliated cells **of** the stomach.

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Abstract

The ultrastructure of the gut of the marine bryozoan *Cryptosula pallasiana* is described. The number of cell types in the stomach is not clearly revealed by ultrastructural morphology alone and caution is made against interpretation of different types of cells from staining results only. Orange-brown inclusions in the stomach wall are secondary lysosomes and residual bodies which stain after the manner of lipofuscin. Caecal cells are strikingly similar to digestive gland cells of some Molluscs. Neurosecretory-like terminals occur on ciliated cells of the stomach.

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Cryptosula pallasiana

PLATE 1

A-C: basal views of zooids growing on a plate of glass.

In A: arrows point to meconium in the recta of three polypides.

B: fluorescence micrograph of an isolated alimentary tract of a live polypide irradiated by UV light, showing inclusions in cells of the stomach and rectum (at top).

C, D, E: the parts of the gut are shown in retracted and everted polypides.

In E: the arrow indicates the position of a wave of contraction passing down the pharynx. an: anus; ca: cardia; cae: caecum; cs: central stomach; e: embryo; lo: lophophore; fp: remains of former polypide in new gut; lr: lophophore retractor muscle; ph: pharynx; py: pylorus; sph: cardiac sphincter; ts: tentacle sheath.

(A, C-E X 55, scale 0,5 mm; B X 265, scale

PLATE 2

A: apices of caecal cells showing digestive vacuoles (heterophagosomes). Notice the fine coating on the cytoplasmic face and the endocytic invaginations. There are a few cilia (arrows).

(X 23,500, scale 1 μm.)

B: cardiac stomach showing the effects of unequal fixation. Two cells appear « normal », the others are empty-looking with burst vacuoles.

(X 2,700, scale 5 μm.)

C: a Golgi body which produces macro-vesicles containing fibrous material.

(X 25,500, scale 0.5 µm.)

D: a Golgi body which produces large zymogen-like vesicles (z).

(X 25,800, scale 0.5 µm.)

PLATH 3

A: pyloric stomach. A phase contrast micrograph of a live animal in optical section. The pyloric sphincter is marked by arrows. Whirling around in the pylorus is the ergatula (e).

(X 430, scale 20 µm.)

B: pyloric cell apices are densely ciliated with microvilli occurring between the cilia (rootlets are much longer than they appear in this micrograph).

(X 13,800, scale 1 µm.)

C: a single rectal cell. These cells are small, hence the nucleus appears relatively large. The brush border and endocytic tubules are characteristic. There is some convolution of lateral membranes between cells. Note the small muscle fibrils (arrows).

(X 15,100, scale 1 µm.)

PLATE 4

Innervation of the gut.

A: pharyngeal nerves, comprising one major nerve and adjacent minor branches. These nerves (seen here in part of a transverse section of the pharynx) are situated at the bases of the pharyngeal cells. Part of the striated circular muscle is seen outside the basal lamina.

(X 24,200, scale 1 µm.)

B: myoneural junction with a myofibril around a cardiac stomach cell. A definitive synapse is not seen. The small vesicles (arrow) have the morphological appearance of synaptic vesicles. Basal lamina occurs at left of the fibril.

(X 40,000, scale 200 µm.)

C: neurosecretory-like terminals on ciliated cells of the stomach. Two vesicle sizes only are apparent. Dense-cored vesicles occur in stomach cells (arrows) as well as in terminals. Apparent exocytosis of a vesicle (exo) is indicated in one terminal (see inset for detail). Outside the basal lamina (bl) is part of a striated muscle fibril (sm).

(X 35,500, scale 1 µm; inset X 77,000.)

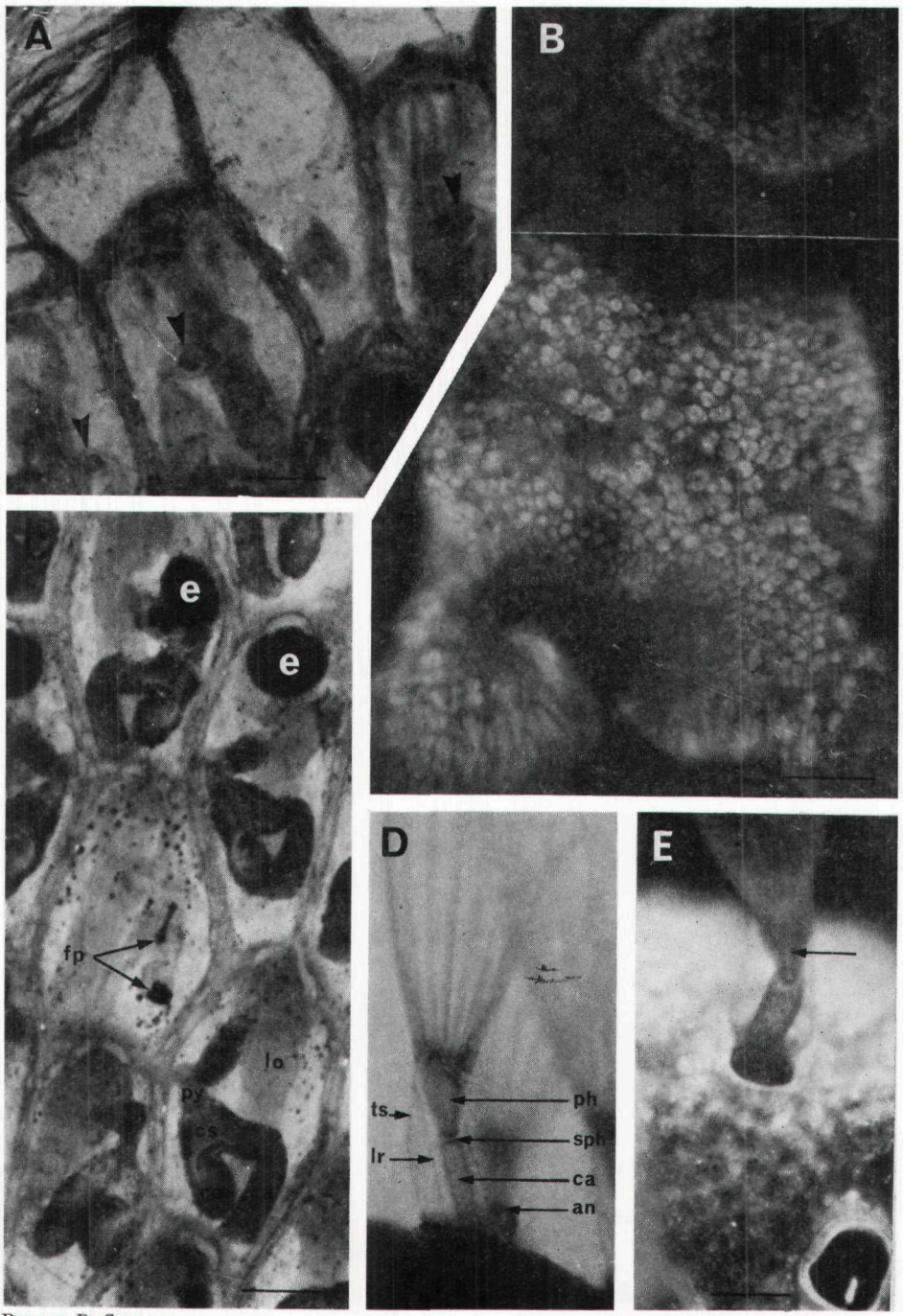

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PLATE 1

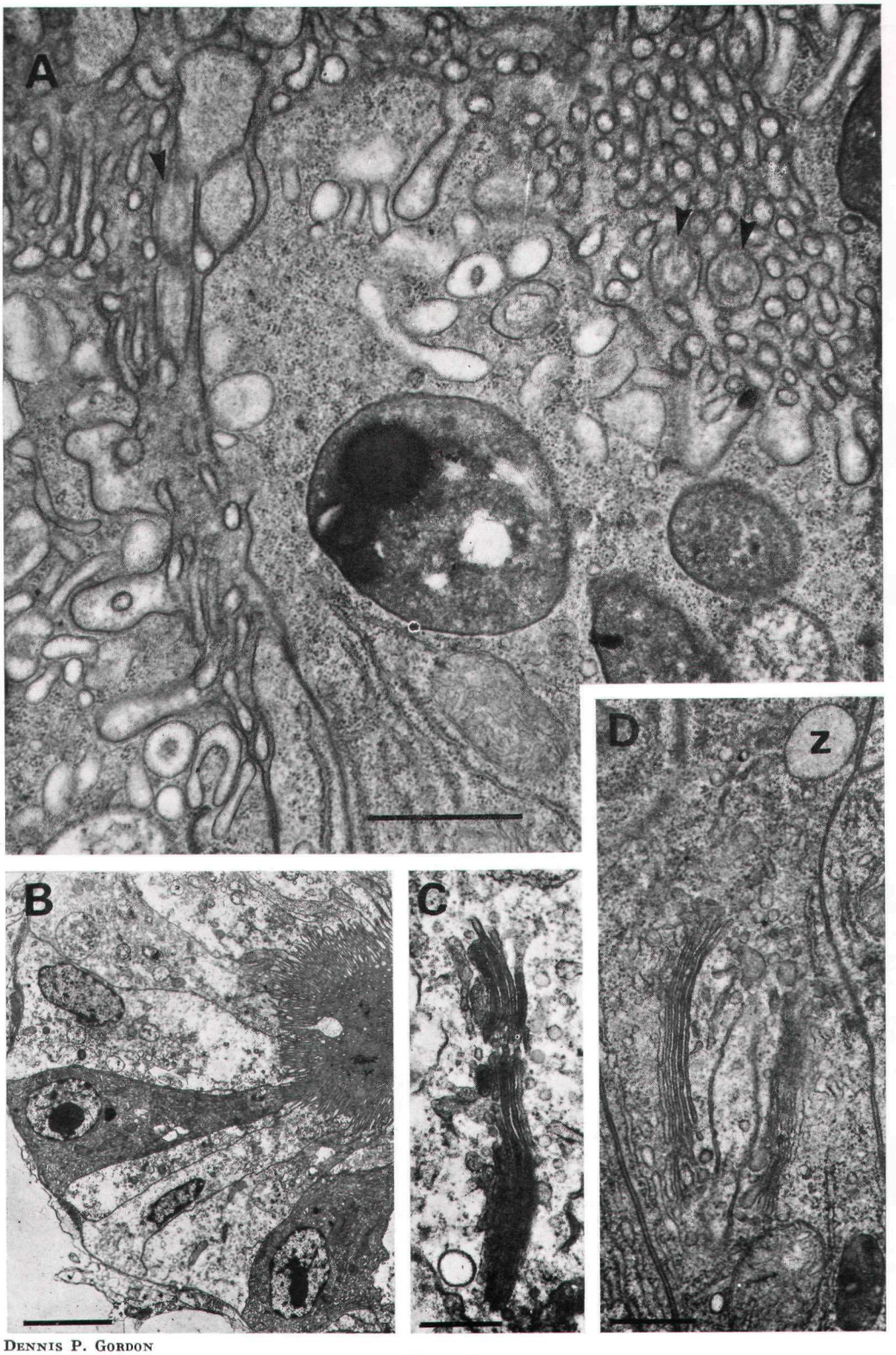

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PLATE 2

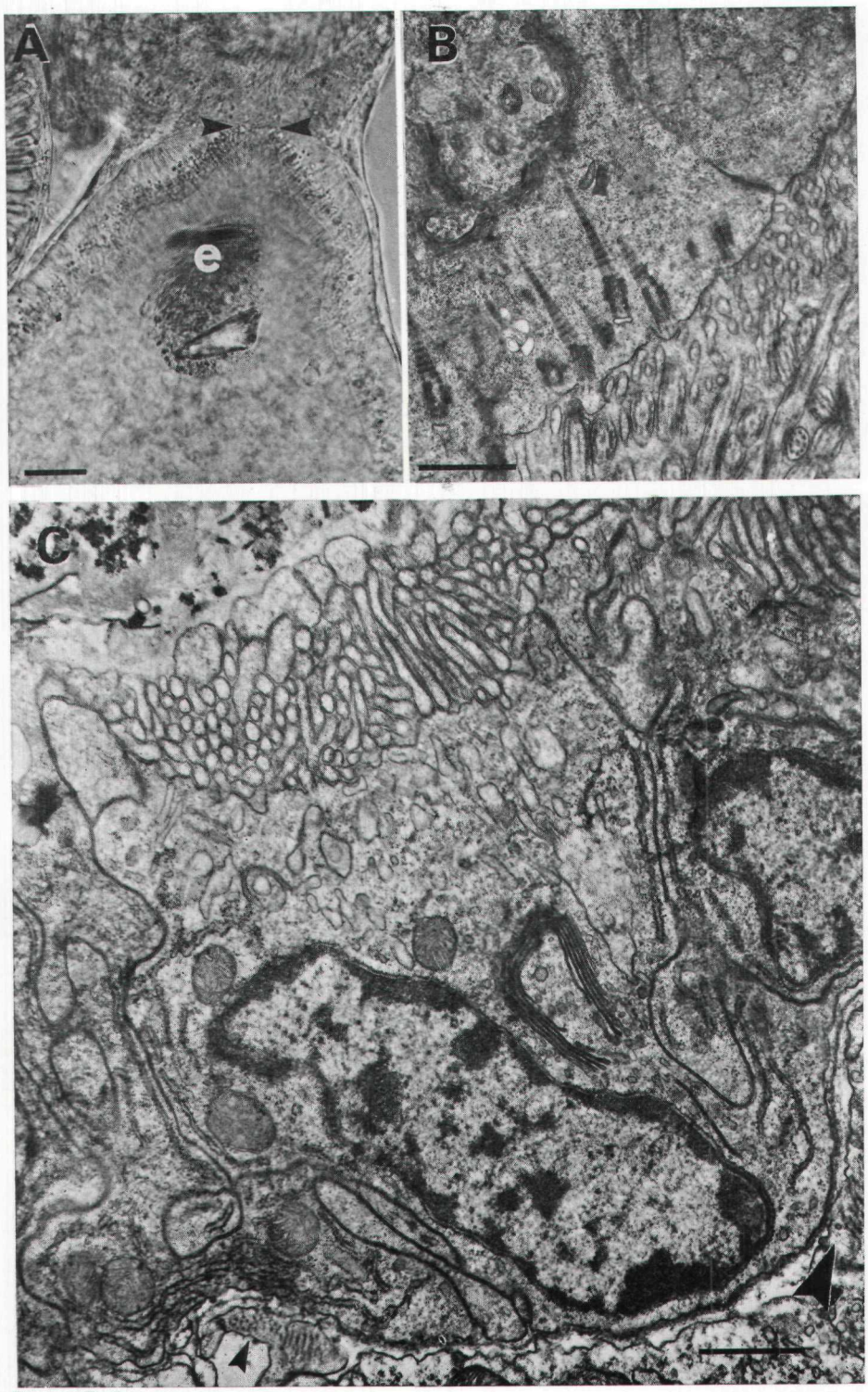

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PLATE 3

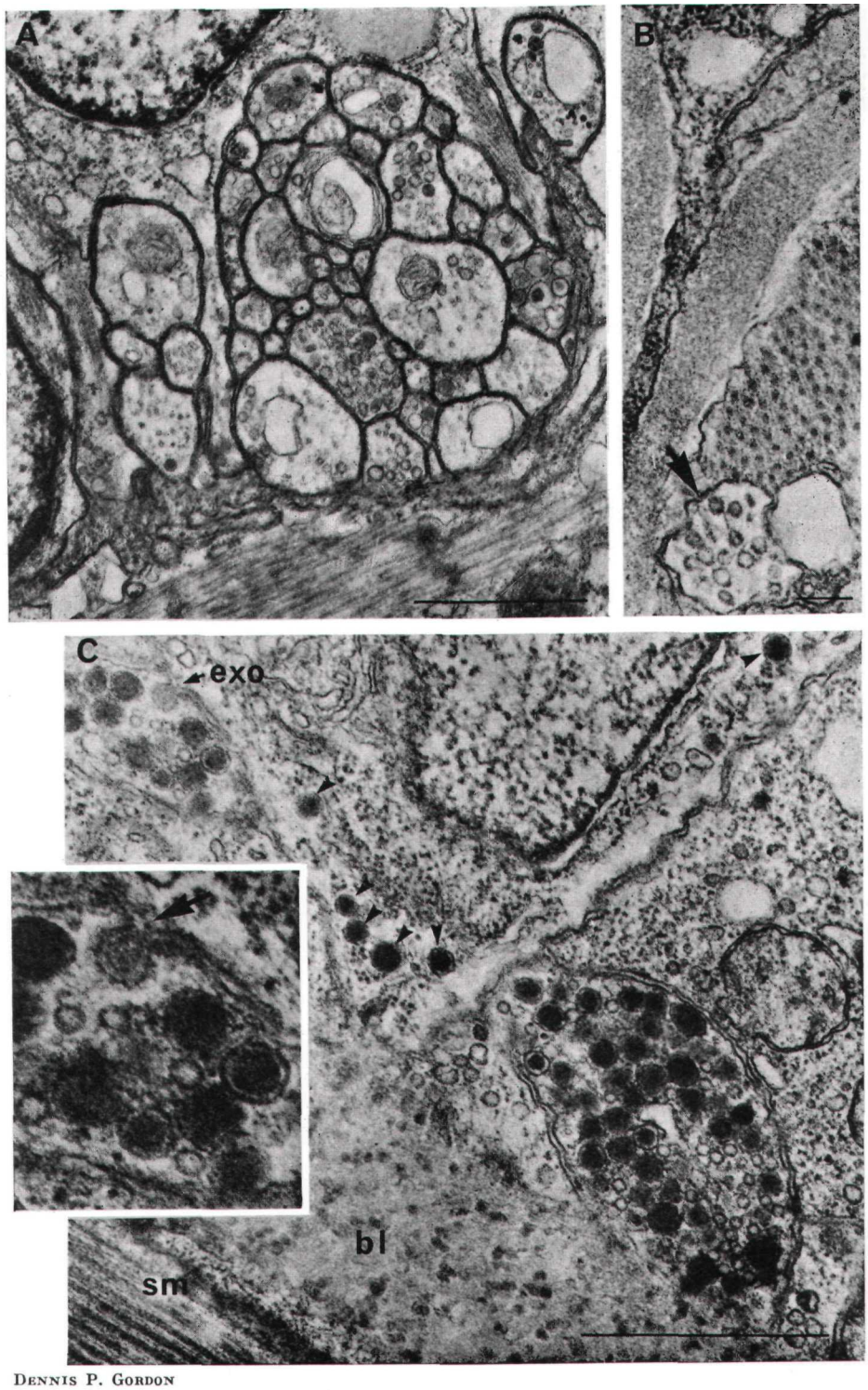

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PLATE 4