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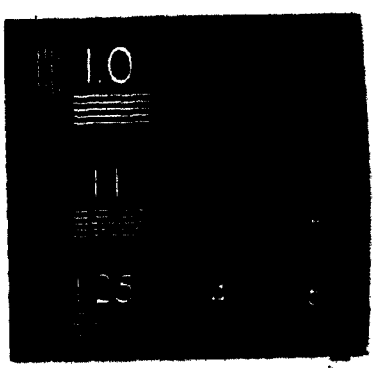

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TITLE

The fine structure of normal and regressing polypides of the fouling bryozoan Cryptosula pallasiana (Moll).

by

Dennis P. Gordon

A thesis submitted in partial fulfillment of requirements for the degree of Doctor of Philosophy in the Department of Biology at Dalhousie University.

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DALHOUSIE UNIVERSITY

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CONTENTS

Abstract:

Tha anatomy and morphology of the polypide of a bryozoan (Cryptosula pallasiana) are described at the ultrastructural level, clarifying doubtful points raised in earlier literature and describing features not previously known in bryozoans.

Tentacles: There are six nerves in each tentacle, not four as previously recorded. The new nerves comprise one additional frontal nerve and an abfrontal nerve. All but the peritoneal nerves comprise a cluster of axons. Neurociliary synapses occur between the frontal nerves and adjacent ciliated cells. Tentacle muscle is shown to be smooth. Tentacles are covered externally by a thin cuticle of mucopolysaccharide. The basal lamina is collagenous, and the fibril diameter of 11.5nm is among the smallest cited for an invertebrate.

Lophophore base: Epithelial cells around the mouth and at the bases of tentacles are blastemic in nature, probably serving to replace cells in damaged tentacles. A new set of muscles is described - the basal transverse muscles of the tentacles, each comprising a single cell containing short myofibrils of both thick and thin filaments. Large dense vesicles up to 270nm diam in large dorsal cells of the ganglion indicates the occurrence of neurosecretion in bryozoans. Lophophore retractor muscle is shown to be smooth, whereas the circular muscle of the mouth is striated. Filament dimesions of all muscles are given for the first time.

Alimentary tract: 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. Caecal cell apices do not fuse into a syncytium. Ingestion is by endocytosis. Orange-brown inclusions in the stomach wall 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.

Polypide regression: Features of regression and necrosis are described, and the transformation of muscle into paracrystalline arrays.

GENERAL INTRODUCTION TO THE THESIS

It is in some ways paradoxical that a phylum of morphologically quite diverse organisms, with numbers of species approaching that of echinoderms, and with no small ecological and economic significance, has continued to be so poorly represented in the literature. Yet such is the case with the Bryozoa in many aspects of their biology, particularly their ecology and fine structure. Since the founding of the International Bryozoology Association (I.B.A.) in 1965, however, and after the first I.B.A. conference in 1968, there has been a focus of interest on aspects of ultrastructure, but this has been almost exclusively on the body wall (cystid) by paleontologists. The polypide (feeding apparatus (lophophore) and alimentary tract) has been sadly neglected.

Prior to the commencment of my research there existed only two published electron micrographs (Fawcett, 1958; Bullivant & Bils, 1968) on any aspect of the polypide (in this case, of a cilium and rootlets and of a pharyngeal cell, respectively). Since this time two more papers have appeared (Renieri, 1970; Matricon, 1973), mainly concerning the pharynx. These contributions comprise the sole literature on the ultrastructure of the gut and feeding apparatus of a bryozoan. Part of the reason for the relative dearth of anatomical literature on bryozoans is no doubt due to the nature of the organisms. The majority of bryozoans (of the order Cheilostomata) are incrusting forms with a calcified body wall. Decalcification

of an incrusting colony results in a fragile item less than 0.5 mm in thickness which is difficult to handle. It is not surprising, therefore, that most anatomical studies have been of bryozoans of the wholly uncalcified order Ctenostomata, and of three of the four fine-structure papers devoted to bryozoans, two described ctenostomes (Bullivant & Bils, 1968; Matricon, 1973) and the third (Renieri, 1970) a lightly calcified anascan cheilostome. Since most living bryozoans are cheilostomes of the suborder Ascophora, however, it would seem preferable to investigate one of these if one desired to study a 'typical' bryozoan. An ascophoran, Cryptosula pallasiana, which is also cosmopolitan and of some economic significance, was chosen for this study.

Apart from the academic interest of exploring the ultrastructure of the polypide to further our knowledge of the anatomy and architecture of the whole animal (a valid enough reason in itself), there are excellent reasons for employing EM techniques in a study of the polypide to answer specific questions imposed by, and unable to be answered by, conventional techniques of light microscopy, viz. in the lophophore:

a. What is the nature of the basal lamina? Is it collagenous as in numerous other metazoans or is there some other protein component?

b. What is the extent of the communications between adjacent coelomic compartments (the mesocoelous compartments of the tentacles and lophophore base, and main body cavity which is metacoelous)? Can fluid movement be deduced from the

architecture of the lophophore?

c. What kinds of muscle are present? What are their filament dimensions and how do these compare with those of other invertebrates?

d. What is the extent of the lophophoral nervous system? Is there a relationship between nerve supply and ciliary beat as Fawcett (1961) has suggested?

In the gut:

a. How many cell types are there in the stomach - two types as many authors have stated (e.g. Calvet, 1900; Rey, 1927; Brien, 1960) or is the distinction doubtful as Bronstein (1939) suggested?

b. Do caecal cell apices fuse into a syncytium during cellular ingestion of food material as Ries (1936) stated?

c. What is the nature and origin of certain orange-brown inclusions that accumulate in the stomach wall?

d. Do bryozoan stomach cells resemble molluscan digestive gland cells at the EM level as they do at the level of light microscopy (Morton, 1960)?

In addition to considering these questions a general study of polypide ultrastructure was undertaken in order to characterize the 'normal' appearance of cells and tissues in order to interpret their regression as the whole polypide undergoes a process called brown body formation. This phenomenon has for more than a century captured the interest of bryozoologists who have speculated as to whether it is primarily excretory or senescent in nature. This question and the

following were specific considerations of my research, in which it was felt that a study of ultrastructure would provide some of the answers.

a. What is the relationship between the accumulation of orange-brown inclusions in the stomach wall and polypide regression?

b. What is the fate of the regressing cells and tissues and what is the role of phagocytosis in regression?

c. Of what value is polypide regression to a bryozoan?

These considerations, then, are the rationale behind this thesis, which is also intended to serve as a general documentation of basic ultrastructural data from which other more detailed studies can arise. The findings are presented in four sections: the tentacles; lophophore base; alimentary tract; and regression of the polypide.

SECTION 1 THE ULTRASTRUCTURE OF TENTACLES

1.1 Introduction

The apparent simplicity of the bryozoan lophophore is deceptive for it performs a multiplicity of functions. In addition to the obvious purpose of food collecting, which has been well described by Atkins (1932) and Bullivant (1968), the lophophore is said or implied to perform the function of a gill (Van Beneden, 1845; Hincks, 1880; Mangum & Schopf, 1967), excretion (Harmer, 1898; Calvet, 1900; Marcus, 1926), gamete release (Silén, 1966, 1972; Bullivant, 1967) and detection of mechanical stimuli (Hincks, 1880; Lutaud, 1955). Clearly, if the lophophore can perform all of these functions it is an organ system of some interest, and a useful model to be employed for correlating form with function. Many of these functions, however, can be investigated effectively in such small animals only at the ultrastructural level, but there have been only three published ultrastructural studies to date concerning the polypide (Bullivant & Bils, 1968; Renieri, 1970; Matricon, 1973) and these did not consider the lophophore.

The aim of this section (and the following on the lophophore base) is to relate lophophore structure and function and to consider specific points based on areas of disagreement about structural details in the literature. For example, the tentacle muscles have been described as both striated (Silbermann, 1906) and smooth Brien (1960). Presumed sensory cilia on frontal and abfrontal faces of each tentacle have been de-

signated setae or bristles (Lutaud, 1955; Bullivant, 1968) and syncilia (Nielsen, 1971:320). Knight-Jones (1954) studied the ciliary beat in a number of invertebrates and observed that bryozoans exhibit laeoplectic metachronism of the lateral cilia. What is the nature of the innervation of the tentacles and is there a relationship with ciliary beat?

This study will consider questions like these as well as lay the foundation, by a study of general ultrastructure, for understanding the events that take place when the polypide regresses at the end of its lifetime.

1.2 Materials and methods

Observations were made on Cryptosula pallasiana (Moll) a monotypic, cosmopolitan, marine-fouling gymnolaemate (Ryland, 1965) which is common intertidally in Nova Scotia. Freshly collected colonies were scraped off 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% glutaraldehyde in seawater with 1% sucrose for 1-3 hours, although Millonig's and 0.1M cacodylate buffers at pH 7.4 in lieu of seawater were fairly satisfactory. After washing in buffer or seawater for some hours specimens were post-fixed in 1% 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% EDTA after

glutaraldehyde fixation and subsequently embedded undissected. Thin (c. 60 nm) sections were cut with glass knives or a Du-Pont diamond knife, mounted on formvar-coated 200 mesh copper grids or on uncoated 300 mesh grids and stained in aqueous 4% uranyl acetate (or saturated in 70% 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. Scanning electron microscopy: Colonies were fixed as above for TEM. Polypides were dissected from zooecia and processed according to the method of Watters and Buck (1971) employing camphene which sublimes at room temperature. Dehydration with acetone led to surface tension problems and shrinkage of tissues. Stubs and polypides were gold or aluminum coated and examined on a Cambridge Stereoscan S 600.

c. Histochemistry and light microscopy: Stains were used on whole mounts, and 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). To visualize distribution of connective tissue epon sections were stained using Sievers' (1971) thionin- acridine orange combination, and whole mounts and paraffin sections of Bouin-fixed material was stained with light green and Mallory's triple stain after the method of Humason (1972). Distribution of polysaccharides in the zooid was determined using the periodic acid-Schiff method on epon sections. Whole mounts of live material were photographed on a Zeiss Photomicroscope II using negative-field phase contrast to

visualize tentacle ciliation, and feeding zooids were photographed with a Zeiss Tessovar macrophotography set-up using Ektacolor S film and Panatomic X.

1.3 Gross morphology and anatomy

There are 17 tentacles in Cryptosula (figs 1-3) ranging in length from 650-1200 µm and tending to be shorter in younger polypides. In transverse section the tentacles are roughly wedge-shaped tubes tapering frontally (orally). There are four longitudinal tracts of cilia, one frontally, one each laterally, and the abfrontal (aboral) surface bears a discontinuous series of inconspicuous cilia. Externally, the tentacle is covered by a cuticle, textured by protruding tips of microvilli, which tends to obscure cell boundaries except when tentacles are slightly contracted and cells show as ripples.

Internally, a tentacle comprises two cell layers and a basal lamina (fig 4). The outer cell layer consists of ten longitudinal rows of epithelial cells and four nerve tracts, and the inner layer comprises two rows of peritoneal cells, two nerve fibers, and two muscle cell tracts. Since the peritoneal and muscle cells overlap longitudinally, more than six cell elements may appear in cross sections. The two cell layers are separated by a relatively massive fibrous tube, the basal lamina. The central tentacular cavity (mesocoelous in nature) opens to the exterior by a pore in the tip of the tentacle. Just above the base of the lophophore the tentacles widen as more cells are interpolated frontally. The tentacles fuse bas-

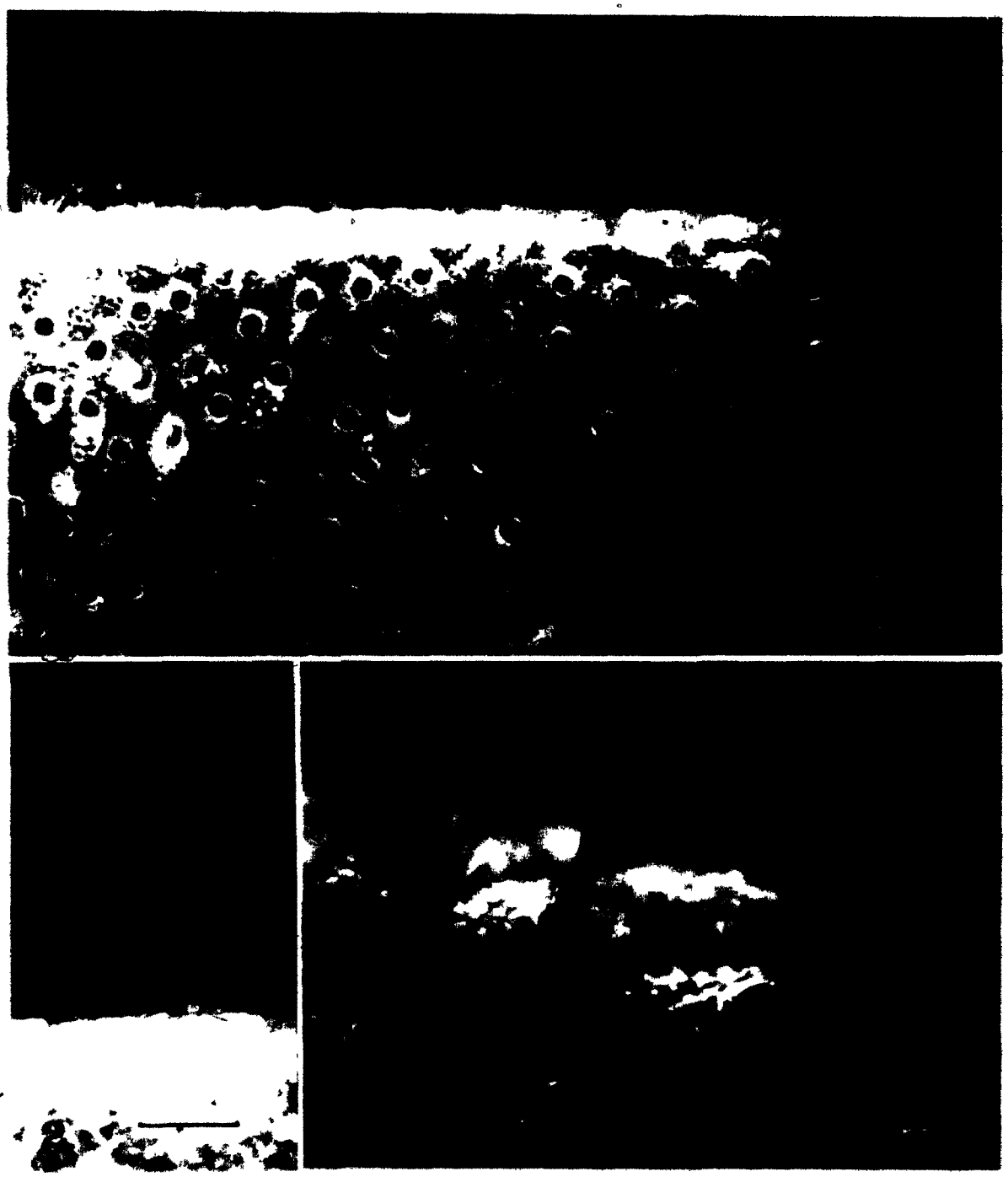

Figure 1. Part of a colony of the cheilostome bryozoan Cryptosula pallasiana (Moll) growing on a glass plate, showing zooids with evaginated lophophores in surface and side views. x 17 (scale 1 mm).

Figure 2. A single lophophore of Cryptosula showing an eccentricity of tentacle length, a phenomenon of frequent but not universal occurrence. x 34, (scale 0.5 mm).

Figure 3. Young zooids near the growing margin of a colony. There is some variation in colony colour but pale orange is predominant, the colour residing in both cystidial and polypidal tissues. x 55 (scale 0.4 mm).

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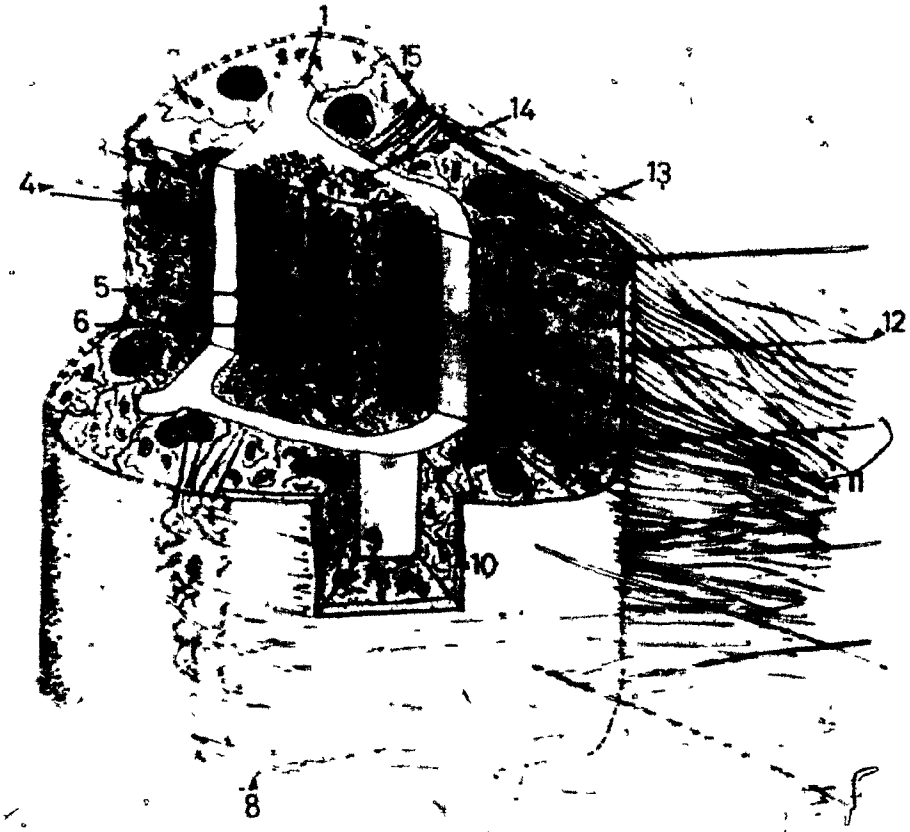

Figure 4. A tentacle depicted in cut-away view showing its anatomy based on electron micrographs.

Key:
1. basal lamina
2. epidermis
3. abfrontal nerve
4. tuft of short abfrontal cilia
5. longitudinal tentacle muscle
6. lumen of tentacle (mesocoel)
7. solitary abfrontal cilium
8. lateral cilia
9. long lateral rootlet of a cilium of a frontolateral cell
10. row of lateral rootlets in cross section
11. frontal cilia
12. cilium of a laterofrontal cell
13. frontal nerve
14. peritoneal nerve
15. cuticle

ally and their coelomic lumina become confluent with a coelomic ring around the mouth which is the main part of the mesocoel.

1.4 Fine structural anatomy and histochemistry

1.41 Cuticle

The cuticle covering the tentacles is a 0.3-0.6 µm wide layer of fibrous material in a clear matrix (fig 5). Numerous epidermal microvilli, occasionally forked, penetrate the cuticle, each terminating in a knob bearing antennular processes. These are continuous with a darker surface deposit on the outside of the outside of the cuticle apparently of the same nature as the cuticular fibrous material, and which in some micrographs appears to slough off from the surface. Histochemically, the cuticle is weakly PAS-positive and exhibits gamma metachromasia with toluidine blue, indicating a mucopolysaccharide (Pearse, 1968).

1.42 Epidermis

The distribution and dimensions of the ten epidermal cell tracts are shown in figure 4. If one designates the broad outer face of the tentacle as abfrontal, and the tapering inner face as frontal, then the ten cells seen in transverse section comprise three abfrontals, four laterals, and three frontals. Ultrastructurally, the ciliated cells (figs 6, 13) are distinguishable from the non-ciliated cells by the possession of not only cilia but more and larger mitochondria, and Golgi bodies which are typically located near the ciliary


b

Figure 5. Tentacle cuticle: Longitudinal section of a frontolateral cell of a tentacle showing the fibrous cuticle (cu), ciliary rootlets (r) in transverse section, lateral cilia (l) and microvilli (m). Note that the central pair of tubules in each axoneme is parallel to the cell surface, i.e. normal to the plane of the stroke. The tips of the microvilli bear antennulae which are continuous with a dark surface deposit. x106,000 (scale 200 nm).

Figure 6. Tentacle epithelia: Longitudinal section showing half the width of a tentacle, from the outer cuticulated epidermis, through the basal lamina (b) and peritoneum (p) to the longitudinal muscle (m). Many rootlet profiles occur in the epidermal cell, and small dense granules are seen in the mitochondria. x 31,500 (scale 1 µm).

rootlets and whose long axis is often perpendicular to the surface. In all cells small lytic vacuoles may occur, and pigment granules are sometimes prominent in frontal cells.

1.43 Basal lamina

A prominent feature of tentacle construction is the fibrous basal lamina. In the lophophore base (described later) a ring of basal lamina is continuous with the lamina of each tentacle forming an interconnected 'basket' of connective tissue. At the tentacle tip the fibrous tube is open. In thickness it varies from 0.75 µm laterally to 2.5 µm frontally. Abfrontally, the tube bears two longitudinal ridges, more or less demarcating the abfrontal cells from the laterals. Frontally, a minor keel is sometimes apparent (fig 7). Histochemically, the basal lamina is stained green in paraffin sections by light green (fig 9), blue by Mallory's triple stain and yellow in Sievers' epon stain for connective tissues, indicating the presence of collagen. In EM sections the collagen occurs as fibrils c. 12 nm wide with a periodicity of 55-65 nm, where each period is represented as a 'node' (fig 8). Within the clear matrix the fibrils are disposed in two layers, the one perpendicular with respect to the other, viz. an outer longitudinal layer over an inner transverse layer.

1.44 Peritoneum

Peritoneal cells are long, tapered, and overlapping with a conspicuous bulge marking the presence of the elongate nucleus. They are flattened against the inside of

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Figure 7. Basal lamina: Scanning electron micrograph of part of the basal lamina of a tentacle from which epidermal cells and nerves have been removed. Frontally, there is a minor keel (arrow). With cells removed the lamina is still seen to be a fairly rigid structure. x 2240 (scale 5 /um).

Figure 8. Basal lamina: Fibrils in the basal lamina of a tentacle are characterized by a nodular 60 nm periodicity (arrows). x 120,000 (scale 200 nm).

Figure 9. Basal lamina: Phase contrast micrograph of a light green stained paraffin section showing the basal lamina (arrows) of a tentacle cut longitudinally. x 925 (scale 10 /um).

the basal lamina and may partially cover the muscle and nerve cells in this region. Mitochondria are of the same dimensions as those of the unciliated cells and structures resembling multivesicular bodies are sometimes found.

1.45 Musculature

Two rows of myoepithelial cells traverse the length of each tentacle, one disposed abfrontally, the other frontally (fig 4). They overlap to an extent so that in transverse section the tapered end of one cell may be seen to be overlain by the broad surface of another. Within each cell are one or more bundles of filaments whose insertion points are in dense bodies on the basal lamina, although occasional isolated dense bodies are found. There is a more or less regular arrangement of thick and thin filaments, with a ratio of about 1:7, and one thick surrounded by 9-13 thin filaments (fig 11). There are no Z bands. Center to center spacing of thick filaments is 42-75 nm. A slightly diagonal periodicity of 15-20 nm is also apparent (fig 10). As in the peritoneum, the nuclei are elongated. Mitochondria are not numerous except near the tentacle base and are smaller than those of the ciliated cells of the epidermis. Multivesicular bodies like those of the peritoneum may also occur in muscle cells, and there are occasional centrioles with radiating microtubules. Although muscle occurs throughout the tentacle it is absent from transverse sections through places where the myofibrils do not extend to the cell extremities.

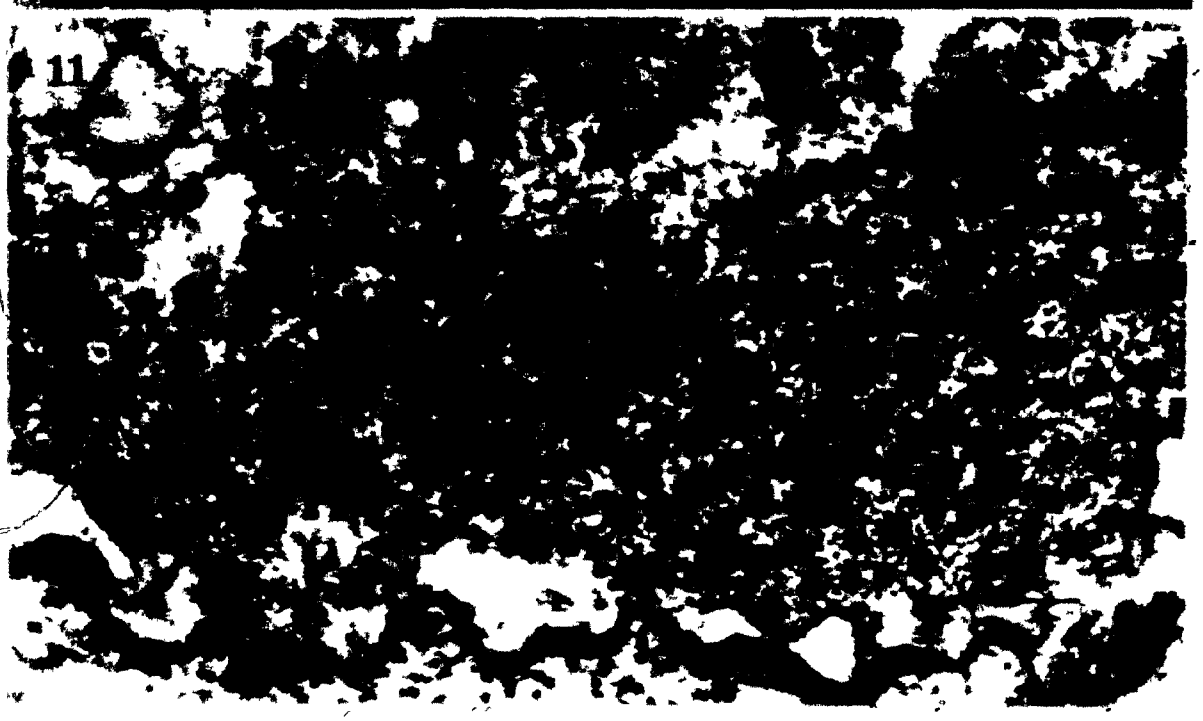
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Figure 10. Longitudinal tentacle muscle: Longitudinal section of part of a tentacle myofibril showing a slight 15-20 nm diagonal periodicity (arrows) in the thick filaments. x 108,500 (scale 200 nm).

Figure 11. Longitudinal tentacle muscle: Transverse section of part of a tentacle myofibril showing thick and thin filament arrays. There is some variation in thick filament diameter. Collagen fibrils (f) occur outside the sarcolemma. x 108,500 (scale 200 nm).

1.46 Mesocoel

When the tentacles are in an extended feeding position the lumen is more expansive than in retracted tentacles where it is frequently seen to be absent in sections. When tentacle muscles contract, shortening the tentacles, the basal lamina buckles inwardly in many places thereby squeezing the peritoneal and adjacent cells together. This necessitates displacement of the mesocoelic fluid proximally to the lophophore base. (This will be discusssed in more detail in the second section, on the lophophore base).

1.47 Ciliation

The general disposition of all cilia is shown in figures 4 and 12. The lateral cilia arise from two cell rows on each side of the tentacles, and while externally there is no apparent difference between the cilia of these cells, either in beating or in length, they are distinguished internally by their rootlets. Those in the two frontolateral cells have two rootlets disposed at right angles to each other, those in the two abfrontolaterals have single rootlets (fig 13). In the former case, one rootlet penetrates straight into the cell, the other runs frontally just under the cell surface for the full length of the cell. These rootlets are striking for their length which can reach 13 µm. Longitudinal sections cut perpendicularly to these rootlets reveal how many there are in each cell. Since sections in this plane also reveal the total number of lateral cilia, subtracting the number of rootlets from the total value gives an average number of 48 cilia for each frontolateral cell and 63 for each abfrontolateral cell.

Figure 12. Tentacle ciliation: Negative-field phase contrast micrograph of a live tentacle seen in optical section laterally. Small arrows indicate the long solitary cilia of laterofrontal cells, beneath which are seen curving the frontal cilia. On the abfrontal surface are seen tufts of short cilia alternating with long solitary cilia (big arrows). The basal lamina is seen as two light parallel lines running through the tentacle. x 910, (scale 10 μm).

Figure 13. Tentacle ciliation: Transverse section of a tentacle showing the ciliated lateral cells. The abfrontolateral (left) cell is marked by single rootlets, the frontolateral (right) is characterized by two rootlets, one parallel to the surface (lr). Arrows indicate satellite bodies). x 29,900 (scale 0.5 μm).

Figure 14: Tentacle ciliation: A vertical rootlet showing periodicities of 12.5 nm (top arrows) and 70 nm (bottom arrows). x 90,000 (scale 100 nm).

Each rootlet is characterized by a major period of 70 nm, the band width at each period being 38 nm. There are minor periodicities of 13 and 18 nm; the rootlet filaments are about 1.5-2.0 nm wide. The rootlets fray terminally, and usually insert on the basal lamina or nuclear envelope. A small satellite body lies adjacent to the basal body. In transverse section the rootlets appear as in figure 5. Lateral cilia are approximately 25 /um long and in the forward position of the effective stroke generally obscure the frontal cilia.

Frontal cilia are of two kinds. From each cell of the center cell row arise about a dozen cilia c. 15 /um long whose plane of beating is normal to that of the lateral cilia. Their rootlets are single and traverse the cell as far as the basal lamina. Flanking the frontal cells on either side is a row of laterofrontals bearing single cilia 5 /um apart and c. 20 /um long (fig 12). These correspond to the 'frontolateral setae' of Bullivant (1968) and 'soies tactiles' of Lutaud (1955). These cilia are not rigid but are capable of occasional flicking movements. There is a long major rootlet as well as a very short lateral rootlet, both arising from the basal body, and an associated basal centriole. At the tentacle base more cilia are interpolated into the laterofrontal cells and these beat in the manner of regular frontal cilia.

Abfrontal cilia are also of two kinds - short tufts of about ten immobile cilia 10 /um long alternating with solitary

cilia comparable to those of the laterofrontal cells (fig 12). A tuft of short cilia occurs at the tentacle tip. All cilia possess the 9+2 axonemal configuration. Near ciliary tips reduced numbers of tubules (domino tip images) were found. There is a transition from the 9D+2 pattern (D = doublets) to a 9S+2 pattern (S = singlets). The smallest number seen was 5S+0 and at the very tip no tubules were encountered.

1.48 Nerves

In each tentacle are six unsheathed nerves of which four are subepidermal and two subperitoneal (fig 4). The most prominent of these are three frontally disposed subepidermal nerves (fig 15) comprising a cluster of axons whose profiles are irregular in both transverse and longitudinal section. Variation in axon diameters in cross section seems to be due to varicosities which are seen plainly in longitudinal sections, in which it is difficult to follow individual axons far due to their apparent snaking around each other. Vesicles which are found in the axoplasm are mostly small clear vesicles 50 nm diam (range 36-60 nm) resembling synaptic vesicles, the remainder being a few larger clear vesicles 65-100 nm, large dense vesicles c. 100 nm diam and dense-cored vesicles c. 60 nm diam. Nerve profiles containing predominantly small agranular vesicles with a few large granular vesicles are said to represent cholinergic nerves (Burnstock & Iwayama, 1971).

Synapses are made with ciliated cells and seem to be

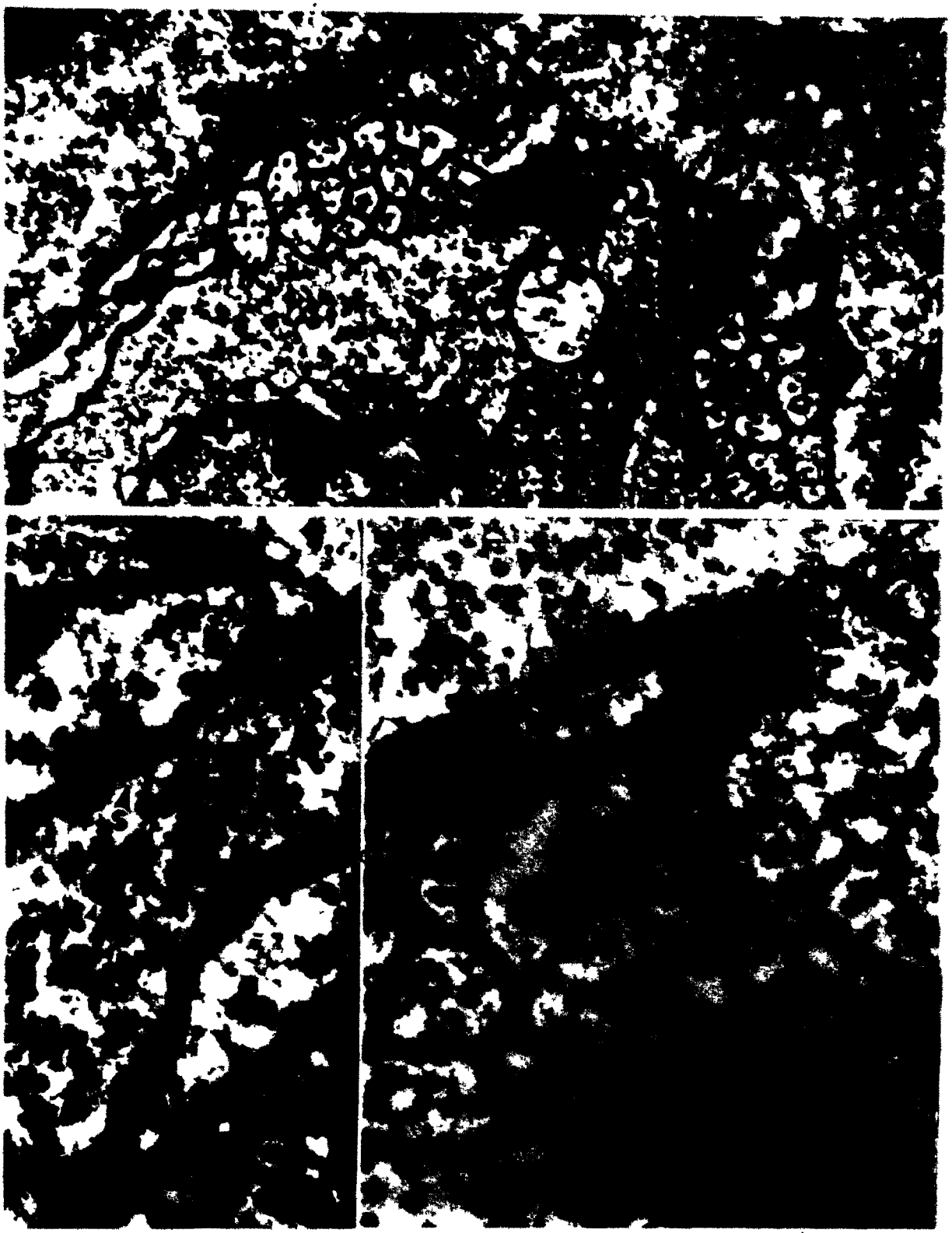

Figure 15. Frontal tentacle nerves: Transverse section through the three frontal nerves of a tentacle, sandwiched between ciliated cells and the basal lamina. Most axons contain microtubules (t), some contain small clear vesicles (v) and neurofilaments (f). x 26,800 (scale 0.5 µm).

Figures 16, 17. Neurociliary synapses between axons and ciliated cells. The postsynaptic side in each case is a ciliated cell (right hand side of synapse). In figure 17 rootlet fibers from a cilium (r) attach by the basal lamina. Small clear vesicles (small arrows) are scattered in the ciliated cells.
16. x 77,100 (scale 200 nm),
17. x 76,000 (scale 200 nm).

of the en passage type. There are, however, no areas of membrane specialization such as one normally associates with synapses, and in these nerves there is only a slight fuzziness and increase of density in the area of contact. Such synapses are seen in figures 16 and 17 where vesicles occur on both sides of the contact and the presumed post-synaptic side is a ciliated cell. Vesicles on both sides are of the same diameter. These synapses are of the neuro-ciliary type.

Sensory cells are said to occur in bryozoan tentacles. Gerwerzhagen (1913), Graupner (1930) and Brien (1960) have depicted presumed sensory cells with axons in gymnolaemates and phylactolaemates after methylene blue staining. I have only once seen an axon leading away from a ciliated cell. This was a cell near a tentacle base containing many rootlet profiles and which could have been either a frontal or front-olateral cell. In either case, if a cell from which an axon arises is truly sensory, as is believed, then the three frontal nerves comprise both afferent and efferent fibers.

The remaining subepidermal nerve is a bundle of some 4-15 axons running along the abfrontal side of the basal lamina (fig 18). Axoplasmic contents are as in the other epidermal nerves. In addition, relatively large lipid droplets occur and sparsely granular ER. This nerve is associated with the presumed sensory abfrontal cilia.

The two subperitoneal nerves are small, each comprising one axon of variable diameter (fig 19). No vesicles have


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Figure 18. Abfrontal tentacle nerve: Transverse section through the abfrontal nerve of a tentacle, which is separated by the basal lamina from a myoepithelial cell (longitudinal tentacle muscle). Small clear vesicles and a single dense vesicle, as well as mitochondria are seen in one axon. x 45,200, (scale 0.5 µm).

Figure 19. Peritoneal tentacle nerve: Transverse section through one of the two single-axon peritoneal nerves abutting against the basal lamina. This profile shows microtubules and smooth ER. x 58,200, (scale 200 nm).

yet been encountered in the axoplasm but there are numerous microtubules and some SER. Nor were synapses seen with the myoepithelial cells which these nerves are believed to innervate.

1.5 Discussion

1.51 Tentacle construction

A tube with a fluid core surrounded by a subepidermal fibrous lamina is a common structural entity among animal phyla, analogues of which are seen in pogonophoran, pterobranch, phoronid and brachiopod tentacles, serpulid branchiae, and molluscan and teleost gill lamellae. Variations on this plan are related to function and size and where these two parameters correspond in different groups the tentacles or whatever are likely to be very similar in most respects. A notable example is seen in the hemichordate Rhabdopleura whose feeding organs closely resemble those of bryozoans (vide Dilly, 1972). In the expanded feeding position the epithelia of the tentacles flatten against the basal lamina and the coelom comes to occupy the greatest volume of the tentacles at this time. It has thus been reasonably suggested that the lophophore serves as a respiratory organ, comparable to structures mentioned above for other organisms (Mangum & Schopf, 1967), although there are reasons for believing this function to be over-rated (Ryland, 1967). Among other lophophorates a comparison has been made between the lophophore of brachiopods and bivalve gill lamellae by Rudwick (1970:118) who points out that brachiopod tentacles are

not joined as in bivalves and this limits the force of water that can be directed against them. Coelomic pressure in brachiopods must be responsible for providing rigidity to the very long tentacles, as in bryozoans, and similarly in phoronids.

Direct comparison of tentacle fine structure within the three lophophorate phyla is not yet possible. On the basis of light microscopy, however, construction is seen to be comparable (Selys Longchamps, 1907; Richards, 1952), and as in Cryptosula the tentacles possess a substantial basal lamina. It is not clear how much the basal lamina provides support and rigidity to lophophorate tentacles. While the tentacles are flexible, and thus also the lamina, in damaged tentacles of Cryptosula where the epidermal cells have been removed, the basal lamina remains as a stiffly pointing tube (fig 7). By comparison, in the Entoprocta, whose feeding apparatus is similar to that of bryozoans, there is apparently no basal lamina (Mariscal, 1965) and the fluid core is evidently sufficient for tentacle support.

1.52 Cuticle

In the groups of animals mentioned above where tentaculated or filamentous feeding organs may double as respiratory structures, one of the requirements is a surface permeable to oxygen, so it should not be surprising to find that a cuticular surface, which is common to most invertebrates, is, where it occurs on these respiratory epithelia, very thin and ultrastructurally very similar. For example, the cuticle of Cryptosula tentacles is nearly identical in

appearance to that of the pinnules and metameric papillae of pogonophorans (Gupta & Little, 1969, 1970), mussel gill filaments (Gibbons, 1961), entoproct tentacles (Mariscal, 1965) and the branchial crown of Sabella (Kryvi, 1972). Similar microvillous cuticles occur on echinoderm podia (Menton & Eisen, 1970; Souza Santos & Sasso, 1970), Harmothoë (Polychaeta) trochophores (Holborow et al, 1969) and Aeolosoma (Oligochaeta) epidermis (Potswald, 1971), where cutaneous respiration is important.

Histochemically, results indicate that the cuticle of Cryptosula is composed of neutral and acid mucopolysaccharides. This is also the case in Thyone, Asterina, Sabella, pogonophorans and Aeolosoma (l.c.), differences lying in the relative amounts of neutral to acid carbohydrate cited by the various authors.

1.53 Basal lamina

The filaments in the basal lamina appear to be collagenous. They are, however, characterized by being considerably thinner than collagen fibrils of most other invertebrates. Among metazoans collagen is widely distributed, occurring from Porifera to Chordata. Elder & Owen (1967) examined connective tissue fibers in a range of invertebrates and found that collagen fibril diameters ranged from 20 nm in Polyphysia (Polychaeta) to 350 nm in Peripatoides (Onychophora). Doyle & MacNeill (1964) found collagen fibrils 20 nm wide in the basal lamina of Cucumaria respiratory trees and

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smaller diameters have been found in neural connective tissue of insects viz. 15-40 nm in young locusts (Ashhurst, 1965) and 13 nm in a lepidopteran larva (Locke & Huie, 1972). 13-20 nm seems to be among the smallest diameters cited for invertebrates, while in Cryptosula diameters are about 11.5 nm (13-14 in regressing tissues).

Axial periodicities in Cryptosula are represented by 'nodes' 60 nm apart (range 55-65 nm). This figure is comparable to the 64 nm periods common to vertebrate collagen (Banga, 1966). There are no unequivocal infraperiods in Cryptosula except in regressing tissue when the 60 nm periods disappear and a periodicity of 13-14 nm is apparent. The nodular appearance of the major periods is not seen in published micrographs of invertebrate collagens with the possible exception of Thyone (Holothuroidea) (Menton & Eisen, 1970:fig 15) where the major period is 55 nm but the fibril diameter 30 nm. Meek (1966) has commented on the variations in invertebrate collagens compared to those of vertebrates and feels that as well as morphological differences, there may be chemical differences. Certainly collagen should be regarded as a group of proteins, related by the possession of the amino acid hydroxyproline.

Morphologically, the fibrils in Cryptosula most closely resemble the fibrils of the vitreous body (vitrosin) and the zonular fibrils of vertebrate eyes. The dimensions in Cryptosula (55-65 nm period and 11.5 nm diam) closely correspond to the dimensions cited for zonular fibrils of human and

rodent eyes (50-64 nm period and 10.9 nm diam; range 6-25 nm) (Gärtner, 1970a, b). In addition, nodularities resembling those in Cryptosula, with 60 nm spacing, have been induced in vitreous body collagen (Smith & Serafini-Fracassini, 1967) by precipitating hyaluronic acid (one of the two mucopolysaccharide components of this tissue) as hyaluronate. Possibly the nodularities in Cryptosula represent polysaccharide. Since the nodularities do not occur in regressing tissues of Cryptosula, fixed as for normal tissues, it seems their occurrence depends on the physiological state of the organism. Such is the case in earthworm body wall, where fibrils change their band length with changing physiological conditions (Rudall, 1968).

Although this is the first demonstration of basal lamina collagen in bryozoans, collagen has been previously discovered in bryozoan cuticle (Krishnan & Rajulu, 1965; Schopf & Manheim, 1967) based on staining reactions and the occurrence of appreciable amounts of hydroxyproline. Renieri (1970) said that there was collagen in bryozoans but his statement seems to have been based on the mere presence of a basal lamina.

In vertebrates basal laminae are said to be a specialized form of connective tissue whose cheif functions are support and participation in the differential permeability to molecules across capillary walls and epithelial surfaces (Kefalides, 1970). Very little is known about tentacle physiology in bryozoans, but it seems reasonable to assume that the basal lamina would not act as a barrier to passage of metabolites

from the coelom to the outer epidermis and vice versa. With respect to its mechanical function, collagen is a relatively inextensible though flexible substance (Harkness, 1968). In the body wall connective tissue of polychaetes, for example, collagen serves the function of providing a base on which the muscles can act, and of imposing a limit to the extensibility of the system. In Cryptosula it seems certain that the basal lamina performs these same two functions, although it is not clear how the opposing fibril layers work in relation to one another.

1.54 Muscle

In transverse sections the myofibrils are seen to be made up of a more or less regularly disposed arrangement of thick and thin filaments, reminiscent of striated muscle. There is, however, no longitudinal division into regular sarcomeres. The groups of filaments do not insert onto the basal lamina at the same level, so there is no approximation of a Z band. A few dense bodies (isolated bits of Z band material) have been seen at different levels among the filaments, and this type of muscle approaches that reported among various molluscs, where scattered dense bodies occur among thick and thin filaments. In the buccal retractor muscle of Monodonta (Trochidae) where this arrangement occurs, Nisbet & Plummer (1968) refer to it as striated. They observed a less organized arrangement, in which dense bodies were fewer, in pulmonate collar muscle, which they called smooth. Richardot & Wautier (1971) observed muscle

more organized than that of Monodonta in an ancylid, where the dense bodies were aligned but not contiguous, and regarded it as a type of muscle intermediate between smooth and striated. Both groups of authors agree that lack of striation is due to lack of transverse contiguity of dense bodies. In Cryptosula, where dense bodies are few, the tentacle muscle is best regarded as smooth.

The diameter of the thick filaments (20-42 nm) in Cryptosula is considerably greater than the 11 nm diam cited for thick filaments in mammalian striated muscle (Huxley & Hanson, 1960) but compares with 18-40 nm in mammalian smooth muscle (Devine & Somlyo, 1971), as well as 15-120 nm in gastropod smooth muscle (Hanson & Lowy, 1960). No figures are available for phoronids or brachiopods. Thick filaments from entoproct socket muscle are 35-50 nm diam (my determination from micrographs of Reger, 1969). Thin filament diameters in Cryptosula of 4.5-7.5 nm are near the 5 nm diam quoted for a range of invertebrates (Hanson & Lowy, 1960).

1.55 Ciliation

The distribution of cilia on the tentacles of a marine bryozoan was most recently described by Lutaud (1955) and Bullivant (1968). Lutaud recognized that the lateral cilia arose from two cell rows on each side, despite the difficulty of determining cell boundaries by light microscopy. The cilia from both cells beat as a unit, lying along the surface of the tentacle in the forward position of the effective stroke. Sections of cilia in this position (fig 5) are seen to

have the two central tubules of the axoneme aligned more or less parallel to the cell surface i.e. at right angles to the plane of the stroke. The same alignment is true of the frontal cilia also. This arrangement seems to be general in ciliated tissues (Fawcett & Porter, 1954; Gibbons, 1961; Horridge, 1965; Tamm & Horridge, 1970).

The rootlets in Cryptosula are long, as is common for invertebrates (Fawcett, 1961). The lateral rootlets of the frontolateral cells attain 13 µm while the basal rootlets range from 2.25-3.50 µm in length. The major periodicity in the rootlet fibers is 70 nm which is identical to the 70 nm periods which are generally encountered in ciliated tissues (Afzelius, 1969). According to Fawcett (1958), in the lophophore of the freshwater bryozoan Pectinatella three rootlets arise from each basal body, one vertical, and the other two roughly horizontal but diverging in opposite directions. From his micrograph I estimate the rootlet periodicity to be 72-75 nm. As his micrograph is cropped it is not possible to see whether the lateral rootlets are of comparable length. One has a smaller diameter. The cells bearing these cilia are not described but these are probably lateral cilia. This is the only information pertaining to the rootlet structure (indeed, any fine structure) of the freshwater class Phylactolaemata. The significance of the differences between rootlet structure of adjacent lateral cells and of the two classes cannot even be guessed at, particularly as there is not yet a known correlation between presence and absence of rootlets and rootlet

orientation and the character of ciliary movements. Gibbons (1961) feels that rootlets are not essential to function but merely play a mechanical role such as support.

Fawcett & Porter (1954) summarized views on the function of rootlets extant from 1880 to 1954, which included nutrient pathways, contractile elements, specialized nerve endings and impulse-conducting structures. Dorey (1965) found Gibbons' (1961) opinion that support is the only function of the rootlets unsatisfying, and suggests that one function may be to preserve the various uniformities of orientation that occur in different types of ciliary field. Fawcett (1958) suggested that if the rootlets were contractile, their shortening in a particular sequence (in Pectinatella for example) would impart pivotal movements to the basal body which would affect the shaft. However, that the rootlets are contractile is in doubt as nobody has ever reported narrowing of the band widths or a possible sliding of the component filaments. The 60-70 nm period was regarded as an identifying characteristic of collagen (Afzelius, 1969) and until rootlets are characterized chemically, their proper nature remains in doubt.

1.56 Innervation of the tentacles

The nervous system of bryozoans has recently been described in reviews by Hyman (1959), Brien (1960) and Ryland (1970), and Lutaud (1969) verified the presence of a colonial nervous system as well as giving further details of the distribution of polypide nerves. On the basis of light microscopy

there are said to be four nerves in each tentacle, two motor and two sensory. Six nerves were found in Cryptosula. The two motor nerves reported in bryozoans correspond in Cryptosula to the subperitoneal nerves. These nerves were detected by previous authors in methylene-blue stained preparations and were seen to arise at the tentacle base to ascend each tentacle on the luminal side of the basal lamina (Graupner, 1930; Bronstein, 1937). That Graupner should also have detected these nerves in transverse sections of the tentacles is quite amazing. In Cryptosula the tiny peritoneal nerves comprise single axons 0.5-1.0 µm diam. After hypotonic fixation, however, they often appear larger (3 µm diam) and this may account for their detection. Graupner's illustration (1930:61) depicts these nerves in transverse section in the same position they are found in Cryptosula, so there is no doubt that he saw them. The assumption that these nerves were motor was based on their proximity to the tentacle muscles (Bronstein, 1937:164).

The two sensory nerves reported in bryozoans correspond to the three frontal subepidermal nerves in Cryptosula. These were regarded as sensory by Gerwerzhagen (1913), Graupner and Bronstein because there seemed to be connections to apparent sensory cells in the tentacle epithelium, most notably at the base and tip of each tentacle. It is unlikely that the newly discovered abfrontal nerve that occurs in Cryptosula is not found elsewhere, and its not having been discovered before by light microscopy is hardly surprising because of its

small size.

What light does electron microscopy throw on the supposed functions of the tentacle nerves? The subperitoneal nerves in Cryptosula have not yet been seen to make contact with the tentacle muscles so their motor function is still in doubt. The muscles lie about equidistant from both the peritoneal and epidermal nerves, but are separated from the latter by the basal lamina. It is not unknown for nerves to traverse basal laminae to innervate muscle. This occurs in the hemichordate Saccoglossus (Dilly, 1969) (though not in Rhabdopleura (Dilly, 1972) which is structurally very similar to bryozoans in tentacle anatomy). While occasional membrane bounded spaces are found within the basal lamina of Cryptosula, none have been identified unequivocally as axon profiles. Cobb (1970) reported a possible myoneural junction in an asteroid, separated by a basal lamina, there being no apparent direct connections between nerves and muscles, and Rosenbluth (1972) saw 90 nm gaps filled with basal lamina material in certain earthworm junctions.

Of greater interest perhaps, is the innervation of the ciliated cells. Neurociliary synapses are known in few phyla - Ctenophora (Horridge & Mackay, 1964; Hernandez-Nicaise, 1973), Annelida Polychaeta (Holborow et al, 1970) and Hemichordata Pterobranchia (Dilly, 1972). In the ctenophore Pleurobrachia there are synaptic clefts 10-12 nm wide against which lie clear vesicles 30-45 nm diam. In the polychaete Harmothoë and the pterobranch Rhabdopleura dimensions were

not given. In Cryptosula the vesicles are 36-60 nm diam and the gap between opposing membranes is 20 nm which is not different from the space seen in normal membrane apposition. An obvious synaptic cleft is lacking in some of the micrographs of Horridge & MacKay (1964), none are apparent in Harmothoë and Rhabdopleura and only in Beroë (Hernández-Niçaise, 1973) are they well developed. The identification of these areas as synapses has mostly been based on the accumulation of vesicles against the supposed presynaptic side. The addition of the Bryozoa to the small list of disparate phyla known to possess neurociliary synapses suggests that this type of synapse may be found elsewhere among the many metazoans which possess ciliated epithelia.

The means by which a nervous impulse might be transmitted from the base of a cell to the cilia has been subject to some speculation. Horridge & MacKay (1964) suggested that this was accomplished by changes in membrane potential from base to apex of the cell. In Pleurobrachia and in Rhabdopleura the nervous effect is believed to be one of inhibition, based on the observations that in the former any part of a comb will develop its own wave of ciliary beat unless inhibited, and in the latter isolated ciliated cells beat rhythmically and faster for a while than on an intact tentacle. On the basis of observed sudden stoppages and resumption of beating of cilia, a nervous inhibitory impulse was also postulated for bryozoans (vide Fawcett, 1961:277) and other animals (Sleigh, 1962). Apparent neurociliary synapses

in Cryptosula therefore provide a morphological basis for such interpretations.

The occurrence of vesicles, often numerous, on the post-synaptic side in Cryptosula is significant, and whether or not they are involved in efferent transmission of signals is debatable. There are three possible explanations for their occurrence, viz.

a. They are Golgi secretions from the ciliated cells whose location is coincidental and which are unrelated to nervous transmission;

b. They are endocytotic vesicles involved in the recovery of exogenous material released by presynaptic vesicles;

c. They are afferent vesicles and the synapse is two-way.

The first alternative is not attractive. The size and location of the vesicles suggests definite involvement in synaptic transmission. The second is a possibility. Waxman & Pappas (1969) observed such recapture of presynaptic secretions to influence events deeper in the postsynaptic cells of the feline oculomotor nucleus. The third possibility would imply that the ciliated cells could be sensory receptors. It is a characteristic feature of sensory cells to contain numerous vesicles which are seen at synapses (Cordier, 1964) but in which a transmitter has yet to be demonstrated. It is more likely, though, that only the laterofrontal cells and those on the abfrontal side of the tentacle which bear cilia are sensory. Vesicles on both sides of a synapse were not seen by earlier workers who discovered neurociliary synapses. Two-way synapses are known in coelenterates but here they are interneural

(Westfall, 1970). More information is needed to account for the vesicles in the ciliated cells of Cryptosula.

1.6 Summary

The anatomy and morphology of the tentacles of a bryozoan (Cryptosula pallasiana) are described at the ultrastructural level, clarifying doubtful points raised in earlier literature and describing features not previously known in bryozoans. In the latter category are the following:

a. There are six nerves in each tentacle, not four as previously recorded. The new nerves comprise one additional frontal nerve and an abfrontal nerve. All but the peritoneal nerves comprise a cluster of axons.

b. Neurociliary synapses occur between the frontal nerves and adjacent ciliated cells, adding the Bryozoa to the small list of phyla known to possess such synapses.

Points which have been clarified include the following:

a. Tentacle muscle is shown to be smooth, thereby confirming Brien's (1960) opinion, based on light microscopy.

b. Tentacles are covered externally by a thin cuticle of mucopolysaccharide morphologically identical to that found in many other invertebrates.

c. Cilia occur on eight of the ten cell rows comprising each tentacle. Abfrontal cilia are of two kinds - short tufts of about ten cilia alternating with longer solitary cilia. There are no syncilia.

d. The basal lamina is shown to be collagenous. The collagen filaments have a periodicity of 55-65 nm. The diameter of 11.5 nm is among the smallest cited for an invertebrate.

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SECTION 2 THE ULTRASTRUCTURE OF THE LOPHOPHORE BASE

2.1 Introduction

Bryozoan body organization is considered to be trimeric as in deuterostomes. The epistome of phylactolaemates, lacking in the Stenolaemata and Gymnolaemata, is a dubious protosome, the lophophore with its cavity represents the mesosome, and the main body of the zooid the metasome. A ring coelom in the lophophore base is the major part of the mesocoel, with extensions into the tentacles. It is known to be in communication with the metacoel so there is probably some reciprocal exchange of fluid between the two compartments. It was implied by Mangum & Schopf (1967) that oxygen rich mesocoelous fluid from the evaginated lophophore could mix with metacoelous fluid upon retraction, an idea partly expressed by Hincks (1880). Ryland (1967), on the other hand, felt that morphological arrangements would not permit such mixing, particularly as the smallness of the openings between adjacent cavities would preclude much flow. One of the aims in this section of my research was to determine the extent of the communication between adjacent coelomic compartments and to deduce fluid movement from the architecture of the lophophore.

Another area of debate is the nature of the lophophore retractor muscles. Once thought to be striated (Nitsche, 1871; Hincks, 1880; Borg, 1926), they were said by Marcus (1926, 1939), Rogick (1937) and Brien (1960) to be smooth. The striations that are often seen in retractor muscles were found by

Marcus to disappear in polarized light, and he attributed the striae to folds in the sarcolemma (1939: 273). According to Brien, though, the retractors contain two fibers which become helically coiled upon retraction. The retractor muscles of the mouth, on the other hand, were said by Brien to be truly striated. Because of the different opinions expressed and the difficulty of obtaining unequivocal evidence on the basis of light microscopy, Neilsen (1971) felt that all 'striated' muscles reported in bryozoans ought to be studied more closely. This section therefore considers the ultrastructure of the muscles of the lophophore.

A fine-structural characterization of the bryozoan nervous system has not yet been published, although research is currently on-going in France. In this section, therefore, nervous fine structure will not be considered in detail, although attention will be called to the possibility of neurosecretion, which should be expected in Bryozoa as it has been reported in every metazoan phylum studied so far (Porifera and Mesozoa excepted) (Kelly, 1967).

2.2 Materials and Methods (as for section 1).

2.3 Gross morphology and anatomy

The lophophore base is structurally the most complex part of the polypide (figs 1 a,b). Here the tentacles are united at their bases and their lumina are confluent with a ring coelom around the mouth. A ganglion occupies the full width of the coelom on the dorsal side, tapering ventrally

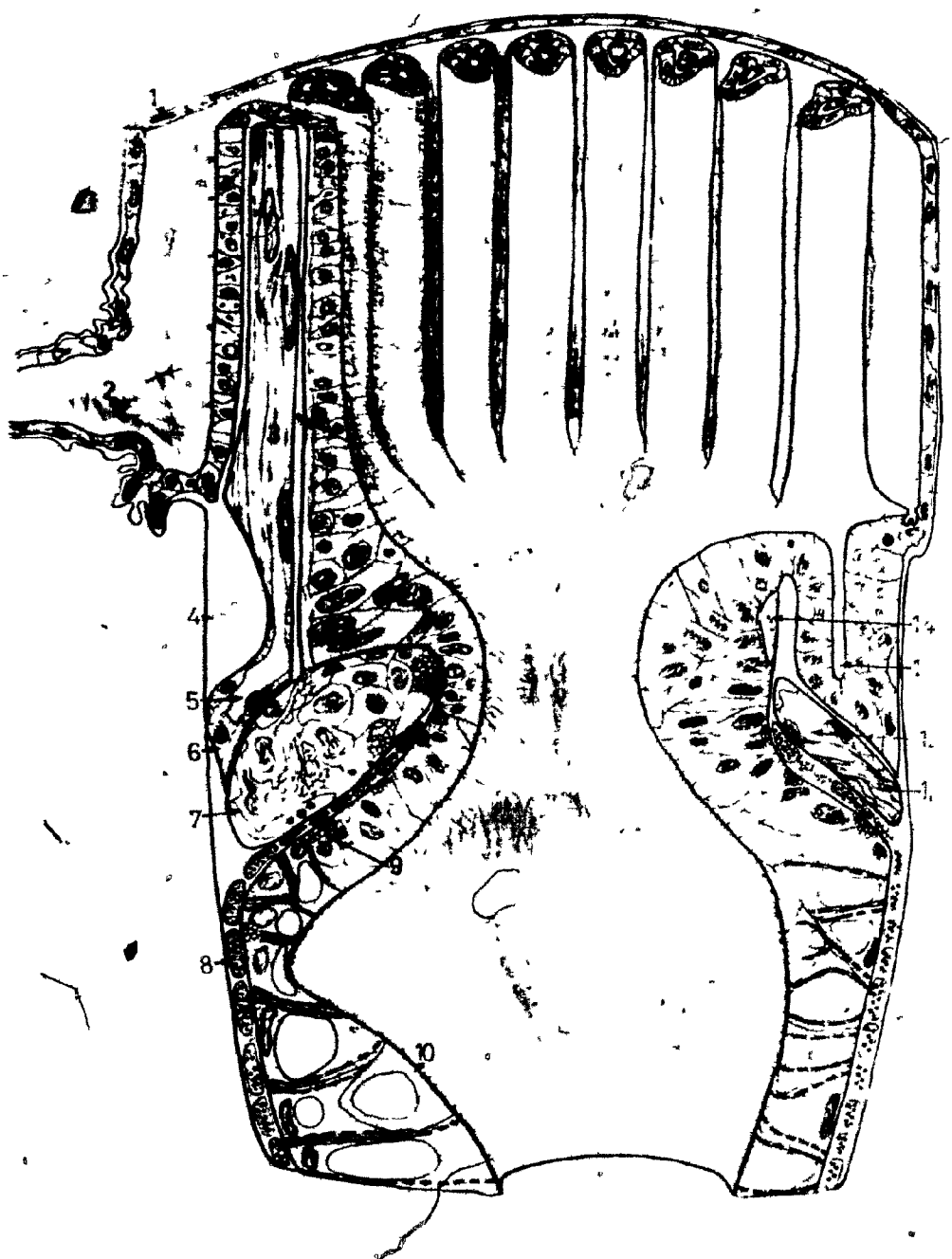
Figure 1. Lophophore base of Cryptosula pallasiana in cut-away view showing its construction based on serial electron micrographs taken through a number of planes.

a. depicts the lophophore base bisected in the dorsoventral plane (the left hand side of the diagram is dorsal, right is ventral).

b. shows a bisection at right angles to a.

Key:
1. tentacle sheath
2. anus
3. lumen of tentacle (mesocoel)
4. basal lamina
5. ring coelom (mesocoel)
6. peritoneum
7. ganglion
8. circular muscle
9. oropharyngeal nerve
10. myoepithelium of pharynx
11. buccal dilator muscle
12. ring coelom
13. ciliated pit
14. basal transverse tentacle muscle
15. lateral nerve tract
16. lophophore retractor muscle

1a



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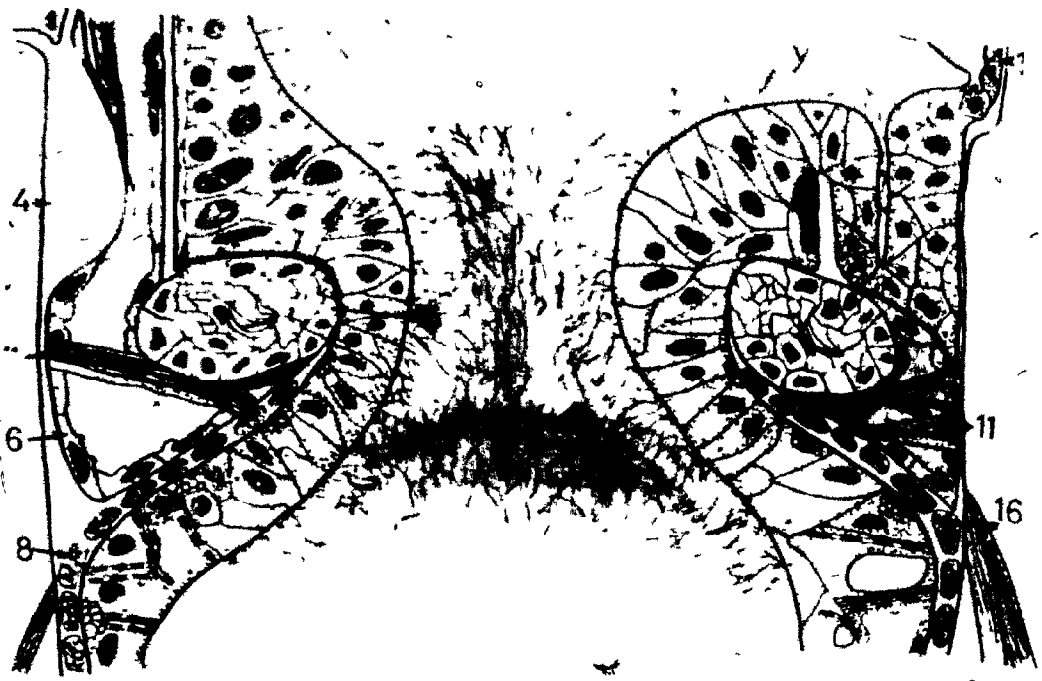

into a nerve tract on either side of the mouth, from which nerves arise to all tentacles. The two tracts do not unite ventrally. Subtending the ring coelom around the mouth are subepidermal circular muscles which are serially continuous with the circular muscles of the alimentary canal. Obliquely crossing the ring coelom at intervals are thin strands of mouth dilator muscles. The inner epidermis, which is one cell thick throughout the polypide is up to three cells thick in the mouth region, tapering to two cells at the level where the tentacle sheath is attached, and one cell beyond this point. Supporting this whole apparatus is a complex fibrous framework of basal lamina which is variously keeled and indented for muscle attachments and other purposes which are somewhat obscure. Either side of the dorsal ganglion are gaps in the basal lamina connecting the main body coelom with the lophophoral coelom. Between the bases of the tentacles are ciliated pits. The mouth is densely ciliated.

2.4 Fine structural anatomy and morphology

2.41 Surface features

The cuticle of the tentacles and tentacle sheath is continuous with the cuticle of the oral region and pharynx where it is 0.05 µm thick and permeated with microvilli. There is no morphological and presumably no chemical difference between tentacle and mouth cuticle and the description of cuticle in the previous section therefore holds for this region also.

Mouth cilia are 20-25 µm long and are like the frontal

cilia of the tentacles in their basal structure. From the basal body of each cilium arises a more or less vertical rootlet about 7 µm long. In the plane of the stroke are two lateral processes from the basal body. Pointing orally is a short rootlet 0.04 µm long and in the opposite direction is a basal foot and satellite body. As with tentacle cilia, the central tubule pair is at right angles to the plane of beat.

Originating in the crotch between every pair of tentacles is a cuticle-lined pit (figs 1, 4). These pits have not previously been recorded in bryozoans, no doubt because of their small size. While they are 2.5-3.0 µm long they are barely 0.3 µm diameter. Their function is obscure. Some of the cells surrounding each pit contain small vesicles 70-100 nm diam resembling vesicles seen in axons, and occasional axons occur at the bases of some epidermal cells surrounding each pit. Cilia occur in the top two-thirds of each pit and as they have the normal 9+2 tubule configuration and possess short rootlets, it is likely that they mobile. Some of the microvilli in the pits are clavate with an invagination near the tip.

2.42 Epidermis

The epidermis of the oral region is thicker than elsewhere in the animal. The cells have an embryonic appearance and mitotic figures are often encountered (figs 2, 4). Nuclei are relatively large, occupying 45% of the cell volume. Centriolar structures, some with radiating microtubules are occasionally encountered. Microtubules are common in

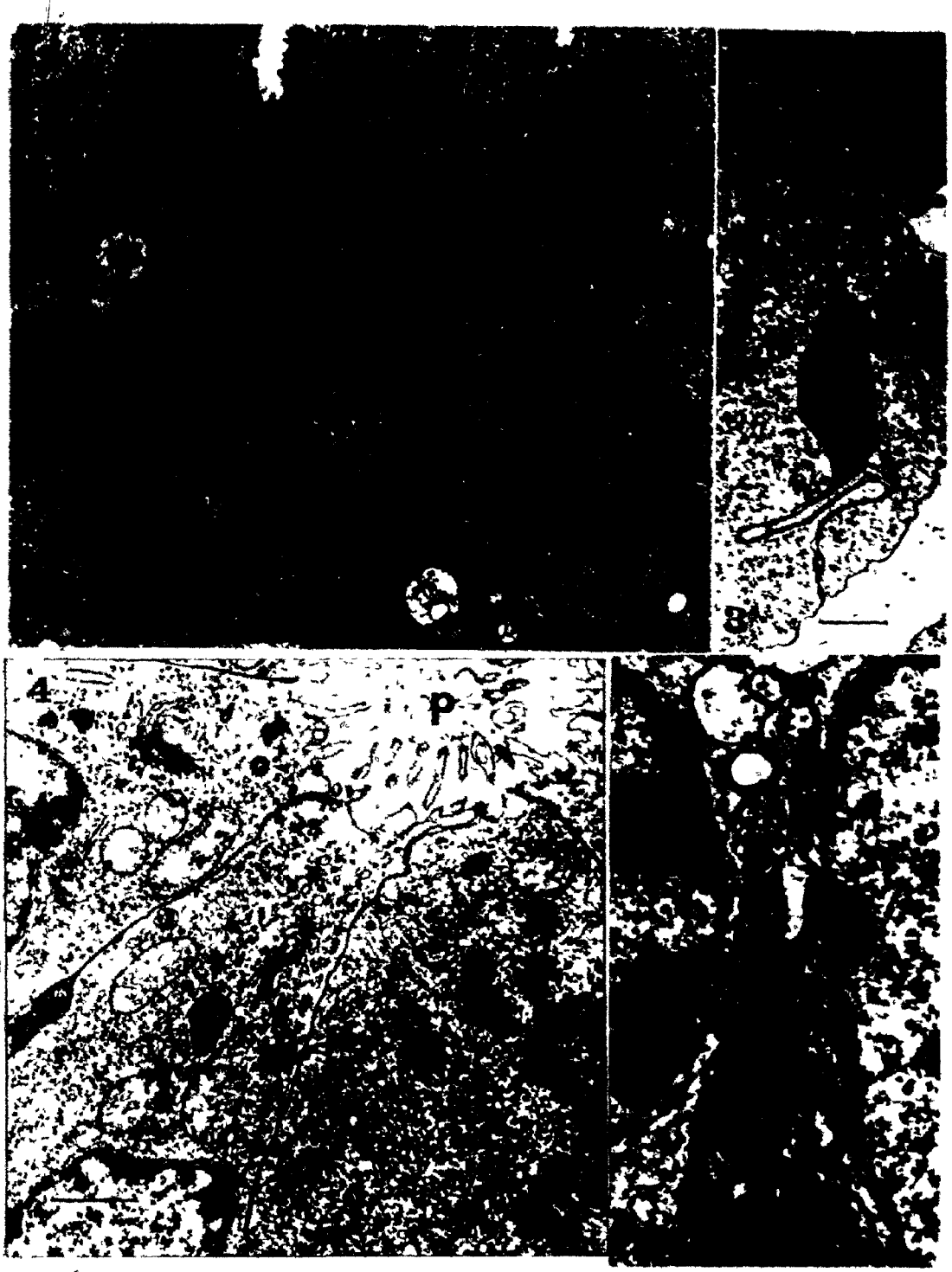
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Figure 2. Buccal epithelium: Transverse section of a lophophore base through the level of the mouth showing the embryonic appearance of most cells. In one (upper right) a mitotic figure is seen, which is probably telophasic judging by the pieces of nuclear envelope forming around the chromatic lobes (arrows). x 5910 (scale 2 µm).

Figure 3. A deposit of fibrous material between epithelial cells around a ciliated pit. x 24,000 (scale 0.5 µm).

Figure 4. Ciliated pit: Transverse section of a cuticle-lined pit (p) at the lophophore base. Cilia are not seen at this level although a centriole pair (arrows) is seen in one cell. The epithelial cells around the pit are blastemic like those around the mouth, and a mitotic figure with microtubules (m) is seen in one cell. x 15,800 (scale 1 µm).

Figure 5. Cells at a tentacle base, in transverse section, showing a lipid droplet in a ciliated cell, adjacent to one of the three frontal nerves. x 22,800, (scale 0.5 µm). (n = nerve; l = lipid droplet).

young and dividing cells. Multivesicular bodies and lipid droplets may also occur in this epithelium (fig 5). Intercellular deposits of fibrous material were occasionally seen between the cells around the ciliated pits (fig 3). These deposits are usually associated with secretions from Golgi bodies in the stomach of Cryptosula, and their function is unknown. As such Golgi bodies were not found in epithelia of the lophophore, the occurrence of these deposits is surprising.

Sensory cells in this region are difficult to identify. Bronstein (1937) reported sensory cells in the crotches between tentacles and Silbermann (1906), Graupner (1930) and Lutaud (1955) saw them by methylene blue staining in the tentacles. In Cryptosula the most likely candidates for sensory cells are ciliated cells containing vesicles at their bases and having elongate processes running adjacent to axons (or merging into them) such as I have seen in the tentacles. Sections rarely, however, capture the irregular course of these processes and cytoplasmic contents are not obviously different from surrounding epithelial cells.

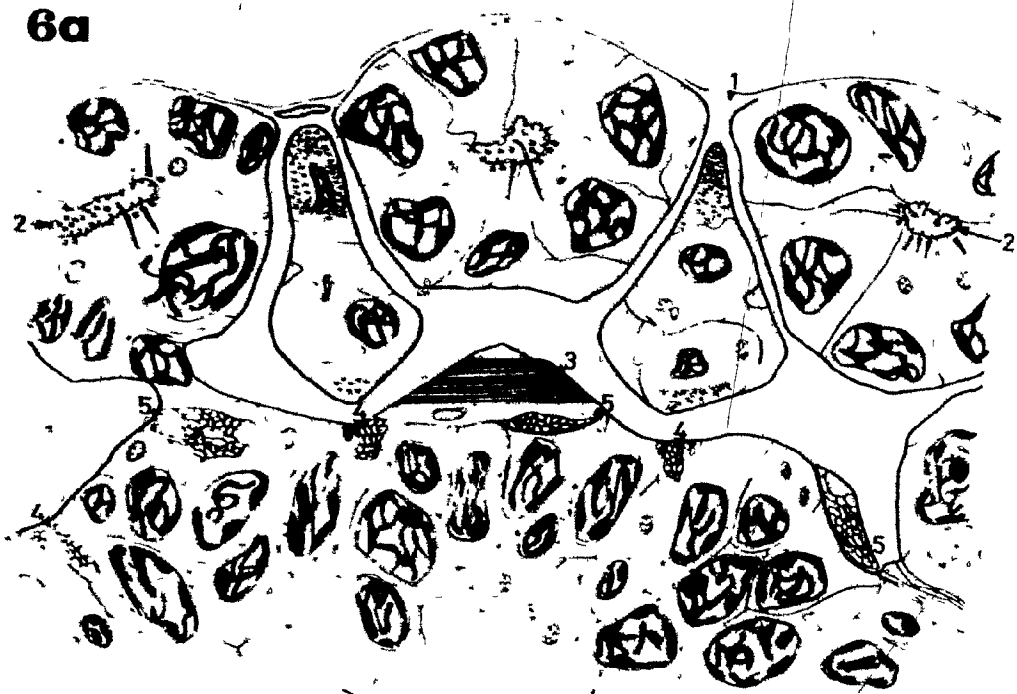
2.43 Basal lamina

The fibrous basal lamina is perhaps the most conspicuous element of the lophophore base. It is much thickened in places, for two main reasons. First, this is the site of attachment of the lophophore retractor muscles, the largest in the body. Second, a strong supporting framework is necessary for the bending movements of the tentacle crown. It is

Figure 6. Diagrammatic representation of slightly oblique transverse sections of the lophophore base at the level of the mouth (a) and where the dorsal tentacles begin to separate (b), showing the different profiles of basal lamina encountered with changing levels up the tentacle bases.

1. basal lamina
2. ciliated pit
3. basal transverse tentacle muscle
4. frontal tentacle nerve cluster
5. abfrontal tentacle nerve

6a



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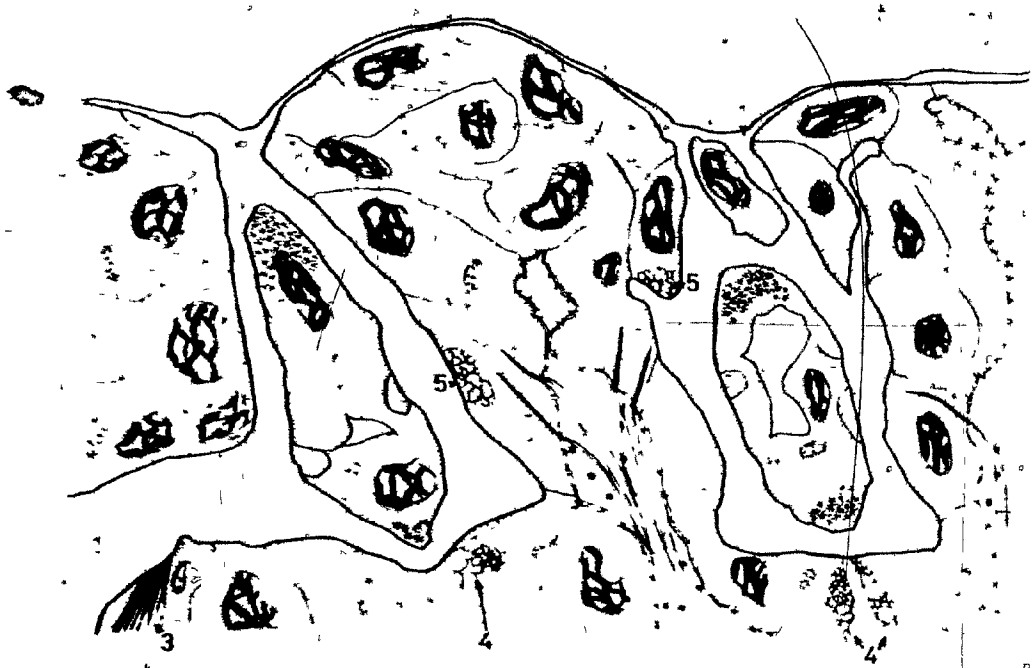

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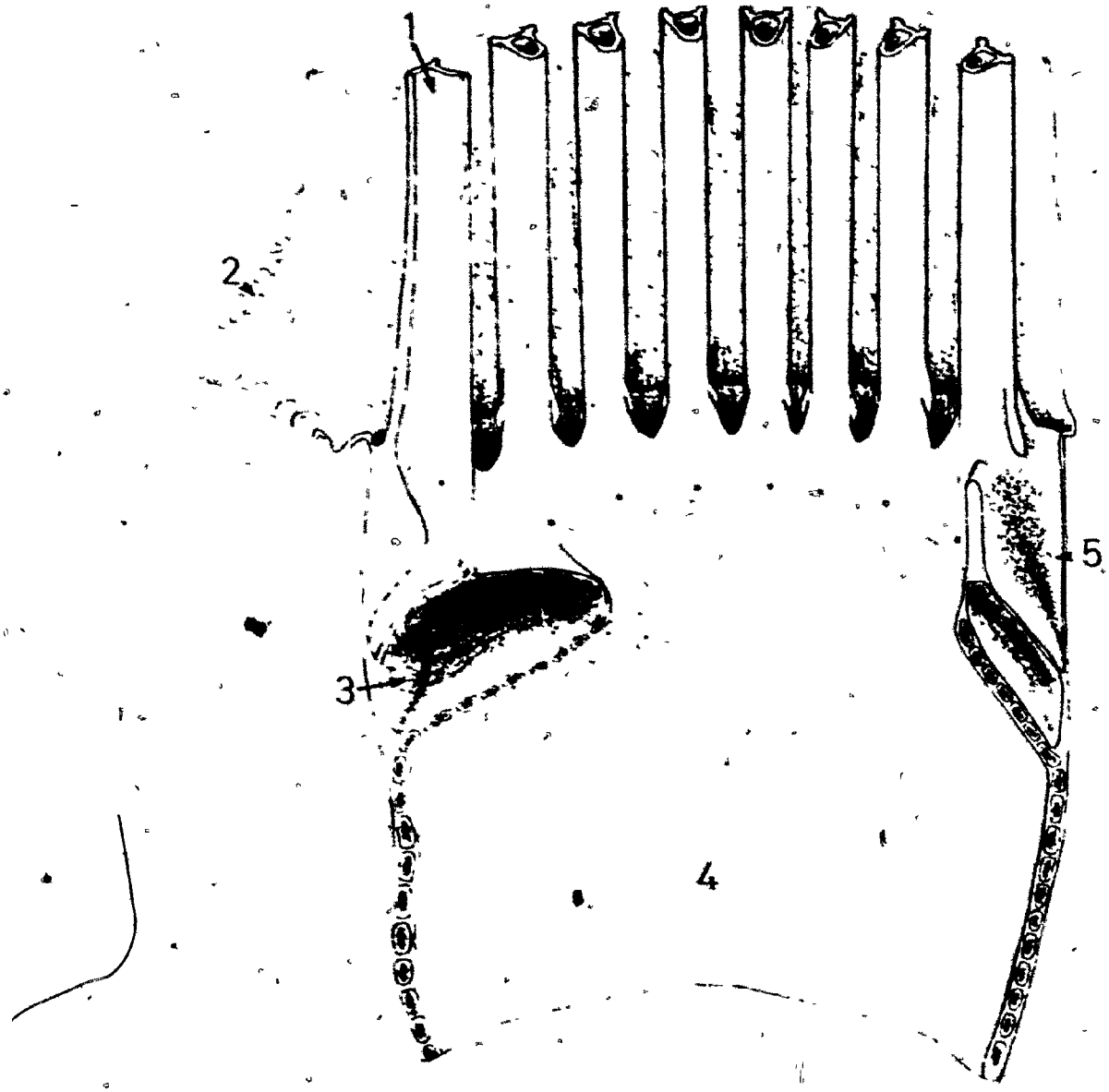

Figure 7. Diagrammatic representation of the basal lamina 'skeleton' of the lophophore base.

1. mesocoel
2. basal lamina of the tentacle sheath at the anus
3. cavity to accommodate the dorsal ganglion
4. pharynx
5. depression which accommodates epithelial cells around a ciliated pit.

striking to watch intertidal bryozoans at ebb tide. Contrary to expectations, lophophores are not retracted as small waves swash over colonies, and with every passing of a wave lophophores are flung back and forth until finally, with the passage of no more water over the colony surface, they are withdrawn. When submersed, bryozoans may also exhibit bending movements of the crowns during normal feeding. The transverse complexity of the basal lamina is shown in figure 6 and a view of the whole structure in figure 7. Over the dorsal ganglion is a thin shelf which tapers ventrally on both sides of the mouth. Basal lamina also separates the ganglion and lateral nerve tracts from the peritoneum. (In the polypide primordium it is the peritoneal cells which secrete the basal lamina, thereby behaving as fibroblasts, but they later lose this function). Basal lamina also ensheaths the circular muscles of the mouth and some of the nerves for a little way beyond their origin.

2.44 Peritoneum and mesocoel

The peritoneum (fig 1) is a layer of single cells completely lining the ring coelom and is continuous with the peritoneum of the tentacles. There is also a peritoneum lining the lophophore base on the metacoel side (fig 14). The cells are flattened with irregular surfaces. Nuclei are likewise flattened, and are relatively large. No organelles other than the usual RER and mitochondria were seen and the peritoneum is not ciliated as in phylactolaemates.

The dimensions of the mesocoel are related to one of the functions of the lophophore which is that of sperm release. This

is an elusive phenomenon which has been witnessed by only two people (Silén, 1966, 1972; Bullivant, 1967). While I have seen sperm loose in the metacoel of Cryptosula, I have not observed actual release from the tentacle tips so I do not know if all or only the two mediodorsal tentacles are involved (as in some bryozoans), but in either case, the following figures are relevant. Sperm of Cryptosula are 45 µm long and 1.25-1.75 µm wide. Entering the ring coelom from either side behind the dorsal ganglion they would find themselves in a chamber (roughly oblong in cross section) 12 µm wide by 16 µm high, or 8-11 sperm diameters at maximum dilation, from which they could easily enter the tentacles. The dilated lumen of single tentacles is equivalent in cross section to 5-14 sperm diameters.

A three dimensional picture of the architecture of the lophophore base based on ultrastructure (figs 1, 14), and observations on live polypides allows interpretation of the movement of coelomic fluid at times of eversion, retraction and feeding of the polypide. In sections of retracted polypides the tentacular lumina are occluded due to contraction of the tentacle muscles, and upon eversion of the polypide stretching of the tentacles can be seen by careful observation. Stretching or relaxation of the longitudinal tentacle muscles can be accomplished only by forcing fluid up the tentacles. This could be achieved by movement of metacoel fluid into the ring coelom and thence up the tentacles upon eversion of the polypide. During feeding, the buccal dilator muscles, which cross

the ring coelom at intervals, contract, thereby opening the mouth wider than normal. The mouth is not normally closed when relaxed but remains open at a diameter of between 25-30 µm, dilating upon contraction of the dilator muscles to c. 36 µm. Complete closure is possible and happens infrequently by maximum contraction of the circular muscles of the mouth. When the buccal dilators contract, the floor of the ring coelom is raised, simultaneously closing the gap connecting each tentacular lumen by occluding it with peritoneal cells (fig 14a). It is normally a narrow passage when it is open. Ring coelom fluid must therefore pass back into the metacoel, the only place which can accomodate it, although some may enter the tentacles before complete closure. Reverse flow occurs when the circular muscles contract. Blocking off the passages to the tentacles should prevent suctional effects from being felt there.

2.45 Musculature

There are four sets of muscles at the lophophore base. One set, the buccal dilators, opens the mouth as mentioned above; another, the circular muscles, close the mouth; a third, hitherto unknown set will be discussed later as to its possible function; the fourth set comprises the lophophore retractors, which retract the feeding polypide into the cystid.

The buccal dilators (figs 1, 12, 14b) cross the lower part of the ring coelom at intervals around the mouth. Each muscle cell may be perforated and splays outward across the coelom from insertion points on the basal lamina on each side.

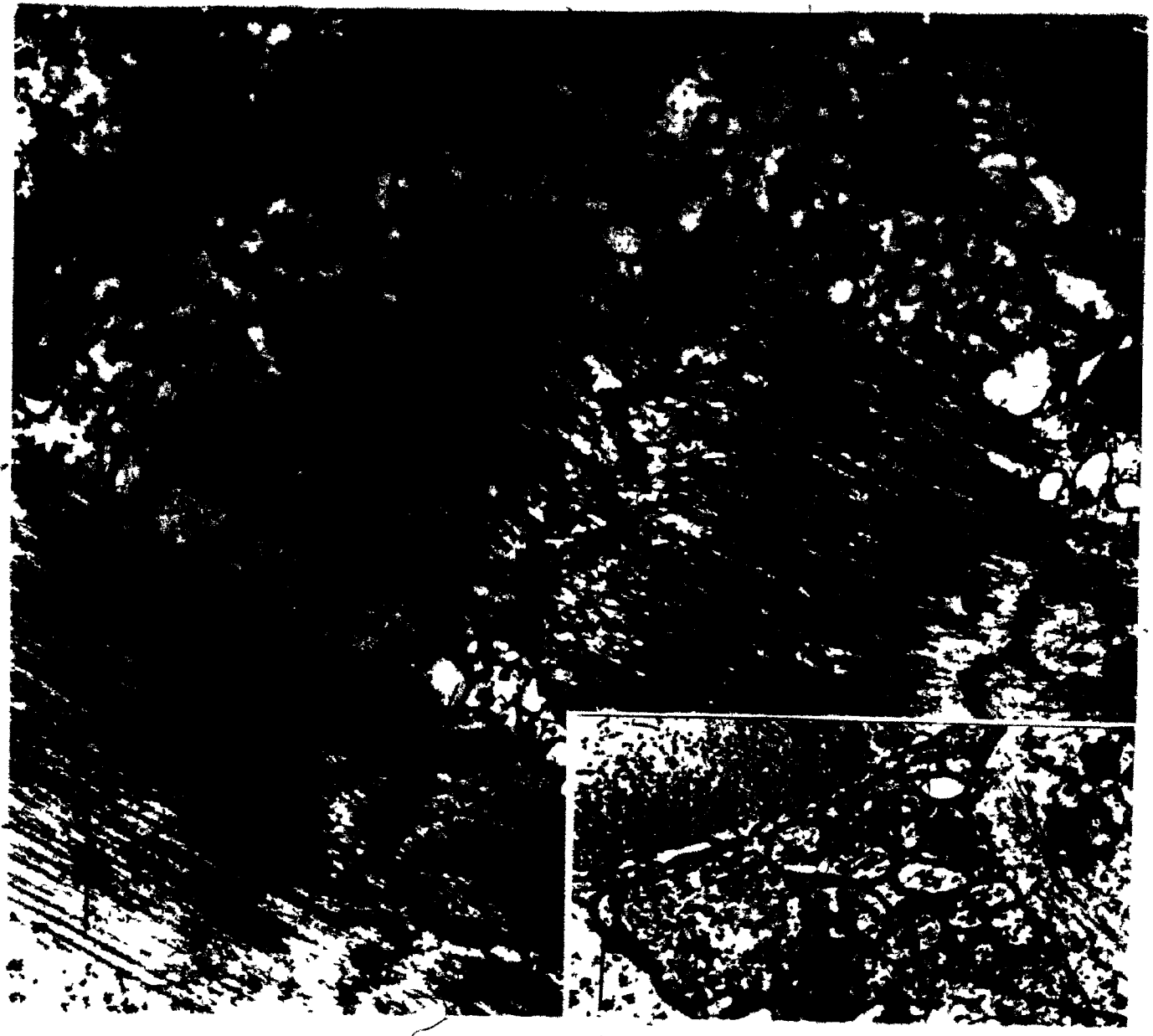

Figure 8. Circular muscle of the mouth: Oblique section through the mouth region in the plane of the circular muscle sheet, showing sarcomeres and irregular Z bands. x 29,600 (scale 1 /um).

Figure 9. Circular muscle of the mouth: Longitudinal section through the mouth region showing the close apposition between a circular muscle fibril and an oropharyngeal nerve. Note the small dense vesicle (arrow) in the pharyngeal cell cytoplasm. x 24,900, (scale 0.5 /um).

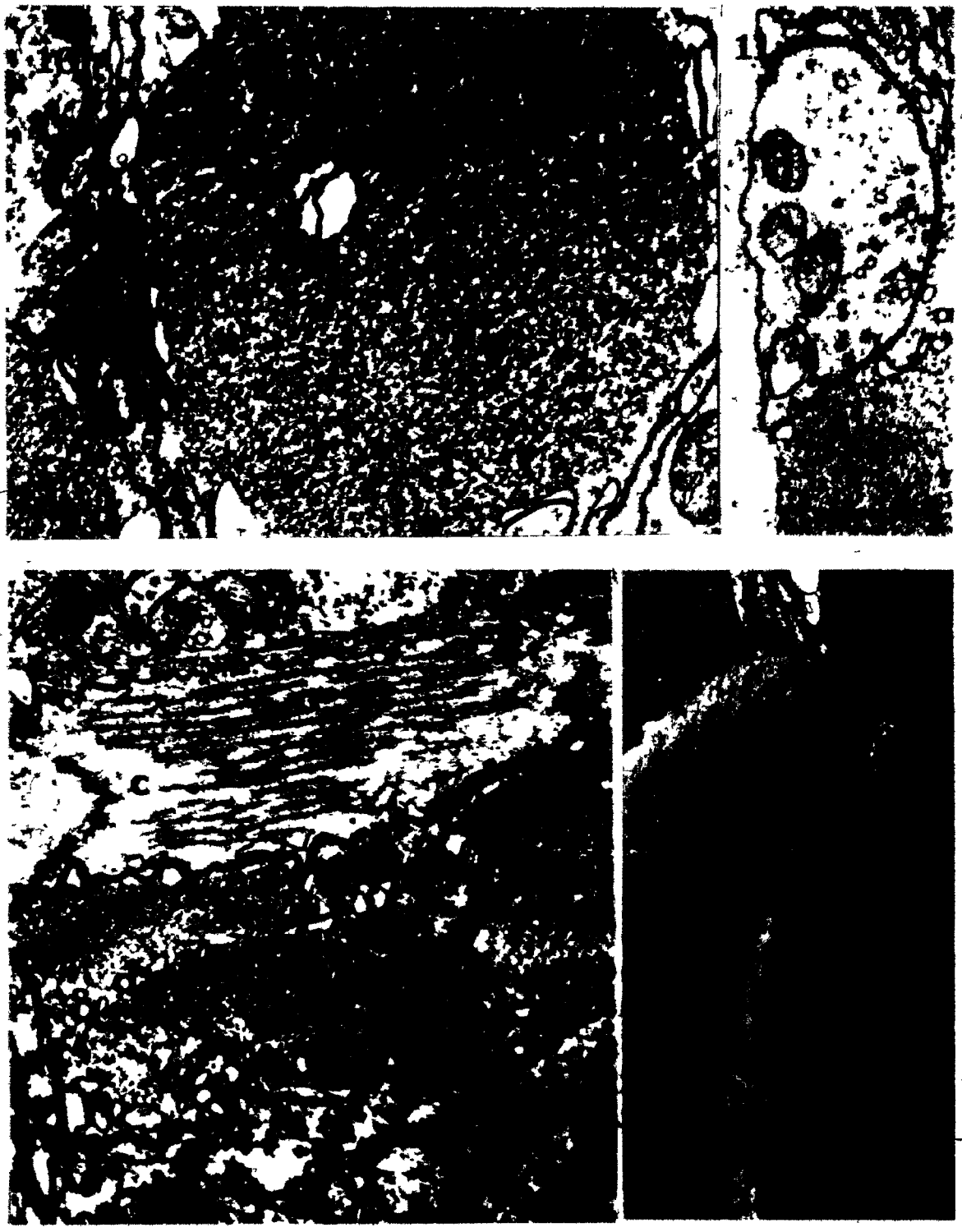
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Figure 10. Lophophore retractor muscle: Transverse section of a muscle cell. There is one longitudinal fibril per cell in which tubules may occur in the center. x 30,600 (scale 0.5 µm).

Figure 11. Myoneural junction: Oblique section of a junction occurring with retractor muscle outside the basal lamina of the lophophore base (left). Arrows delimit an area of parallel membranes possibly indicating a synapse. x 31,900 (scale 0.5 µm).

Figure 12. Muscles of the mouth region: This oblique transverse section shows striated circular muscle sarcomeres (c) adjacent to buccal dilator muscle (d) whose filaments are perpendicular to those of the circular muscle. A thin basal lamina separates the circular muscle from two oropharyngeal nerves (arrows). x 28,600 (scale 1 µm).

Figure 13. Basal transverse tentacle muscle: Longitudinal section through a fibril adjacent to a crest of basal lamina (bl). An abfrontal nerve passes over the muscle en route to the abfrontal side of a tentacle. x 6700 (scale 2 µm).

The cells are relatively short and there is no division into regular sarcomeres. About twelve thin filaments c. 3 nm diam surround each thick filament (20-24 nm diam). The location of these muscles at lower levels of the lophophoral coelom would seem to relate them to the tentacle muscles a little higher up, but filament dimensions are sufficiently different to regard them as a separate set of smooth muscles.

The circular muscles of the mouth (figs 1, 9, 12, 14) are serially continuous with the circular muscles of the alimentary tract and form a muscular sheet beneath the ring coelom. This is striated muscle, occurring in definite sarcomeres about 5.0-6.5 µm long and 4 µm wide. Filament dimensions are the same as for the buccal dilators.

The third set of muscles has not been described in bryozoans before and I shall here call them the basal transverse muscles of the tentacles (figs 1, 6, 13, 18). Each muscle is a single cell up to 10 µm long stretching across the V between the frontal keels at the bases of the tentacles. Thick filament diameters range from 13-75 nm. Thin filaments are c. 3 nm diameter.

Inserting laterally on the basal lamina at the level of the ring coelom are the lophophore retractor muscles (figs 1b, 10, 11). These are bundles of 18-29 cells on each side, each cell being about 3.5 µm wide and up to 910 µm long in the stretched condition. The lengths of contracted muscle are difficult to estimate owing to the folding of the cells upon contraction. Packing of thick and thin filaments is not as regular as in the

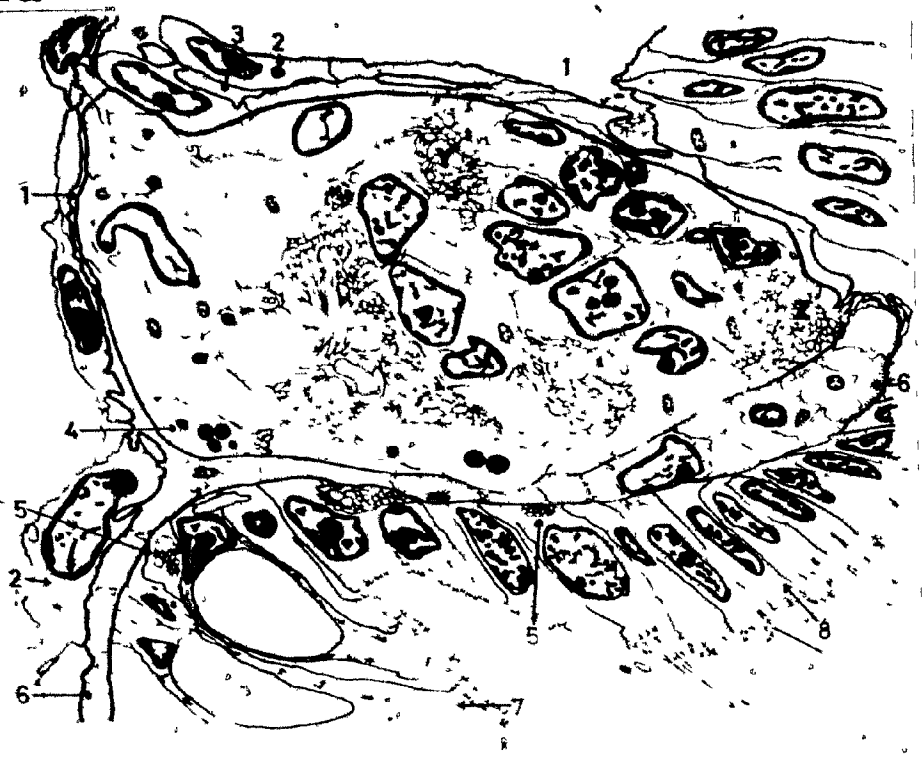
other muscles described. This seems to be due to the variability of the center to center spacing between the thick filaments, but in some sections a more or less regular disposal of about ten thin filaments around one thick filament can be discerned. There are no sarcomeres or scattered dense bodies, therefore the retractor muscles are plainly smooth.

2.46 Nerve center

The dorsal ganglion (figs 1a, 14a) and lateral nerve tracts (figs 1b, 14b, 15) constitute a horse-shoe shaped nerve center in the animal. In the ganglion neurons are arranged in groups presumably responsible for collective innervation of prescribed regions of the zooid. Tracing the nerves from their origins to their destinations is not easy at the EM level without recourse to perhaps hundreds of serial sections of the lophophore base taken through numerous planes in conjunction with vital or special staining of nervous tissue. Such a study is currently being undertaken by Dr Geneviève Lutaud of the Laboratoire d'Anatomie Comparée in Paris, half of which has been published (Lutaud, 1969) and of which the fine structure will be submitted for publication this year (1973). My own description, therefore, will be based on what can be ascertained from micrographs used to determine the three-dimensional construction of the lophophore base and the arrangement of tissues therein, with reference to Lutaud's work, and earlier literature.

The ganglion and lateral nerve tracts are covered by a thin sheath of basal lamina. A median longitudinal section of

14a



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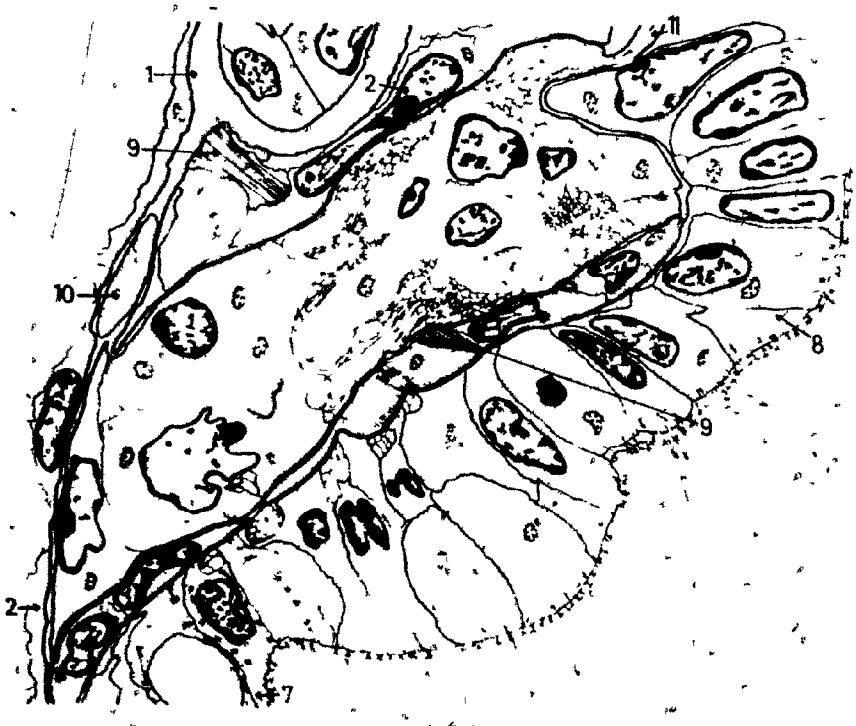


Figure 14. The nervous center: Diagrammatic representation of the dorsal ganglion (a) and a lateral nerve tract (b) at the lophophore base. In a it is seen that the ganglion occupies the full width of the ring coelom which is very reduced at this point. Five axon tracts are seen, of which one at Y will give rise to tentacle nerves (see 11 in b) and Z curves over the lip of circular muscle to run dorsally to oropharyngeal nerves.

In b the lateral nerve tract is quite wide at this point not far from the ganglion. It becomes reduced to half this size. In this diagram (traced from a photo montage) the buccal dilator muscle is contracted and the mesocoel has been obliterated by displacement of mesocoelous fluid. Notice how the nerve tract is lifted upwards causing peritoneal cells to occlude the entrance to a tentacle lumen.

1. basal lamina
2. peritoneum
3. mesocoel
4. glial cell with gliosomes
5. oropharyngeal nerve
6. circular muscle of the mouth
7. pharyngeal myoepithelium
8. mouth cell
9. buccal dilator muscle
10. nerve to lophophore retractor muscle
11. tentacle nerve.

the ganglion slices through 20-25 neurons arranged in 4-5 groups separated by about five axon bundles (fig 14a). The ganglion is solid, not hollow as in phylactolaemates, and is clearly composed of a greater number of cells than the 25 cited for Flustrellidra hispida by Graupner (1930). The neurons of Cryptosula are not all of the same size, the more dorso-posterior cells being somewhat larger as Lutaud (1969) observed in Electra pilosa. Because of the irregular contours of the neurons and their processes it is difficult to determine which are unipolar, which bipolar and which multipolar. All three kinds are said to occur in bryozoans, of which most were thought to be bipolar by Graupner (1930), or unipolar with median ventral multipolar cells continuing into the lateral nerve tracts (Lutaud, 1969). Neurons are shown in figures 15-17. Small myelin figures are sometimes seen and glycogen occurs in some cells (fig 17) but is not common. Small pigment granules were seen in some of the more dorsal cells which are probably glial in nature.

Obvious vesicles occur in most neuronal perikarya, associated with Golgi bodies, but are most abundantly concentrated in axons. Most vesicles are small clear vesicles 30-50 nm diam while in the large dorso-posterior cells dense vesicles c. 80-270 nm diam resembling neurosecretory vesicles occur in the cell body. If these cells are to be equated with what Lutaud calls the direct proximal nerves (in Electra pilosa) then they follow a course towards the distal end of the polypide as far as the diaphragm of the tentacle sheath, where they subdivide

Figure 15. Lateral nerve tract: Showing the disposition of nuclei in a peripheral 'rind' with axons directed inwardly as a 'core'. Part of a perikaryon (arrow) is shown in figure 16. x 4060 (scale 5 /um).

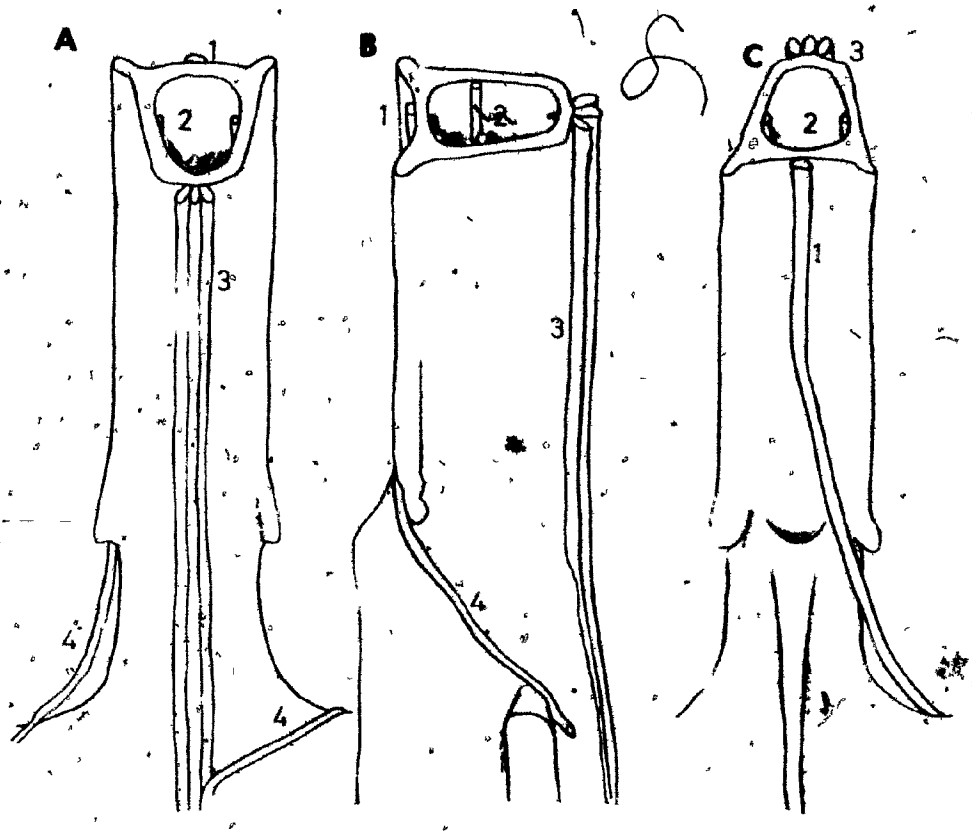
Figure 16. Neuron from a lateral nerve tract: Part of figure 15 showing a number of multivesicular or granular bodies in the perikaryon. x 30,200 (scale 1 /um).

Figure 17. Neuronal glycogen: Two sites of alpha glycogen deposition are shown in a neuron. x 32,100 (scale 1 /um).

into secondary branches whose end-points are not clear. In Cryptosula, the only neuron bundles whose axons I have been able to follow are a central anterior cluster which continue into the lateral nerve tracts and from which arise the tentacle nerves (fig 14a,b) and a ventral group whose axons leave the ganglion from the pointed oral side (fig 14a) to curve backwards under the circular muscle sheet to give rise to the transverse nerves around the oropharyngeal junction.

The lateral nerve tracts in Cryptosula (fig 15) are constructed in a manner similar to that found in invertebrates in general, viz. cell somata are concentrated at the surface with the axons directed inwardly, collectively forming the fibrous core. In transverse section, the lateral tracts taper from about 13 to 8 cell bodies on either side, forming a rough semi-circle of neurons backing onto the mesocoel, with axons on the oral side of the tract.

The frontal and abfrontal nerves of each tentacle appear to arise close together from the CNS. The former can be seen in their frontal positions in oblique transverse sections at levels immediately above the CNS (fig 6a). The abfrontal nerve of each tentacle arises nearby or from the same point, then separates, passing outwards (laterally) over the basal transverse tentacle muscle (which it must innervate en passage as it is the only nerve associated with this muscle), under the lateral flange of basal lamina and around the back of the next tentacle (fig 18). The peritoneal nerves arise directly from the CNS under the tentacles.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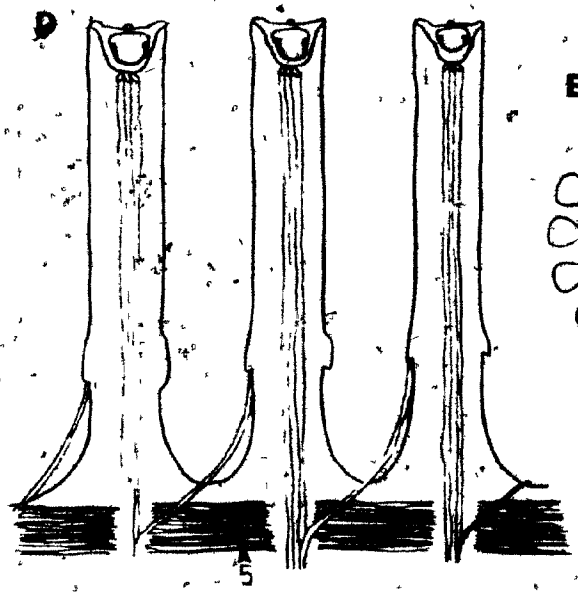
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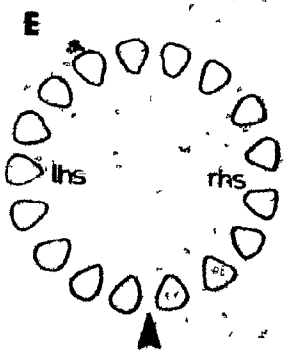
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Figure 18. The origin of the subepidermal nerves: A, B and C show respectively, frontal, lateral and abfrontal views of the basal lamina of a tentacle from the left hand side of the lophophore. Left and right handedness is denoted with respect to the position of the ganglion (arrow in E, which represents the crown of 17 tentacles in cross section). D shows the relative positions of the nerves from tentacle to tentacle.

1,4. abfrontal nerve

2. peritoneal nerves

3. frontal nerves

5. basal transverse tentacle muscle.

2.5 Discussion

One of the new features discovered at the lophophore base is the blastemic nature of the epithelium. Cells surrounding the ciliated pits and mouth cells were sometimes seen to be undergoing mitoses. If Brien's (1960) observation of cell loss from mouth epithelium of phylactolaemates is correct then a blastema would enable cell replacement. This would also be a site from which cells could be mobilized in wound repair, as when tentacles regenerate after amputation (Otto, 1921). The occurrence of blastemic tissue here would also account for Matricon's discovery of a rapidly proliferating epithelium from the vicinity of the ganglion in the degenerating female polypide of Alcyonidium polyoum (Matricon, 1963). The polypide degenerates when ova reach a certain size and the new epithelial layer becomes ciliated and funnel shaped, serving to conduct ova to a distal incubatory pouch.

2.51 Musculature

The variety of muscles and their filament dimensions encountered so far are tabulated below. The tentacle muscles and parietals in the main body cavity which depress the compensation sac are included for comparison. These muscles resolve themselves into three categories, viz.

a. classical smooth muscle with or without dense bodies, thick filaments not greater than 42 nm diam (longitudinal tentacle muscles, buccal dilators and lophophore retractors);
b. paramyosin-like smooth with thick filaments up to 75 nm diam (basal transverse tentacle muscles and parietals);

Table 1. Some muscles of Cryptosula and their characteristics.

Type of muscle	Thick filament diameter nm	Thin filament diameter nm	Type	Function
longitudinal tentacle muscle	22-42	4.5-7.5	smooth	shorten tentacles
basal transverse tentacle muscle	13-75	c.3	paramyosin like	hold fluid pressure?
buccal dilators	20-24	c.3	smooth	open mouth
circular muscle of mouth	20-24	c.3	striated	close mouth
lophophore retractors	13-31	3-7	smooth	retract polypide
parietals	30-72	-	paramyosin like	evert polypide

In Cryptosula muscles of different types act as antagonists e.g. smooth against striated, and classical smooth against paramysoin-like smooth. The dimensions of the very thick filaments in the basal transverse tentacle muscle and the parietals are greater than that encountered in regular thick (myosin) filaments and indicates the presence of paramyosin though lacking paramyosin periodicity. Only the molluscs were known to possess paramyosin (Hanson & Lowy, 1960) although more recently paramyosin has been reported from holothurians (Baccetti & Rosati, 1966; Rosati, 1968), annelids (Baccetti, 1967) and Limulus (Levine et al, 1973). Paramysosin muscles are noted for being able to maintain high levels of tension over long periods without signs of fatigue (Lowy, 1953) and it is clear that this type of muscle would be most efficacious during long intervals of feeding, when the parietals must exert sustained pressure, by their contraction, on the metacoelic fluid. Similarly, the basa

transverse muscles of the tentacles should exert sustained tension during eversion of the lophophore.

These muscles probably act as antagonists of the longitudinal tentacle muscles. During feeding, individual tentacles are capable of flicking their tips, curving inwards and even coiling. In order to behave independently in this manner their fluid connection to the ring coelom should be either cut off or severely restricted, as explained earlier, by occlusion of peritoneal cells. There are two longitudinal muscle tracts, one bending inwards, one outwards. I cannot envisage these restoring the tentacle to the normally erect position without some shortening of the whole tentacle and it is likely that contraction of the basal transverse muscles should restore tentacle shape by displacement of fluid distally. The only other possible function of these muscles would be as accessory mouth-closing muscles which seems pointless in view of the well developed sphincter-type circular muscles.

There has been some doubt in the past as to the nature of the lophophore retractors. Some authors (e.g. Borg, 1926: 231) have taken them to be striated, because under certain conditions they do appear striated. Marcus (1939), however, discovered that in polarized light they appeared smooth, and Brien (1960) observed (in Phylactolaemata) that a kind of helical coiling upon retraction resulted in a striated appearance. In Cryptosula they are certainly smooth, but under phase contrast rippling of the sarcolemma can be seen, and it is likely that effects such as these misled earlier workers.

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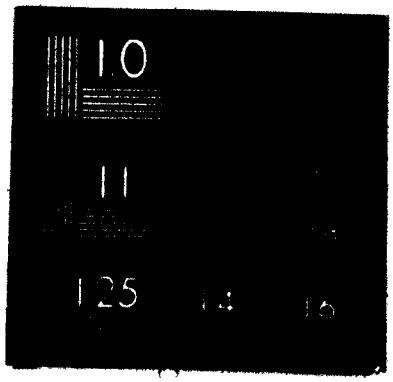

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Attention has been drawn to the structural similarities between sipunculoids and bryozoans (Nichols, 1962). Ignoring for the moment its colonial nature, a bryozoan like the ctenostome Bowerbankia is characterized by a roughly vermiform body with a protrusible feeding apparatus comparable to a sipunculoid introvert, and a set of smooth retractor muscles like those of sipunculoids. The retractor muscles of the latter have been the subject of some physiological studies (Astbury, 1947; Fisher, 1947) because they can undergo a wide range of length changes. This latter is also true of bryozoans. Various disturbances during feeding of Cryptosula will cause partial withdrawal of the polypide such that the lophophore protrudes for varying distances from the orifice. It is unfortunate that the small size of bryozoans precludes ready investigation of physiological phenomena such as these.

2.52 Central nervous system

Most details of the nervous system in bryozoans can be gleaned from a study of the most comprehensive reviews of recent years, viz. those of Hyman (1959), and Brien (1960). In addition, Bullock (1965) neatly summarized the field in relation to other invertebrate nervous systems and his knowledge of nervous anatomy in general. Bryozoans are said to be 'marked by extremely simple nervous systems and no sense organs, but only simple unicellular receptors, in association with the sessile, colonial habit and minute size. There is a distinct brain but no nerve cords'. The histology of the brain is 'typical of small, lower invertebrates' (Bullock, 1965). Most au-

thors more or less agree on the general disposition of the ganglion and nerves in gymnolaemates, although there are some minor differences in the number of cell clusters in the ganglion as it seems that several different histological stains are required to see all of them (Lutaud, 1969). There is no cerebral vesicle as in phylactolaemates (Brien, 1960) and glial cells are few.

In spite of the simplicity of bryozoan nervous organization it should not be surprising to find evidence of neurosecretion. As this phenomenon occurs in most phyla it is reasonable to expect its occurrence in groups not previously examined (Bern & Nagadorn, 1965). The presence of large dense vesicles in Cryptosula neurons does not necessarily prove, however, that neurosecretion occurs. Neurosecretory vesicles are known to range from about 80 or 100 nm diam to 300 nm diam (Hofer, 1968), and synaptic vesicles 30-60 nm diam (Gray & Guillery, 1966) but even regular synaptic vesicles may attain large diameters. In the hydromedusan Gonionemus clear synaptic vesicles are 100 nm diam while dense-cored vesicles are 120-140 nm diam (Westfall, 1970). Furthermore, Newman et al (1968) suggest that in the same organism vesicles of different sizes and density may contain the same secretion. Bern (1962) comments that, in order to establish the occurrence of neurosecretion, one must determine that the secretion is a hormone, not a neurotransmitter. This implies that the secretion must be long-range and long-acting and not directly transmitted but released into circulating or tissue fluids. 'Circulation'

in bryozoans is induced by movements of the polypide and it is not known how effective this is. It would seem to be preferable to deliver the secretion directly, and as neurosecretory neurons are now known to synapse with epithelial cells (Hofer, 1968), themselves of endocrine function, perhaps synapses are involved in Cryptosula. Evidence of such synapses has been seen on ciliated cells of the stomach where neurosecretory-like terminals occur. These will described in more detail in section 3.

2.6 Summary

The architecture of the lophophore base of a bryozoan is described for the first time at the ultrastructural level. New information arising from this study is as follows.

a. The epithelial cells at the tentacle base and around the mouth are blastemic in nature, probably serving to replace cells lost from buccal epithelium or damaged tentacles.

b. There are ciliated pits of unknown function in the crotch between every pair of tentacles.

c. A new set of muscles is described. These are the basal transverse muscles of the tentacles, each comprising a single cell containing short myofibrils of both thick and thin filaments.

d. Thick paramyosin-like filaments up to 75 nm diam occur in basal transverse tentacle muscle and parietal muscle.

e. The discovery of large dense vesicles up to 270 nm diam in large dorsal cells of the ganglion indicates the occurrence of neurosecretion in bryozoans.

In addition, many other features which were only partly known from earlier light microscopical studies are clarified, e.g.

a. Morphological arrangements are such that little or no fluid movement could occur between tentacles and the ring coelom while the lophophore is evaginated, but upon invagination mesocoelic fluid would be forced into the metacoel.

b. The lophophore retractor muscle is shown to be smooth. Individual cells may attain 910 µm in length and they contain only a single fibril in contrast to the two that Brien detected by light microscopy in phylactolaemates.

c. The circular muscle of the mouth is shown to be striated, whereas the opposing buccal dilator muscle is smooth. Filament dimensions of all muscles are given for the first time.

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SECTION 3 THE ULTRASTRUCTURE OF THE ALIMENTARY TRACT

3.1 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 qualified 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 & 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).

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 is the relationship between absorption and the occurrence of orange-brown inclusions in the stomach wall. In addition, then, to characterising general structure and function from pharynx to rectum, at the ultrastructural level, it is the aim of this section 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.

3.2 Materials and methods

(As for section 1 except for light microscopy).

Histochemistry and fluorescence microscopy: Stains were used on whole mounts and 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 more 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.

3.3 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 (figs 1-4). 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, and 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 thesis, and commented on where appropriate. The various sections of the alimentary tract of Cryptosula will be described systematically in the following account.

3.4 Pharynx

The cheilostome pharynx is a vacuolated myoepithelium. It has been adequately described by Bullivant & Bils, (1968) and Matricon (1973). The pharynx of Cryptosula does not


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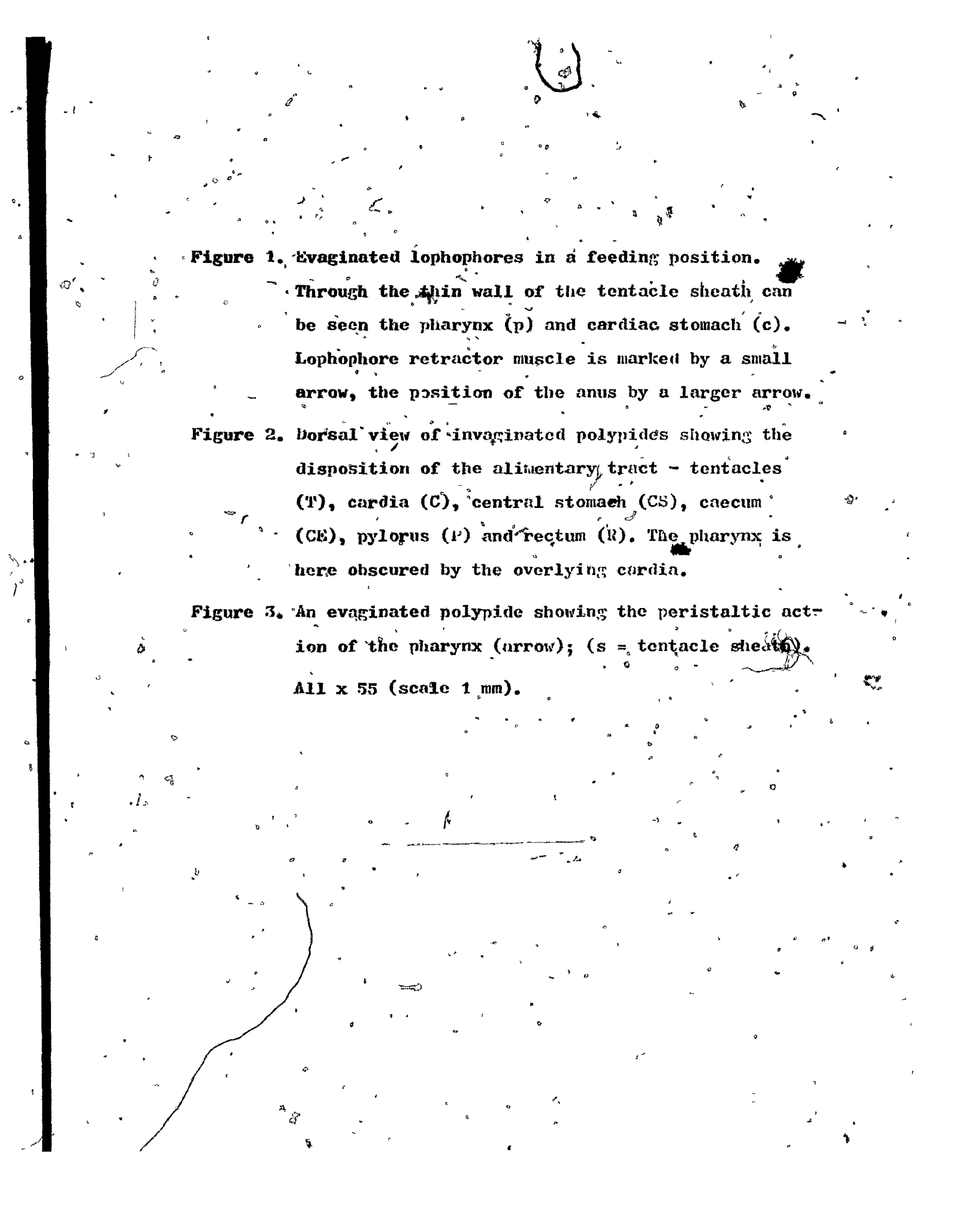
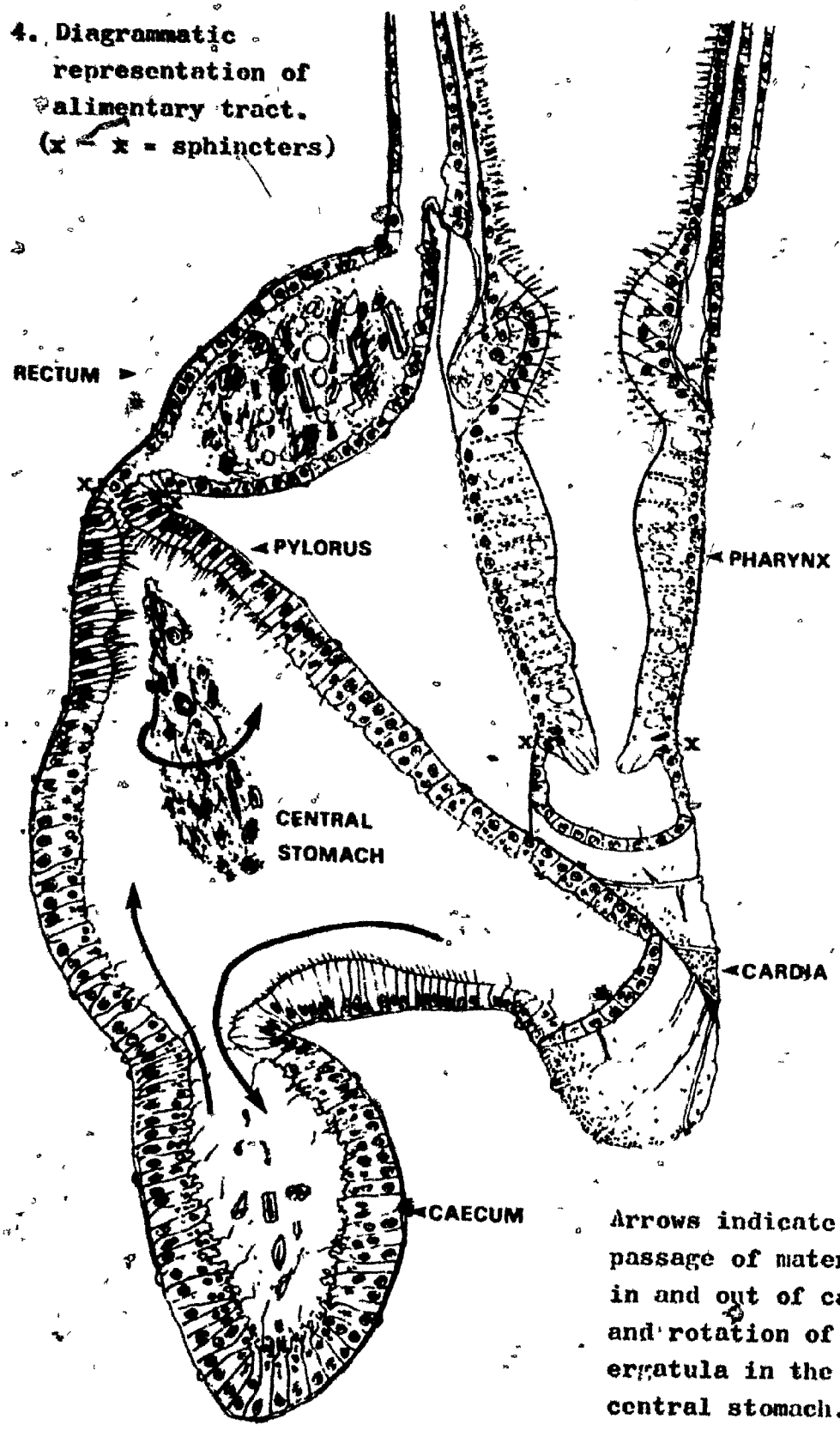


Figure 1. Evaginated lophophores in a feeding position. Through the thin wall of the tentacle sheath can be seen the pharynx (p) and cardiac stomach (c). Lophophore retractor muscle is marked by a small arrow, the position of the anus by a larger arrow.

Figure 2. Dorsal view of invaginated polypides showing the disposition of the alimentary tract - tentacles (T), cardia (C), central stomach (CS), caecum (CE), pylorus (P) and rectum (R). The pharynx is here obscured by the overlying cardia.

Figure 3. An evaginated polypide showing the peristaltic action of the pharynx (arrow); (s = tentacle sheath). All x 55 (scale 1 mm).

Figure 4. Diagrammatic representation of alimentary tract. (x — x = sphincters)



Arrows indicate passage of material in and out of caecum and rotation of the ergatula in the central stomach.

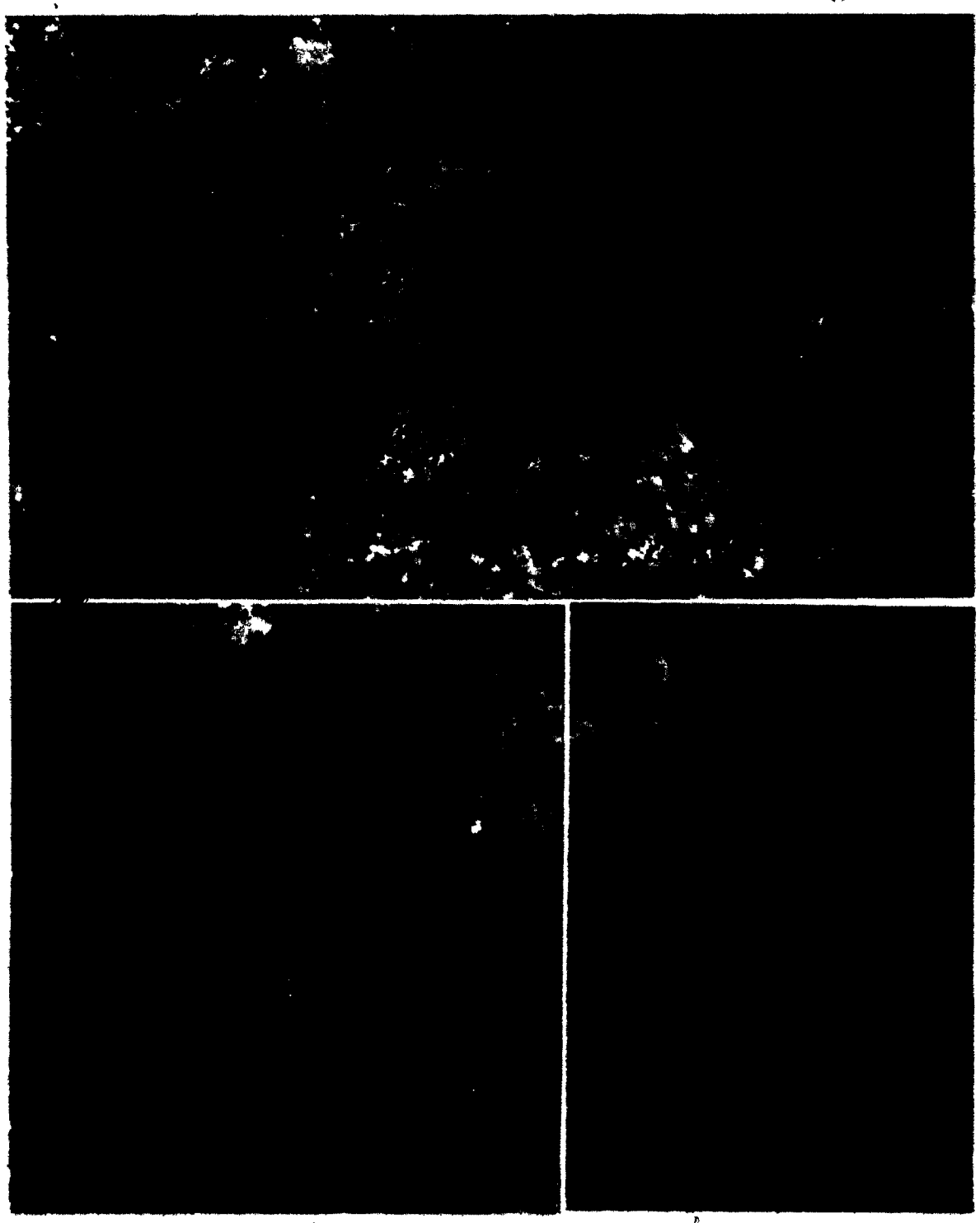
differ except in a few minor points. The sarcomeres in Cryptosula are 2-3 µm long. In the smaller pharynx of Zoobotryon they are only 1-2 µm (Bullivant & Bils, 1968). Surrounding the pharynx in Cryptosula is a thin basal lamina in which the collagen fibrils are aligned in two layers at right angles to 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.

3.5 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.

3.51 Cardia

The cardiac stomach (figs 5-7) of Cryptosula comprises columnar cells 12-15 µm tall from base to apex (q.v. also fig 32). The cell surface is a brush border of slender



Figures 5-7. Cardiac stomach: Tangential and oblique transverse sections of cardiac cells. In figure 5, which is a tangential view near cell apices illustrating a digestive vacuole (v) with many smaller vacuoles in adjacent cells. Myelin figures (m) are not uncommon, as well as cup-shaped mitochondria (arrows). A cell base is shown in figure 6, bounded by a thin basal lamina outside of which is a muscle fibril. In figure 7 the Golgi body is budding off vesicles with dense peripheries (big arrow) although in places vesicles with denser contents are seen (small arrows).

5. x 17,200 (scale 1 μm)

6. x 17,200 (scale 1 μm)

7. x 33,300 (scale 0.5 μm).

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 RER and the well developed Golgi bodies secretion is of some importance. Golgi bodies give rise to vesicles 50-75 nm diam containing a rather dense secretion (fig 7). At the perimeter of the Golgi field the vesicles are mostly 75 nm diam whereas those closer to the saccules are 50 nm 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 (Michael Locke, 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.

3.52 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. By this means, any material from the cardia enters the caecum directly. The upper part of the bipartite stomach, which Silén calls the central stomach, accomodates 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 real 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.

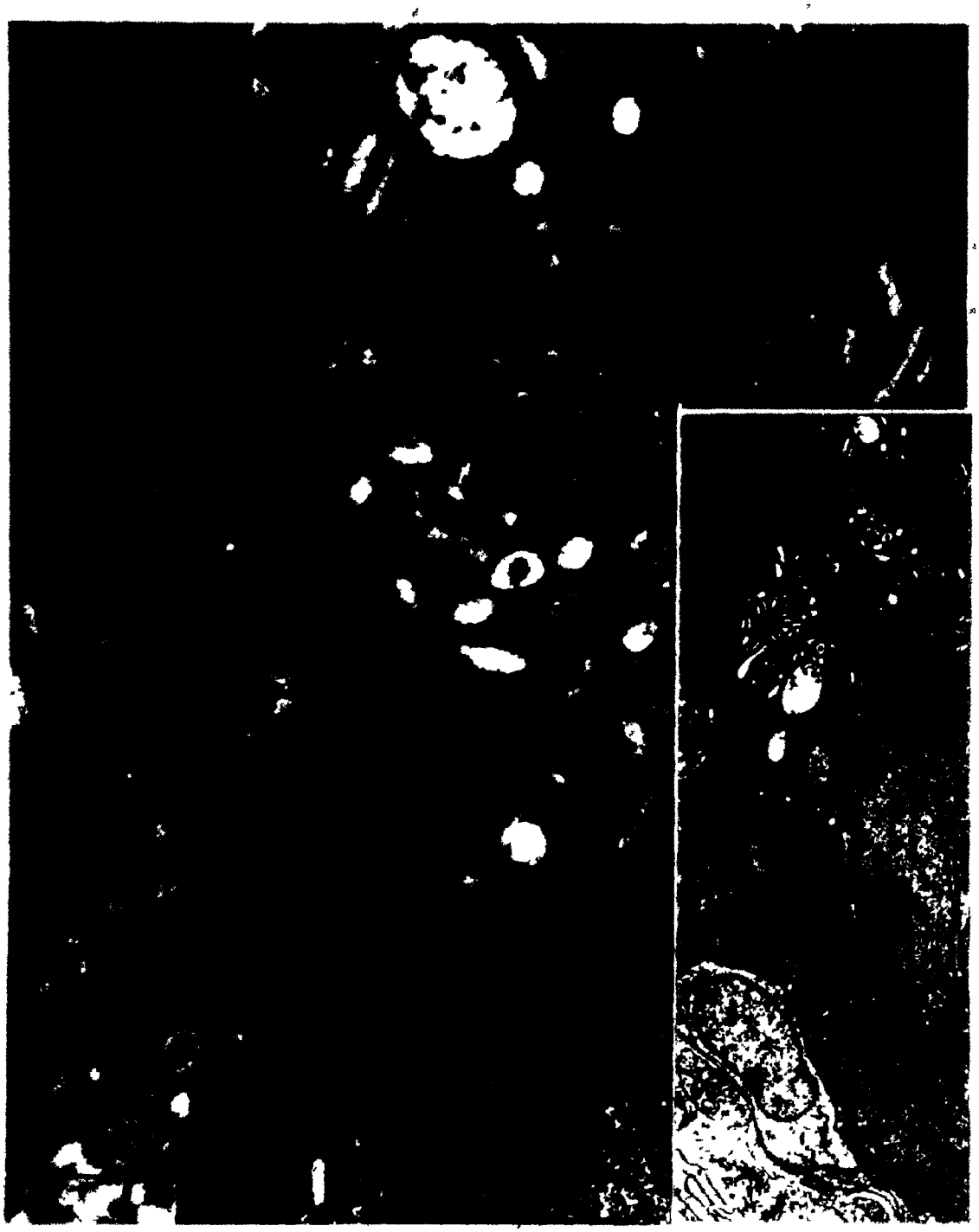


Figure 8. Stomach caecum: The apex of a cell adjacent to that in figure 9, showing digestive vacuoles and extensive canalization. Notice the fine coating on the cytoplasmic side of the endocytotic invaginations. There are a few cilia (arrows). x 18,500 (scale 1/um).

Figure 9. Stomach caecum: A single caecal cell and the apex of the adjacent cell seen in figure 8. Zonation of cytoplasmic activity is very plain. Endocytotic invaginations and heterophagosomes occupy the top third, mitochondria, RER and Golgi bodies the center and the nucleus and more RER the basal third. x 6350 (scale 2/um).

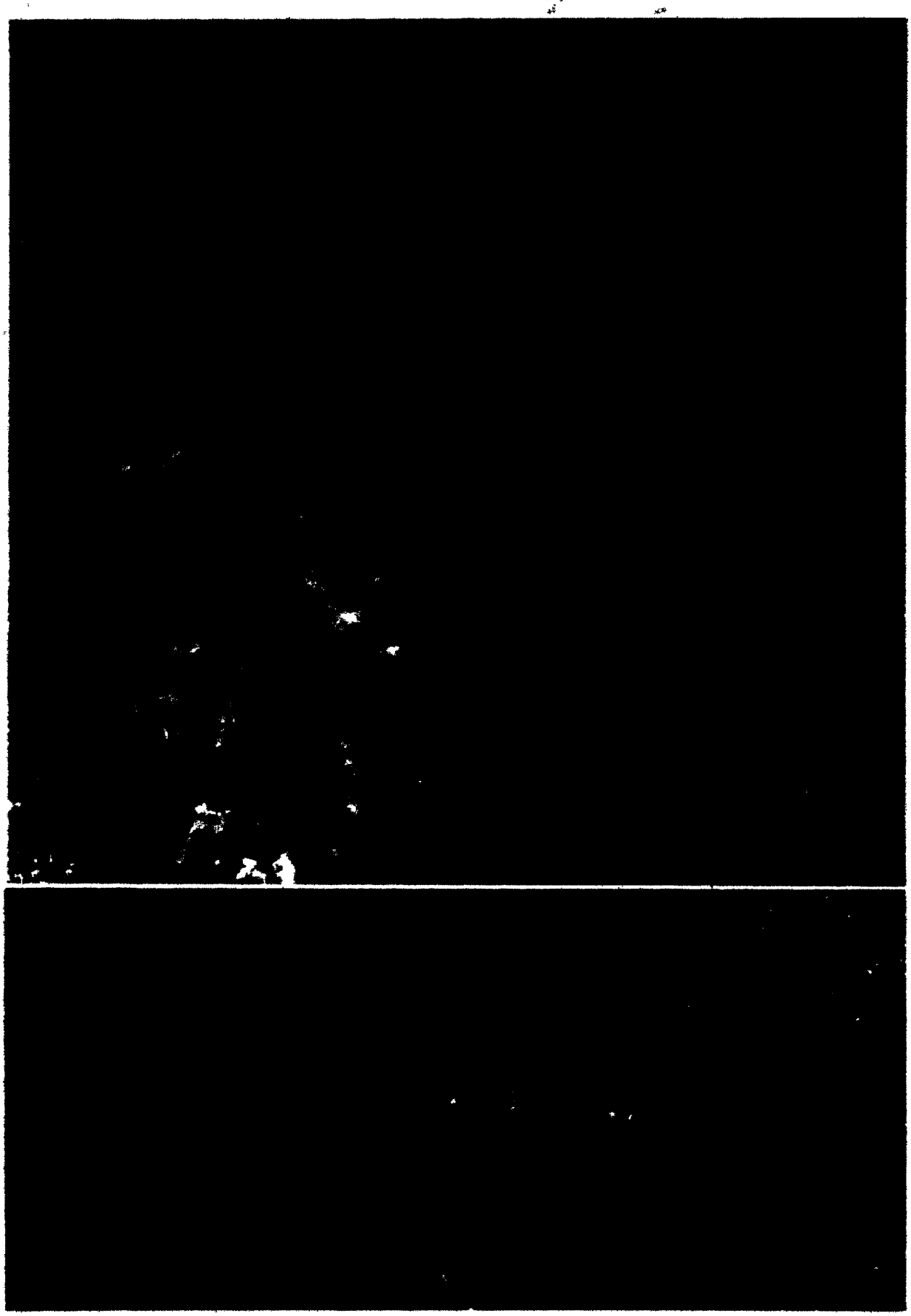
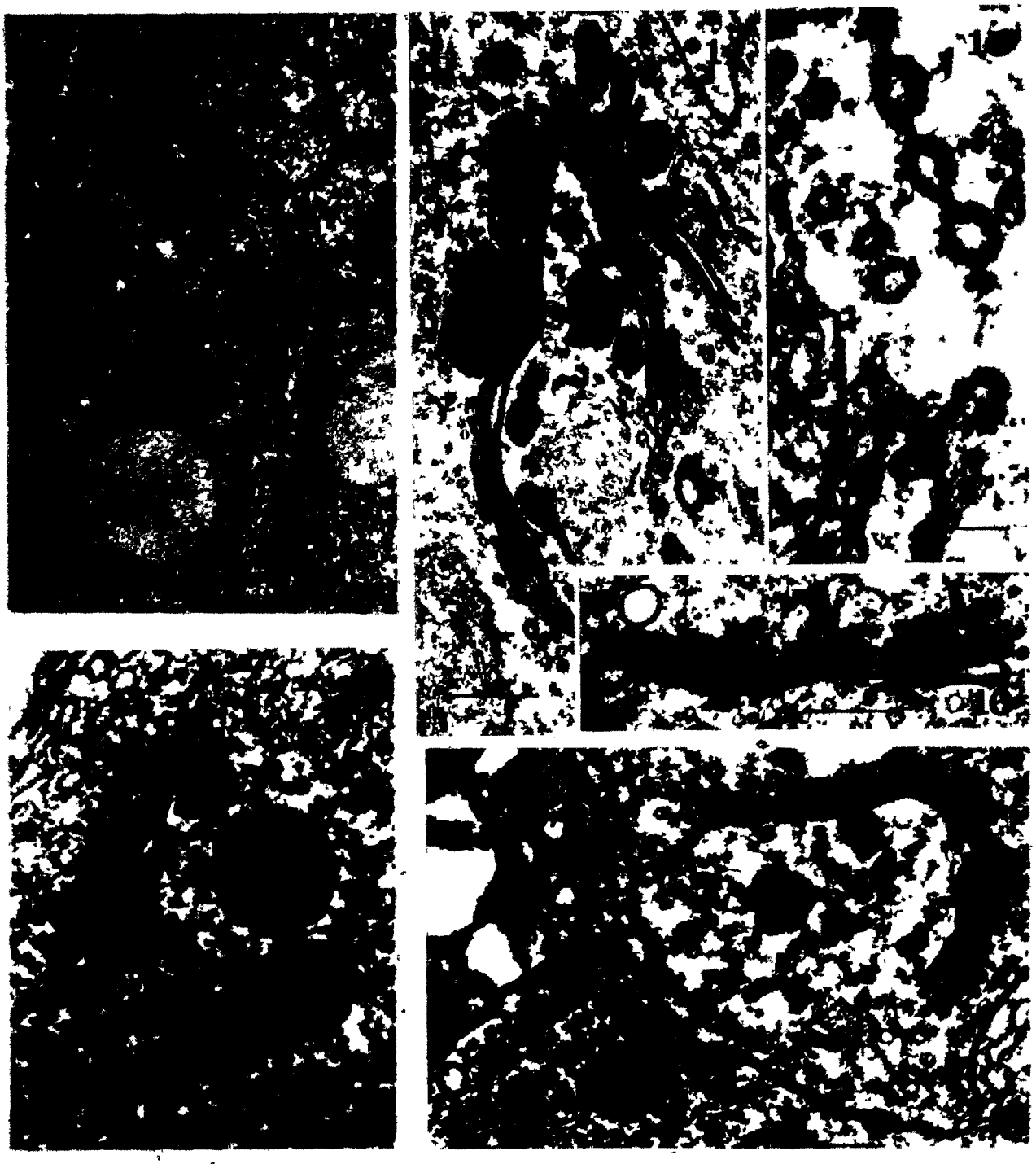


Figure 10. Stomach caecum: Large digestive vacuoles showing skeletal fragments of food organisms. x 33,800 (scale 0.5 µm).

Figure 11. Stomach caecum: A Golgi body which produces large secretion droplets (arrows). x 33,800 (scale 0.5 µm).

Caecal cells are tall and columnar (fig 9) 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 vesicles becoming larger by fusion deeper in the cell (figs 8, 9, 10). 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 (fig 25), including whole diatoms if they are sufficiently small, cell apices do not fuse into a syncytium, as reported for Zoohotryon 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 (figs 11. 12, 13) which appears to leave the cell (fig 12), as well as smaller vesicles 50-75 nm diam 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 (figs 14-17). 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 nm diam are customarily budded from the periphery. These sometimes appear in chain-like form (fig 15) 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 deposited



Figures 12-13. Golgi bodies of the stomach caecum and central stomach: Production of secretion droplets, one of which seems to be leaving a cell (arrow, fig 12).

12. x 35,000 (scale 0.5 /um)

13. x 29,600 (scale 0.5 /um)

Figures 14-17. Golgi bodies of the upper stomach caecum and central stomach: These Golgi bodies produce vesicles of two distinct types - small coated vesicles and macrovesicles containing fibrous elements. In figure 14 (arrow) and figure 15 the small vesicles appear to occur in chain-like formation or the sections have captured surface budding from the cisterns.

14. x 32,500 (scale 0.5 /um)

15. x 121,000 (scale 100 nm)

16. x 34,400 (scale 0.5 /um)

17. x 34,400 (scale 0.5 /um).

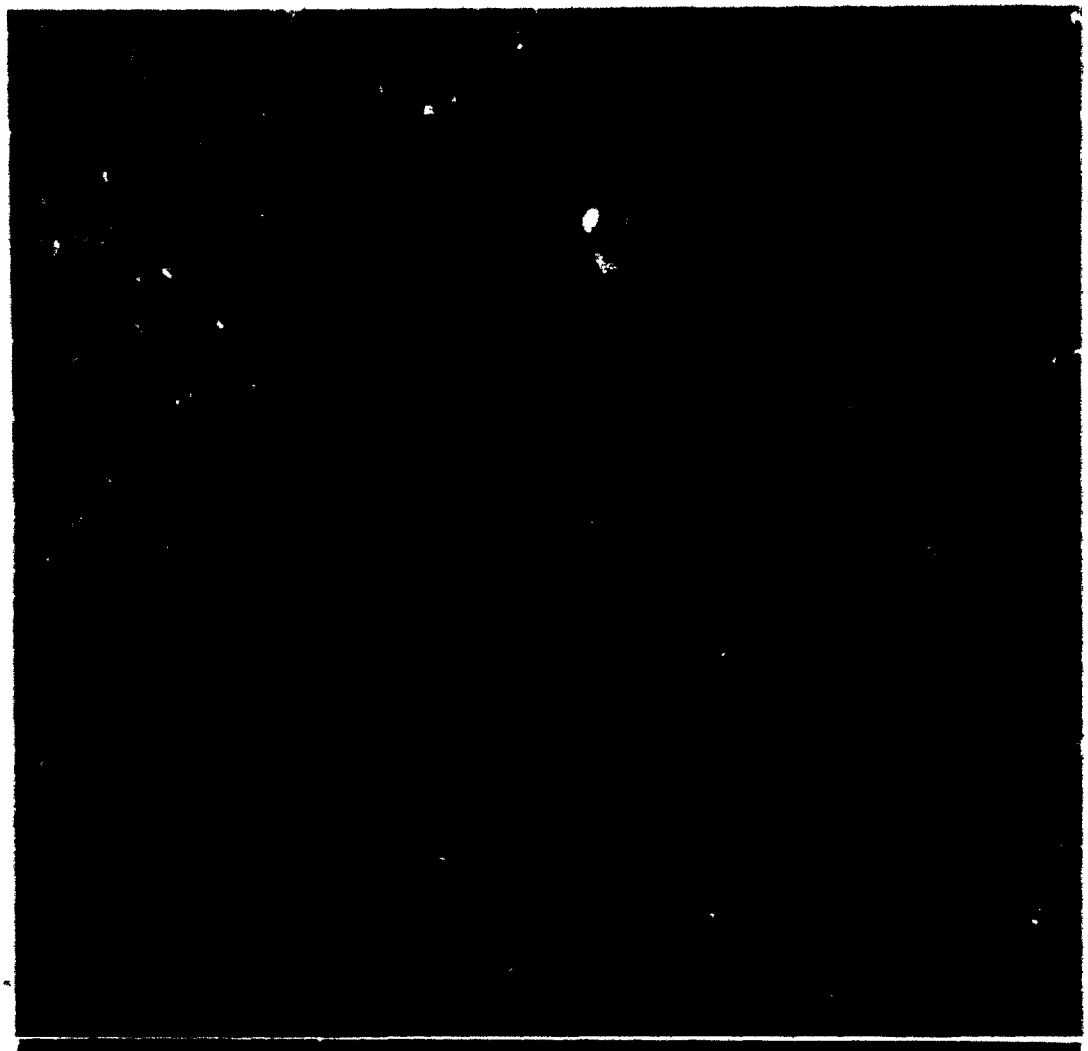
intercellularly somehow, and were not seen to be secreted at the cell apex. This second type of Golgi body occurs mostly in the central stomach and upper caecum. The production of vesicles of two sizes is a common feature of Golgi bodies (Dalton, 1961). Less well-known is whether a Golgi body can produce more than one kind of secretion. It is apparent from the vesicle contents that two kinds of secretion are produced from the second of the two types of Golgi body just described, and recently Ovtracht & Thiéry (1972) have confirmed that Golgi bodies are indeed capable of this.

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 (fig 18). 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.

3.53 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 (fig 19). The pylorus is a dome-shaped structure and its prime function seems to be

Figure 18: Stomach caecum showing basal parts of cells and an underlying muscle strand. Note the small myelin figures (small arrows) and numerous small digestive vacuoles. Note also the opposing filament arrays in the muscle cell (large arrows). (Peritoneal cells have sloughed off during specimen preparation. x 15,800 (scale 1 μm).



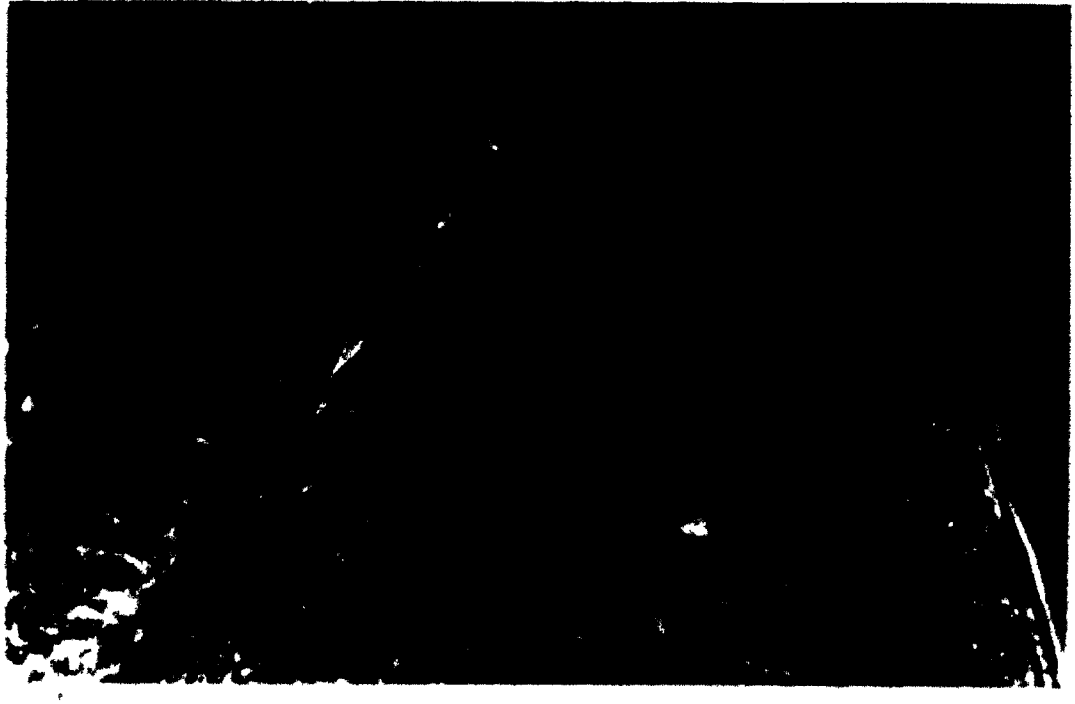


Figure 19. Pyloric stomach: Cell apices are densely ciliated with small microvilli occurring between the cilia. Rootlets are typically much longer than they appear in this micrograph. Endoplasmic cisternae are absent from the actual apex of the cell. Golgi bodies are of the kind that produce vesicles of homogeneous content rather than a fibrous material. x 35,900 (scale 0.5 µm).

Figure 20. Pyloric stomach: A phase contrast micrograph of a live animal in optical section. The pyloric sphincter is marked by arrows. At left are the vacuolated cells of the pharynx (p). Whirling around in the pylorus is the ergatula (e). x 860 (scale 10 µm).

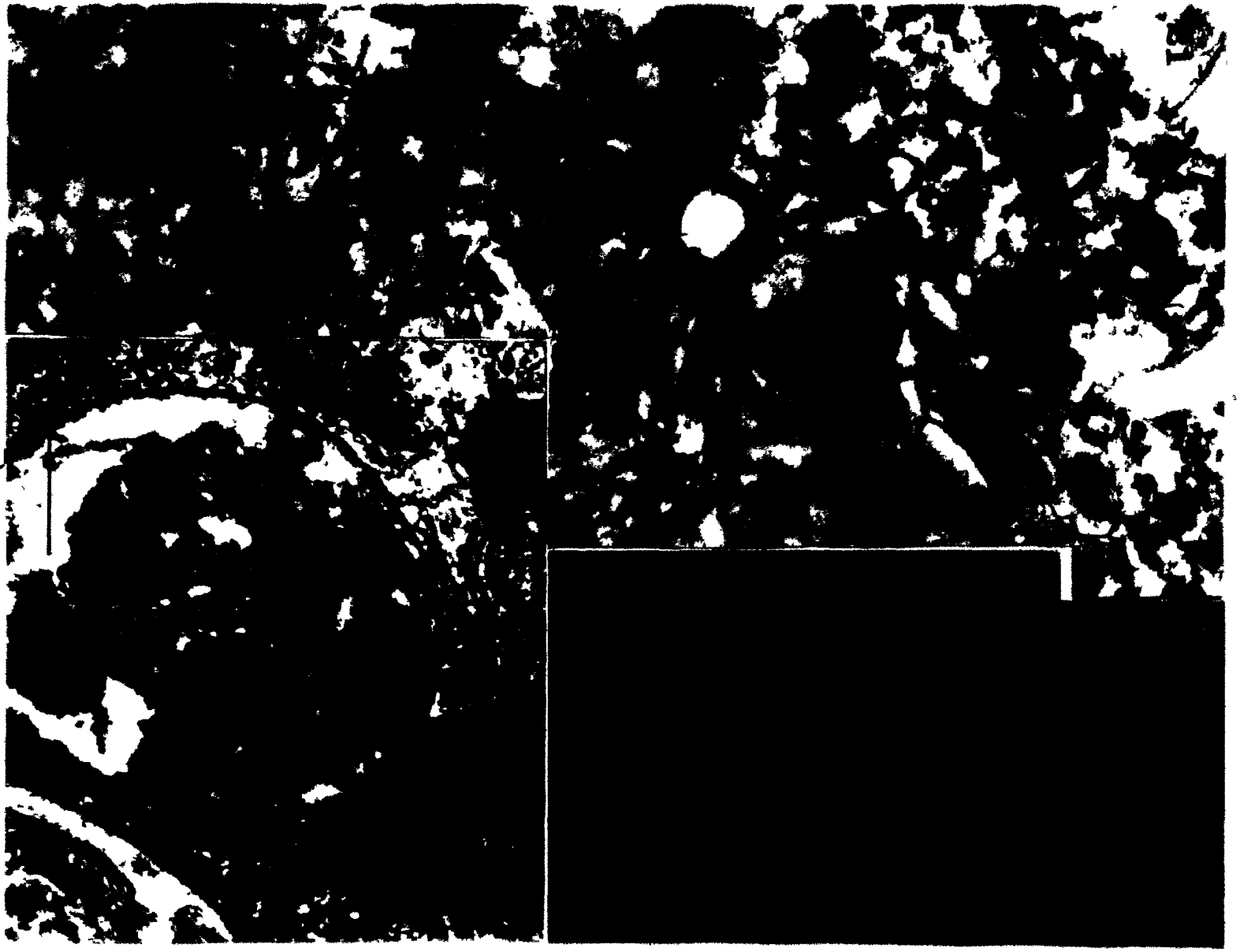


Figure 21. Pyloric stomach: Bacterial cells in the ergatula. x 35,400.

Figure 22. Autofluorescence of granules in the stomach and rectal cells. The greenish-yellow fluorescence is of the orange-brown inclusions. Note the red fluorescence of the ciliated tracts of the central stomach and pylorus and the orange fluorescence of the rectum. The bright red objects in the rectum are diatoms. x 265 (scale 0.1 mm).

Figure 23. Central stomach: One of the granules which contributes to autofluorescence. Membranous elements are of frequent occurrence in the orange-brown inclusions. x 34,400 (scale 1 μm).

Figure 24. Rectum: A single rectal cell. These cells are small, hence the nucleus appears relatively large. The brush border and endocytotic tubules are characteristic. There is some convolution of lateral membranes between cells. Note the small muscle fibril at bottom (arrow). x 24,800 (scale 1 μm).

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 (fig 21) and fragments (collectively termed an ergatula by Morton, 1960) to revolve with its free end projecting into the central stomach (fig 20).

3.54 Orange-brown inclusions

A conspicuous feature of the stomach excluding the ciliated tracts is the relatively large inclusion bodies (secondary lysosomes and residual bodies) which appear in the light microscope as orange-brown granules and give the stomach its distinctive brownish colour (fig 2). 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 autofluorescence (fig 22) when excited by UV light, suggesting that the granules are lipofuscin. Koenig (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 (fig 23) 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 digestive 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.

3.6 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 (figs 24, 25) 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 paracrystalline contents (figs 26, 27). 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 (fig 22).

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). 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 (fig 28).

3.7 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 (fig 29). In Electra pilosa Lutaud (1969) pictures three nerves descending the pharynx. In Cryptosula there are a number of vesicle types, comprising small clear vesicles 40-80 nm diam, small dense vesicles 50-80 nm diam, large clear vesicles 115-150 nm diam and large cored vesicles 100-160 nm diam. 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 ending were found. In figure 30 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.

Figure 25. Rectum: Microvilli and endocytotic canals. x 30,700 (scale 0.5 µm).

Figures 26-27. Rectum: Paracrystalline formations in rectal cells.
26. x 97,000 (scale 100 nm)
27. x 201,000 (scale 100 nm).

Figure 28. Rectum: Interference micrograph of a paraffin section of the rectum opening onto the tentacle sheath. Note the diatoms in the main part of the rectum (d) and the longitudinal muscle fibrils (arrows). At left tentacles (t) are seen within the tentacle sheath (s). x 820 (scale 20 µm).

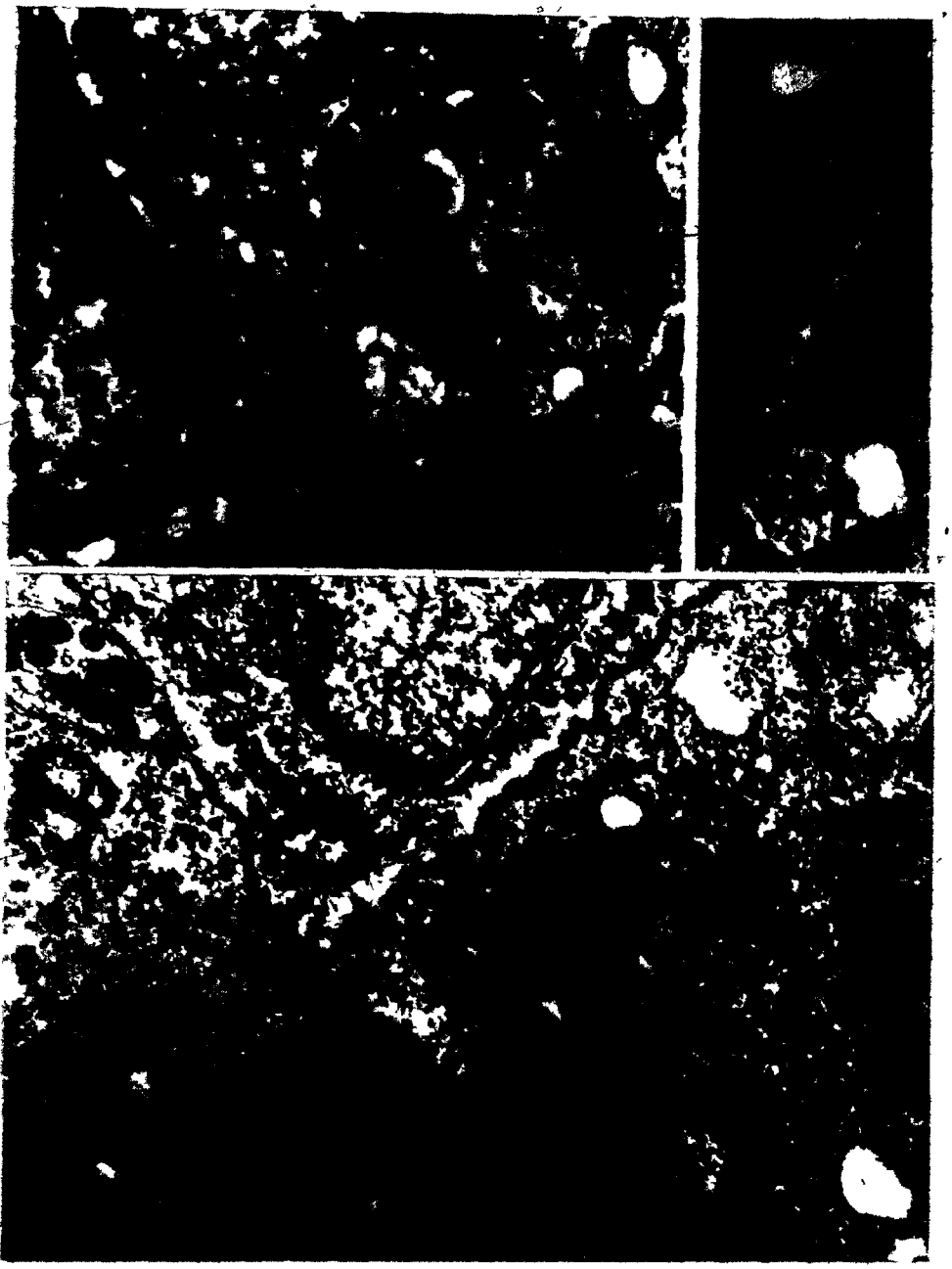


Figure 29. Pharyngeal nerves: Comprising one major nerve and adjacent minor branches. These nerves (seen here in a transverse section of part of the pharynx) are situated at the base of a pharyngeal cell. Part of the striated circular muscle sheet is seen outside the thin basal lamina. x 24,200 (scale 1 /um).

Figure 30. Myoneural junction: Of a myofibril around a cardiac cell. A definitive synapse is not apparent. The small vesicles have the morphological appearance of clear synaptic vesicles. Basal lamina occurs at left of the fibril. x 40,000 (scale 0.5 /um).

Figure 31. Neurosecretory-like terminals on ciliated cells of the stomach. Two vesicle sizes only are apparent. Dense vesicles occur in both the stomach cells (arrows) and terminals. Apparent exocytosis of a dense vesicle (see text for explanation) is shown at 'e'. x 55,500 (scale 1 /um).

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 vesicle - large dense-cored neurosecretory-type vesicles 100-160 nm diam and small clear vesicles 50 nm diam (fig 31). Terminals of this kind containing two such vesicle sizes are typical of neurosecretory terminals and 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 endocytosis of the dense vesicles from the nerve terminals in Cryptosula takes place (fig 31). 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.

3.8 Discussion

In historical accounts of the stomach a feature given some prominence is the occurrence of the orange-brown inclusions in the wall. It seems that the colour of the stomach 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). 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". Farre (1837) also attributed the brown colour of the stomach to a secretion from 'hepatic follicles'.

On the other hand, Van Beneden (1845) stated that the colour of the stomach depended on the nature of the food ingested as Bronstein (1939) and Jebram (1968) 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) rather felt that incomplete elimination of undigested material from the cells in which intracellular digestion takes place leads to accumulation of this material. From EM observations of Cryptosula Bronstein's conclusions would seem to be correct. In addition to the accumulation of material in food vacuoles there is probably the added contribution of autophagy. The occurrence of membranous elements in the vacuoles indicates a local origin of these rather than their elaboration from digested material.

Apart from areas of the stomach where brown inclusions

have been observed to be either present or absent, it has generally been regarded that there are two cell types, one basophilic, the other acidophilic - in gymnolaemates (Calvet, 1900; Silbermann, 1906; Rey, 1927; Soule, 1954; Brien, 1960), in stenolaemates (Borg, 1926), in phylactolaemates (Müller, 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. Basophilic cells were common in newly feeding polypides but disappeared as the polypide became older, and that as there were gradations between the two cell types, the basophilic cells changed in their stainability. The stainability of stomach cells depends heavily on methods of fixation. In my experience fixation of even adjacent cells can be vastly different, leaving one intact and a neighbour with some precipitation of cytoplasm (fig 32) 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,

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Figure 32. Cardiac stomach showing the unequal effects of fixation. Two cells appear 'normal', the others are empty-looking and some vesicles show ruptured membranes. Cilia are few (arrows). Peritoneal cells have sloughed off from the outside during specimen preparation. (r = retractor muscle). x 6900 (scale 5 µm).

the number and size of inclusions in the stomach wall, while intracellular digestion is occurring will cause variations in staining characteristics. There are no mucus-secreting cells in the alimentary tract of Cryptosula. Although two types of Golgi body occur in different cells these are not detectable by light microscopy.

Digestion in bryozoans is recognized to be both extracellular 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 a lysosome marker (Mulnard, 1961) and lysosome autofluorescence and orange fluorescence af-

ter acridine staining is attributed to lipofuscin (Allison & Young, 1969; Koenig, 1963; Weglicki et al, 1968). Furthermore, lipofuscin is viewed as originating in the manner of residual bodies (Daems et al, 1969).

A further similarity between Cryptosula and other invertebrates is seen in the Golgi secretions. The Golgi body which produces macrovesicles containing fibrous material (and small vesicles from the periphery of the sacculés), 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 has 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). In Cryptosula the contents of the macrovesicles are liberated from the cell (how this is achieved was not observed) to lie intercellularly in the peritoneum and funiculus (mesenchyme) around the caecum. Rarely these intercellular deposits have been seen in the lophophore base and tentacle epithelium, 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).

Meulcman (1972) has observed 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 & 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 in the stomach cells reaches a point beyond which no more digestion can take place presumably and the whole polypide regresses. There appears to be no cell replacement in the

stomach of Cryptosula as in Biomphalaria digestive gland, as no mitoses were observed.

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) regards compaction for defaecation as not the prime function of the rotation mechanism, but to serve as a means of further reducing organic material by enzymic digestion and perhaps also mechanical dissolution. The whirling rod in the bryozoan stomach is known 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) discusses 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, but 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, hemichordates, 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.

One further feature noticed by Silén (1944) is worth mentioning. In very young polypides of Membranipora membranacea that had barely begun to feed he saw in the pylorus a glisten-

Figure 33. Edge of a colony of Cryptosula pallasiana. In the recta of three young polypides which have not yet evaginated, a translucent meconium (arrows) is seen.

ing brown rod that appeared to be composed of some kind of secretion. It was later moved into the rectum and in older individuals it was never seen. In recta of polypides of Cryptosula which had not yet fed I saw a semitransparent structure apparently comparable to that observed by Silén (fig 33). After the first defaecation of the polypide this object is no longer seen. It is believed to be meconium (Harmer, 1891).

The rectum in Cryptosula appears to be primarily absorptive in function with associated intracellular digestion on a minor scale.

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 (1941:34) 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. Neuléman (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.

3.9 Summary

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

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.

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

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.

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.

Muscle fibrils of the stomach are striated circular muscles from which thin longitudinal fibrils branch, a condition that is elsewhere known in the crayfish hepatopancreas.

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

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SECTION 4 POLYPIDE REGRESSION (BROWN BODY FORMATION)

4.1 Introduction

"Ces corps bruns ont bien intrigué les observateurs". So wrote Joliet in 1877 (p.195) after reviewing the opinions of previous authors. The cyclic phenomenon of polypide regression and renewal is a characteristic feature of bryozoans which is to an extent paralleled by some other sessile, colonial metazoans. The products of regression (one or two ovoid or spherical brown bodies) puzzled many early workers as regards their nature, because it was not always understood that they were regression products. They were thought to be ova or ovaries (Thompson, 1830; Farre, 1837; Van Beneden, 1845), developing embryos (Loven, 1842), the equivalent of phylactolaemate statoblasts (Allman, 1856; Redfern, 1858), germ capsules (Smitt, 1863, 1865; Hincks, 1871, 1873) and an endocystal secretion (Claparède, 1870). It is amusing that free rein was given to such speculation as the origin of brown bodies from withered polypides had already been recognized by Ellis in 1755 and Grant in 1827. "These black spots are nothing but the dead polypes, or remains of the animals once inhabiting these cells. Of which I had evident proof in my last journey to the sea-coast. For after I had examined this coralline, with its polypes alive in sea water I laid this specimen aside; and upon examining it again sometime after, I found the lifeless contracted animals exhibited the appearance above-mentioned". While the latter part of Ellis' explanation is slightly ambiguous it is clear that he recognized the true nature of brown

bodies, which he illustrated in some gymnolaemates (e.g. Menipea ternata, Bugula plumosa) and a stenolaemate (Crisia eburnea).

Part of the subsequent difficulty in recognizing the true nature of the brown body was due to the observation of a polypide bud often in intimate contact with it. Hincks (1871, 1873), supporting Smitt's (1863) germ capsule theory, held that the brown body was not derived from the withered polypide but was a special formation produced at its expense by sudden condensation of the stomach caecum which became detached. The body thus formed served as the origin of a new polypide. Nitsche (1869, 1871) seems to have been the first author after Grant (1827) to have realized that the brown body was derived simply as a regression product of the withered polypide.

Next, Ehlers (1876) described histolysis in Alcyonidium and Repiachoff (1876) discovered the incorporation of the brown body into the stomach caecum of the backwardly growing new polypide. Hincks (1880) was persuaded of the correctness of Repiachoff's observations after reading Joliet's 'Histoire' (1877), which was supported by Waters (1878). For those of this period and subsequent authors who accepted the polypide origin of the brown body opinions varied as to its nutritive value to the developing polypide. There were those who held that the residue was useful (Repiachoff, 1876; Hincks, 1880; Zschiesche, 1909; Waters, 1913; Gerwerzhagen, 1913) and those who held it to be inert (Nitsche, 1869, 1871; Joliet, 1877; Marcus, 1926) although Joliet felt that mesenchyme cells incorporated with the brown

body into the new gut could be broken down, and Marcus observed that the incorporated brown body became smaller. Waters (1913), seeing mesenchymal strands between the brown body and ovicell in a zooid thought the one supported the development of the other.

Incorporation of the brown body into the new gut is known in Cryptosula pallasiana (Repiachoff, 1876), Scruparia chelata (Joliet, 1877), Carbasea papyrea (Harmer, 1891), Carbasea carbasea (Gerwerzhagen, 1913), Flustra foliacea and Electra pilosa (Marcus, 1926), Chartella papyracea and Callopora lineata (Rey, 1927), Watersipora cucullata (Nawatari, 1952), and Crassimarginatella papulifera and Fenestrulina malusii (Gordon, 1968). It may, however, remain in the cystid as is said to occur in Bugula neritina and B. avicularia (Harmer, 1891), B. simplex and Scrupocellaria scruposa (Calvet, 1900), Bugula turbinata (Römer, 1906), Flustrellidra hispida (Rey, 1927), Alcyonidium gelatinosum (Bobin & Prenant, 1957) and Victorella argilla (Banta, 1967). Alternatively, the brown body was said to be totally destroyed by phagocytosis (Korshalt & Heider, 1912), and Hincks (1880:89) and Prouho (1892:589) saw it eliminated through the intertentacular organ of some species in the reproductive season.

The nature and origin of the brown body having been established, causal factors in its formation and reasons for its existence were put forth. Brown body formation has been attributed to unfavourable environmental conditions (lack of oxygen; low food supply; extremes of temperature, pH and salinity; mechanical (e.g. sediment) and chemical inhibition of evagination;

detachment of colonies) (Marcus, 1926; Rey, 1927) and reproductive activity (e.g. oogenesis and embryo maturation) (Calvet, 1900; Römer, 1906; Borg, 1947; Nawatari, 1952; Bobin & Prenant, 1957; Braem, 1957; Chrétien, 1958; Matricon, 1963; Banta, 1968). Accumulation of residual material in stomach cells has also been thought to be a prime cause, and this has been tied in with the lack of excretory organs in Bryozoa. Brown body formation has been therefore regarded as having an excretory function, an idea first expressed by Ostroumoff (1866), and acknowledged by Harmer (1891, 1896, 1931), Delage & Hérouard (1897), Prouho (1892), Calvet (1900), Marcus (1926), Rey (1927), Bronstein (1939) and Cori (1941). Calvet, however, felt that as in some bryozoans the brown body is not eliminated, excretion may not be the prime function. Marcus (1926) and Gordon (1970) noted that brown body formation tides zooids over periods when adverse conditions prevail temporarily, and Prouho (1892) felt that the "bryozoite bénéficie d'un rajeunissement périodique".

In the literature polypides are generally referred to as 'young' when newly feeding and 'old' when stomach walls are laden with residual material but how and whether natural senescence is related to accumulation of this material has not been clear. Thus, knowing that brown bodies are regression products and that their formation is induced by both extrinsic and intrinsic factors, can brown body formation be regarded as an excretory or an ageing phenomenon? What is the nature of the granular material that comprises the regression product and is it likely that it can be further digested in the gut of the new

polypide? Furthermore, what is the role of phagocytosis in polypide regression? An electron microscopic study of brown body formation was undertaken to elucidate some aspects of this noteworthy phenomenon.

4.2 Materials and methods

(As for section 1).

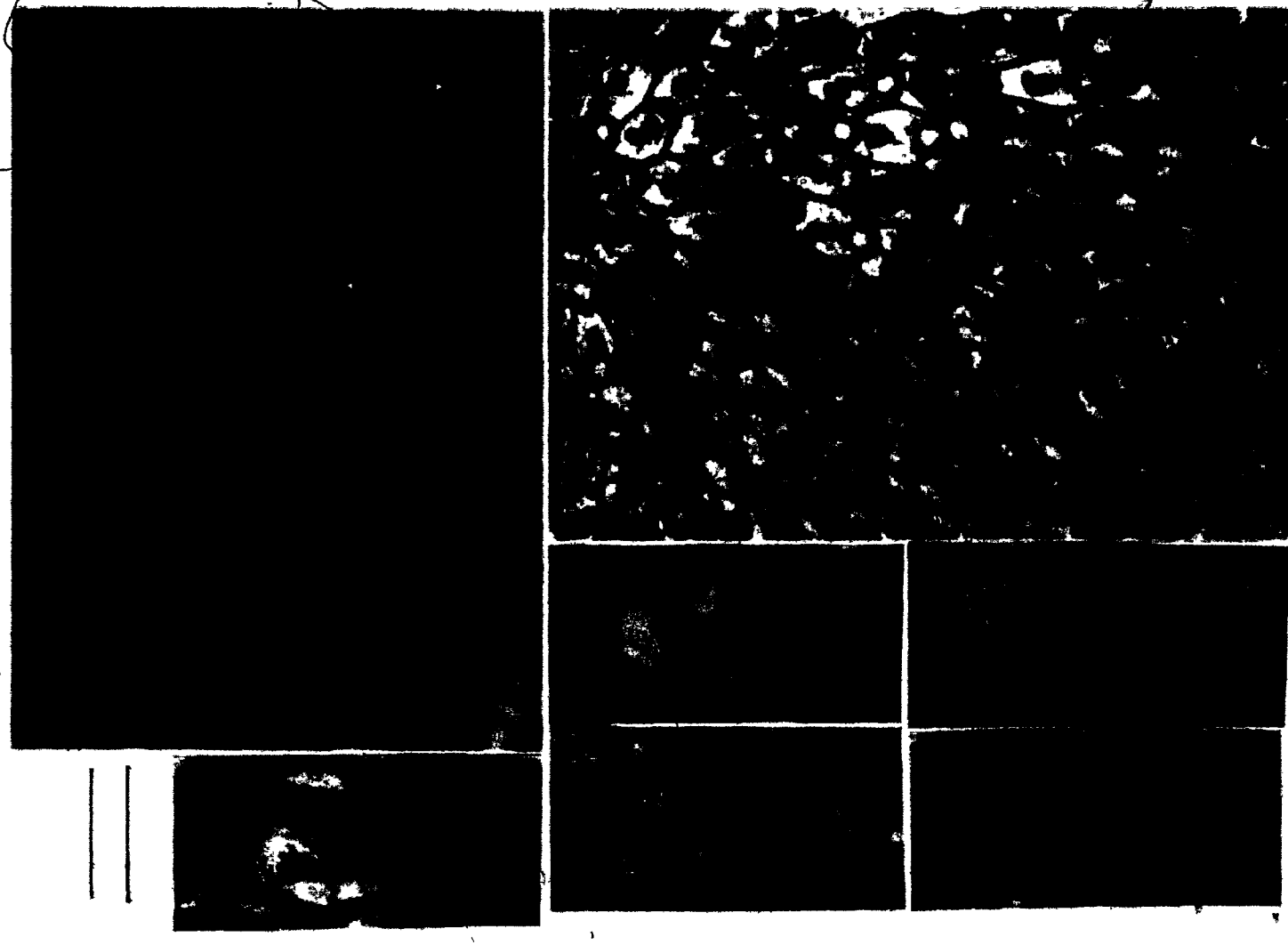
In addition, fluorescence microscopy of live unstained and stained (acridine orange) polypides was carried out on a Zeiss Large Fluorescence Microscope in order to locate nuclear DNA and detect changes in autofluorescence during regression.

Growth of colonies: Timing of polypide regression and other events was carried out on colonies maintained over a period of several months. These colonies were established in the following manner. Knowing that Cryptosula larval release can occur from May to December in Europe with summer maxima (Ryland, 1965), glass plates were fixed adjacent to presumed breeding colonies under intertidal rocks at Graves Island, St Margaret's Bay, in July, 1972. This is a sheltered shore with only moderate wave action. After two months the plates were collected and examined. By serendipity several colonies had become established. The plates were transported to an aquarium of circulating seawater. Colonies of Cryptosula pallasiana and Electra pilosa (L.) were checked at intervals by microscopic examination of their basal (dorsal) sides through the glass plates. The calcified frontal wall of Cryptosula precludes examination by any other means.

4.3 General features of regression at the level of light microscopy

Polypides of Cryptosula were found to survive for periods ranging from 15-72 days (2-10 weeks) in aquarium conditions. After the final emergence of the lophophore for feeding the polypide is retracted and undergoes tissue regression (figs 1-6). Observations of paraffin sections and live colonies reveals that the lophophore starts degenerating from the ends of the tentacles backwards, meanwhile condensing, until it has become a roughly conical or spherical orange mass. Simultaneously, the alimentary tract shrinks in size as the gut lumen diminishes and the stomach becomes compacted. The parts of the stomach containing orange-brown inclusions in life become very dark. The rectum and pharynx are incorporated with the regressing lophophoral mass and the remains of the polypide take on a bipartite appearance, with an anterior reddish part and a posterior dark-brown body.

From paraffin sections the lophophore base and its basal lamina are seen to be the last parts of the lophophore to regress. The orange-brown inclusions of the stomach become a prominent part of the brown body, and lose their ability to fluoresce as regression proceeds. If the polypide does not defaecate prior to regression then a faecal pellet with skeletal fragments of food organisms becomes incorporated. Sometimes the regressing anterior part (lophophore, pharynx, rectum) remains separated from the regressing stomach portion so that



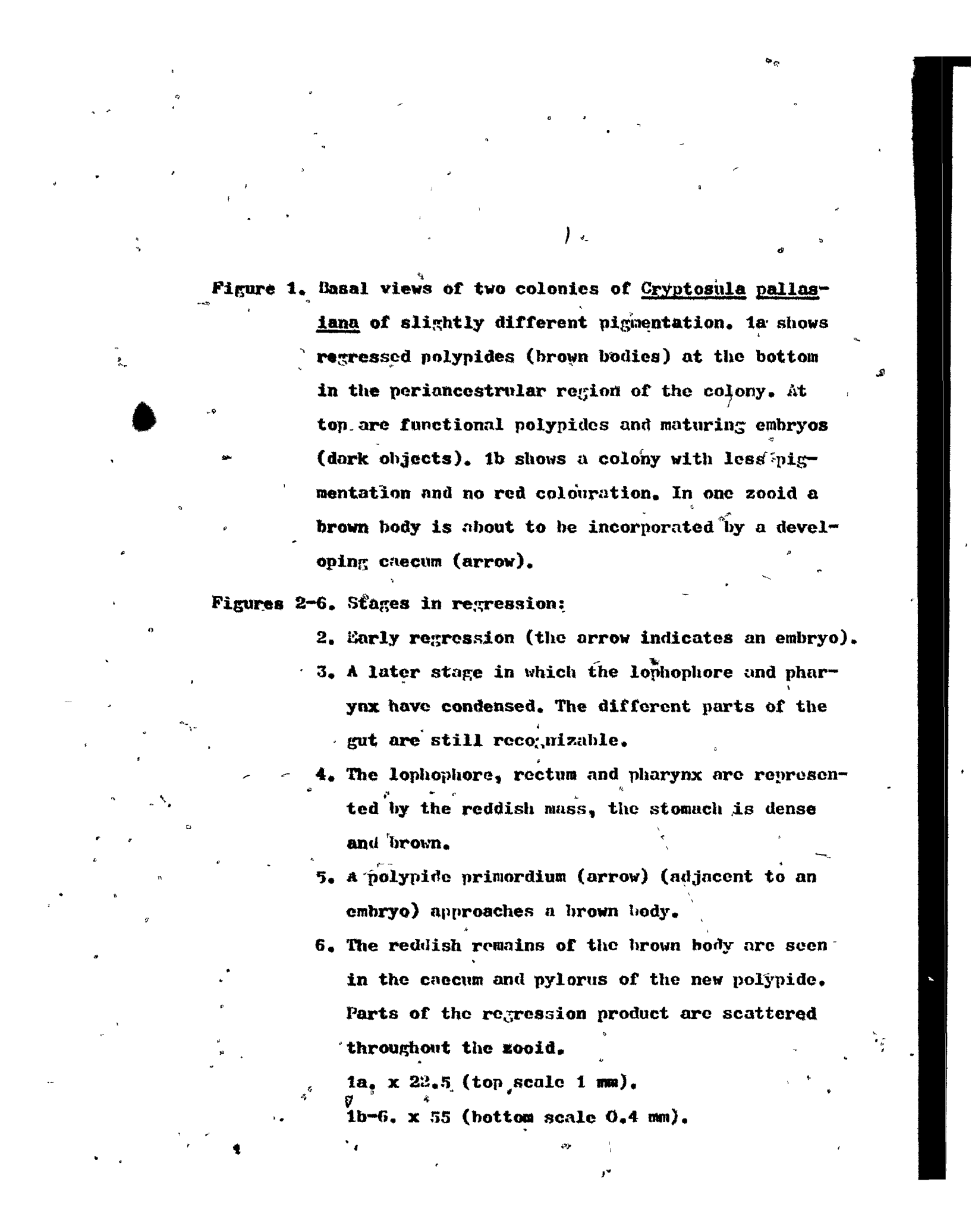


Figure 1. Basal views of two colonies of Cryptosula pallasiana of slightly different pigmentation. 1a shows regressed polypides (brown bodies) at the bottom in the periancestrular region of the colony. At top are functional polypides and maturing embryos (dark objects). 1b shows a colony with less pigmentation and no red colouration. In one zooid a brown body is about to be incorporated by a developing caecum (arrow).

Figures 2-6. Stages in regression:

2. Early regression (the arrow indicates an embryo).
3. A later stage in which the lophophore and pharynx have condensed. The different parts of the gut are still recognizable.
4. The lophophore, rectum and pharynx are represented by the reddish mass, the stomach is dense and brown.
5. A polypide primordium (arrow) (adjacent to an embryo) approaches a brown body.
6. The reddish remains of the brown body are seen in the caecum and pylorus of the new polypide. Parts of the regression product are scattered throughout the zooid.

1a, x 22.5 (top scale 1 mm).
1b-6, x 55 (bottom scale 0.4 mm).

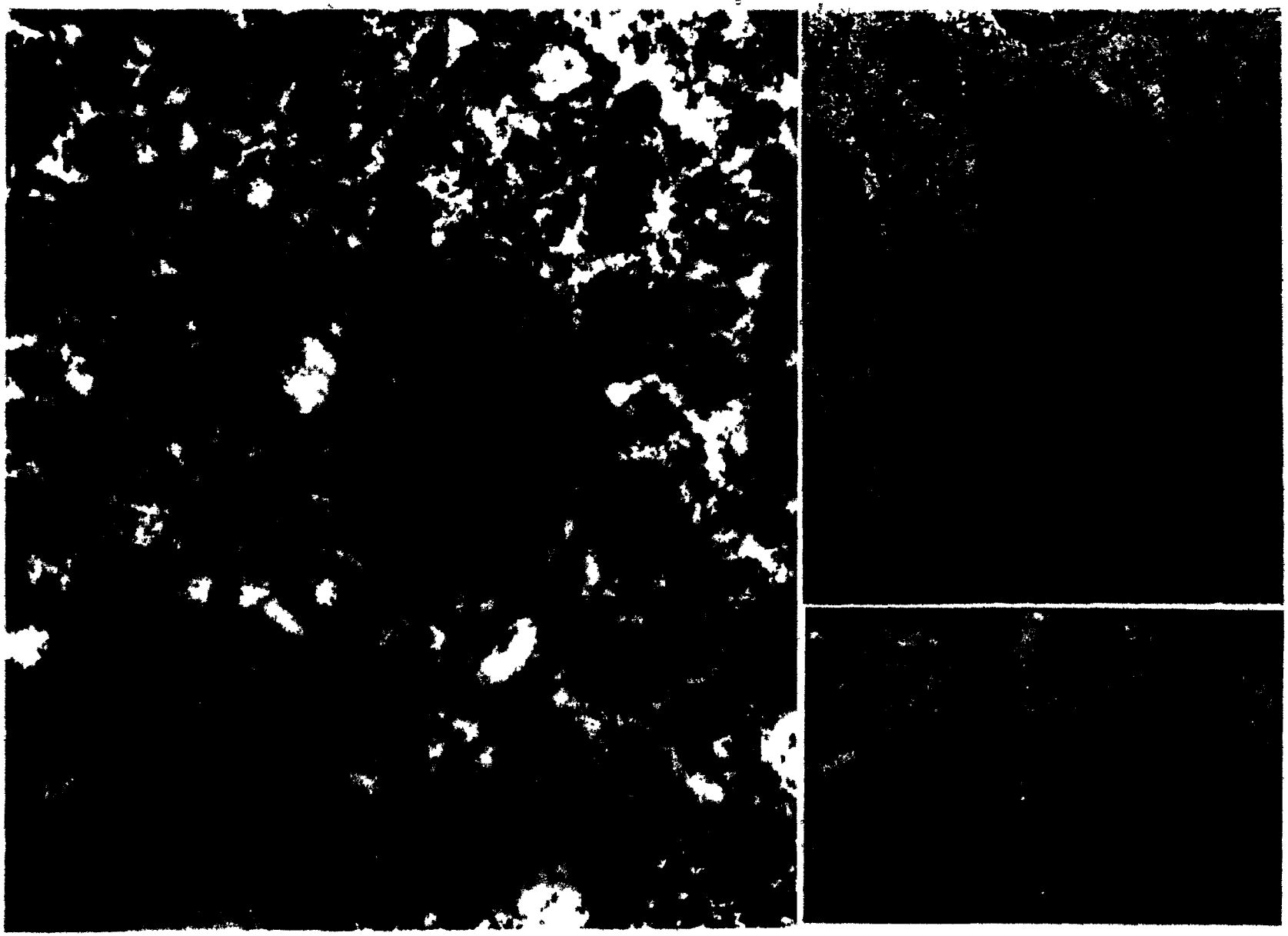


Figure 7. Lophophore regression: A group of cells near the base of a regressing tentacle showing cell displacement and differential cytoplasmic densities. x 6050 (scale 2 /um).

Figure 8. Lophophore regression: Liberated cells - the one with rootlets is clearly of tentacular origin. The upper cell shows signs of necrosis - pyknotic nucleus, pseudopodia and glycogen (arrow). x 8500 (scale 2 /um).

Figure 9. Lophophore regression: A ciliated cell in early regression with a lytic vacuole containing membrane lamellae. Notice the small vacuoles (arrows) which are derived from endoplasmic cisternae. The fibrous bundles are ciliary rootlets cut obliquely. x 99,000 (scale 200 nm).

there are two 'brown bodies', but more often the reddish-orange part appears as a protuberance or less dark region on the brown body proper. After acridine staining fluorescence is confined to a few nuclei (green in UV) in and around the brown body. Complete regression takes 6-17 days from start to finish.

Often during regression large numbers of reddish cells or cell clusters can be seen scattered around the zooid in the mesenchyme, presumably phagocytic in nature. There seems to be some phenotypic variation in colony pigmentation in Cryptosula. Feeding colonies a few centimeters apart on a glass plate apparently on the same diet were noticeably different. The reddish cells scattered in the mesenchyme and similarly coloured degeneration product of the lophophore are lacking in figure 1b. (I have also noticed that estuarine colonies of Cryptosula in New Zealand are lacking in pigmentation whereas Nova Scotian colonies exposed to more or less open sea conditions are dull orange in colour). The occurrence of the scattered cell clusters is irregular in regressing zooids and bears no obvious relationship to the presence or absence of gonads or a regenerating polypide.

Since the development of intrazooecial embryos is said to cause polypide regression in some species of Bryozoa (Marcus, 1926a,b; Bobin & Prenant, 1972) it is worth noting at this point the effects, if any, in Cryptosula (where intrazooecial embryos occur) of reproduction on polypide longevity. Observations on living zooids during the reproductive season revealed the following facts. Colonies are protogynous, ova appearing

about two weeks before testes. Some individuals seem to be monoecious but most are dioecious. In any case, the onset of gametogenesis has no apparent effect on the polypide. After presumed fertilization of ova embryo maturation takes approximately thirty days, near the distal wall of the zooid, during which time a second may be developing on a side wall. Testes also persist in zooids for about thirty days. Throughout all this reproductive activity, including embryo maturation, polypides continue to feed, or regress or regenerate.

4.4 Ultrastructural features of regression

4.41 The regressing lophophore

Among the first features to be seen in tentacle regression are various changes at the cell surface. The cuticle sloughs off including tips of microvilli. In addition, cell contacts weaken and adjacent cells move apart from one another and from the basal lamina. This occurs both in the outer epithelium and inner peritoneum. At this time the collagen fibrils still show a nodular periodicity of 60 nm. Cytoplasmic features vary from cell to cell as not all cells regress simultaneously (fig 7). The abfrontal cells seem to regress more quickly than the lateral and frontal cells, showing more autophagic activity. In the case of the ciliated cells cytoplasm appears almost as in cells prior to regression but as cells slough off from the basal lamina some changes become more apparent. Endoplasmic cisternae become small vacuoles (figs 8, 9) and ribosomes are liberated as a result. At the cell surface abscission of some of the cilia from their basal bodies seems to occur leaving them to degenerate extracellularly or be picked up by heterophagy. Also in detached

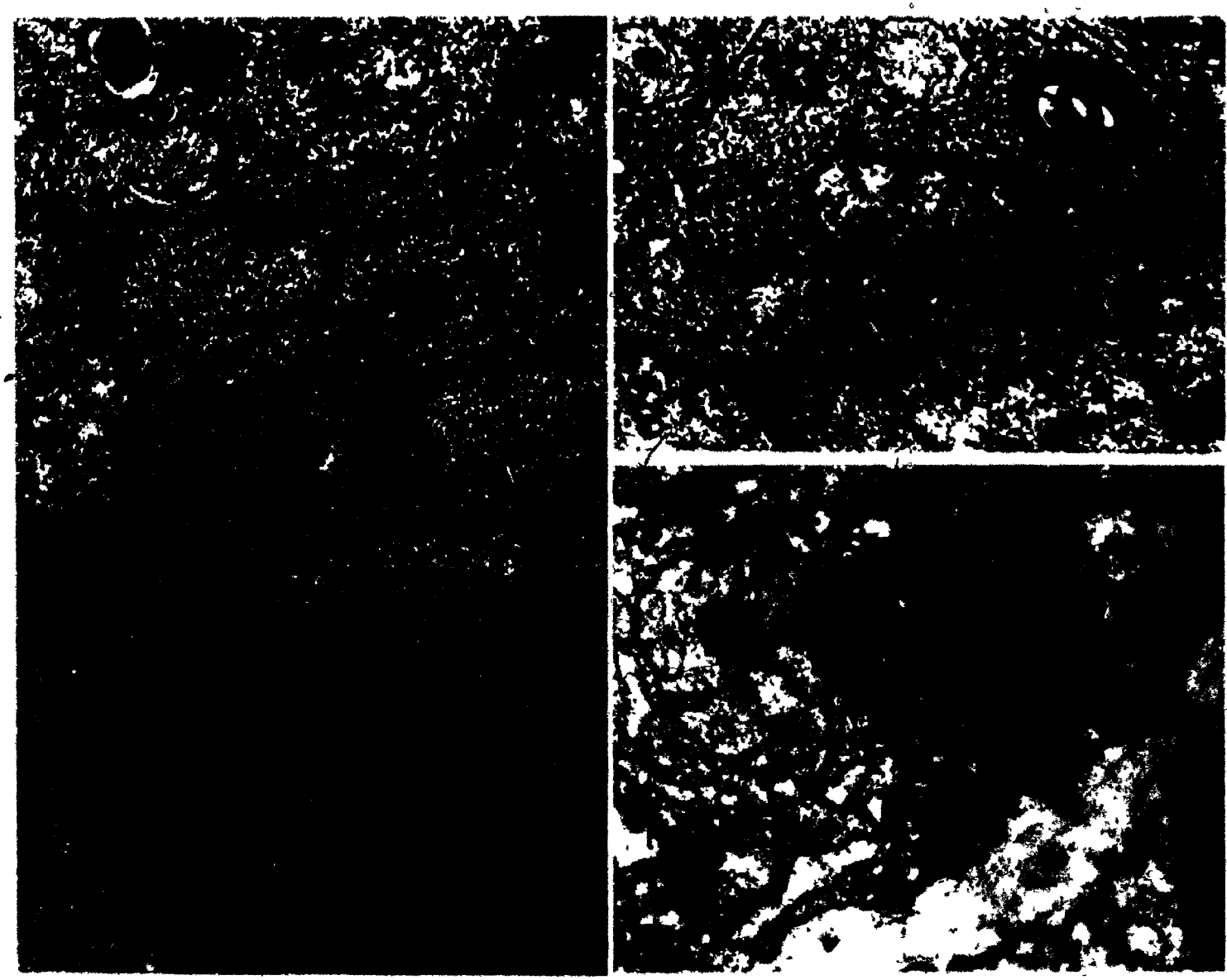


Figure 10. Lophophore regression: A former embryonic cell from the lophophore base with a large myelin figure. x 27,600 (scale 0.5 µm).

Figure 11. Lophophore regression: A cell from the lophophore base with a lytic vacuole and a mitochondrion containing a small myelin figure (l = basal lamina). x 44,600 (scale 0.5 µm).

Figure 12. Lophophore regression: Cells at the lophophore base with pigment droplets (p). There is some condensation of nuclear chromatin. Arrows indicate the basal lamina. x 9300 (scale 2 µm).

cells there is some clumping of nuclear chromatin against the nuclear membrane as incipient pyknosis occurs. Pseudopodial processes may be extended and there is occasional intracellular glycogen deposition (fig 8). Autophagic vacuoles and multivesicular bodies are also present and in some cells lipoidal pigment granules. Mitochondria and Golgi bodies appear 'normal'.

Cells at the lophophore base and mouth regress later than further up the tentacles, and the beginning of regression is marked by the development of myelin figures in the cytoplasm and within and around some mitochondria (figs 10, 11, 16), residual bodies and lipid droplets (figs 12, 13) and finally the moving apart of cells.

Peritoneal cells in the tentacles clump together. These cells undergo marked autolysis compared to the outer epithelial cells. The latter seem to do one of two things. Some which show more signs of regression continue to autolyze while others which appear relatively healthy, having been liberated, seem to take on an amoebocytic role (fig 13).

In cross section at this stage the tentacles are displaced with respect to one another and in many cases are recognizable only by virtue of the irregular profiles of basal lamina that remain. The picture, then, is one of seventeen buckled tubes of basal lamina with aggregations of autolyzing peritoneal cells on the inside, and attached and detached autolyzing and relatively normal epithelial cells on the outside as well as organic debris derived from fragmenting cilia and detached cuticle.

Figure 13. Lophophore regression: A liberated epithelial cell containing axonemal profiles, two nuclei or parts of a former nucleus (one pyknotic (n)) and degenerating mitochondria. Outside the cell are membranous formations derived from shed cilia. The arrows indicate axonemal tubules. x 17,800 (scale 1 µm).

Figure 14. Lophophore regression: A necrotic epithelial cell showing ciliary resorption. The axoneme is separated from the basal body. The arrow indicates a satellite body. x 25,500 (scale 0.5 µm).

Figure 15. Lophophore regression: Cells from the lophophore base in early regression with a lipid droplet (l) and dense residual bodies. x 25,500 (scale 0.5 µm).

Figure 16. Lophophore regression: Cells from the lophophore base in varying states of regression. Mitochondria are showing incipient myelinization and have lost their smooth contours and the nucleus is rather homogeneous, an alternative feature of necrosis. x 33,300 (scale 0.5 µm).

As these cells regress yet further they develop very irregular profiles, appearing to fragment into smaller pieces; in sections of the regressing lophophore mass many profiles of apparently small cells are encountered. Either they represent cell fragments or they are profiles of irregular cell contours.

These changes take place over a period of days during which the regressing lophophore condenses by autophagy, heterophagy and concomitant cell movement. Sections of the condensing mass demonstrate a marked increase in the number of cells showing what Bessis (1964) calls death agony (characterized by the appearance of pseudopods and/or oedema of cytoplasm, nucleus and organelles). Large numbers are also definitely necrotic (involving nuclear pyknosis and/or karyorrhexis and formation of myelin threads or figures). Very few show many signs of 'normality' and, as expected, those that do are the former embryonic cells of the lophophore base, which are the last to succumb (figs 15, 17, 18). Features of cell agony and necrosis are exhibited in figures 17-25.

The basal lamina maintains its structural integrity for some time. At the beginning of lophophore regression it is buckled and bent but this is normal whenever the lophophore is retracted, in order to accomodate its volume in the cystid. What actually causes the collapse of the basal lamina is not known. Possibly liberated hydrolytic enzymes accomplish this as is known in anuran metamorphosis (Eisen & Gross, 1965). At any rate, it does collapse and while much of it seems to become reduced to a fine, fibrous form extracellularly, some is

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Figure 17. Lophophore regression: Regressing and necrotic cells at a tentacle base. Notice the karyorrhexic nuclear cluster (k) and the islands of basal lamina (arrows) some of which are incorporated into adjacent cells. Some cells have herniated. x 5750 (scale 5 µm).

Figure 18. Lophophore regression: Some of the last cells to regress are the former embryonic cells of the lophophore base. These are relatively normal-looking but with minor blebbing of the perinuclear space (arrow), while most contain large autophagic vacuoles. x 7220 (scale 5 µm).

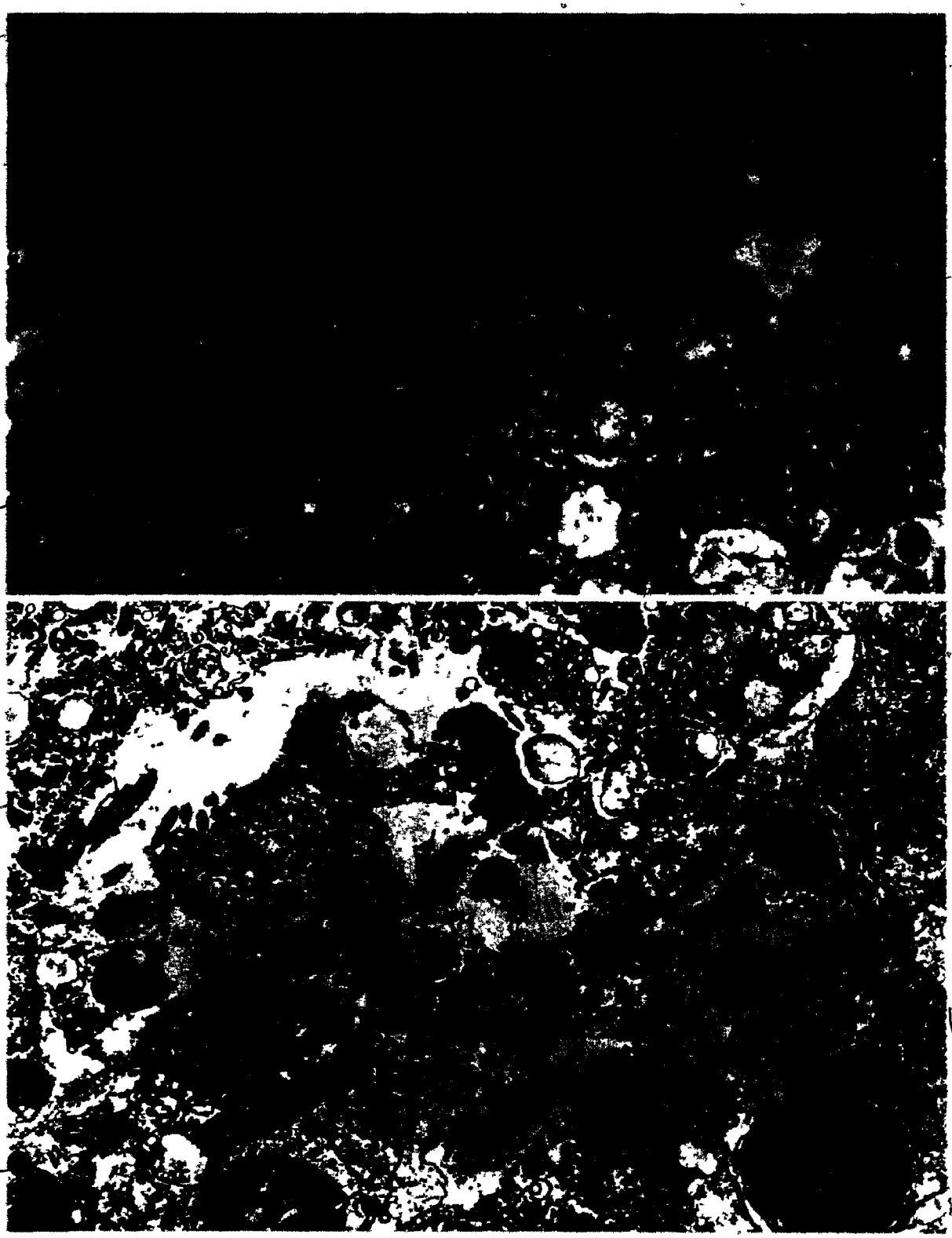


Figure 19. Lophophore regression: Cells from the lophophore base containing secondary lysosomes. The dense vacuoles may contain regressed muscle. Note the intercellular deposits of fibrous material (arrows) derived from certain Golgi vesicles. x 49,600 (scale 0.5 μm).

Figure 20. Lophophore regression: Some cells from the center of the regressing lophophore mass. Here there is much debris from ruptured cells, though some of this is generally incorporated into others. A former ciliated tentacle cell can be recognized by the incorporated axoneme (arrow). x 6180 (scale 2 μm).

B1

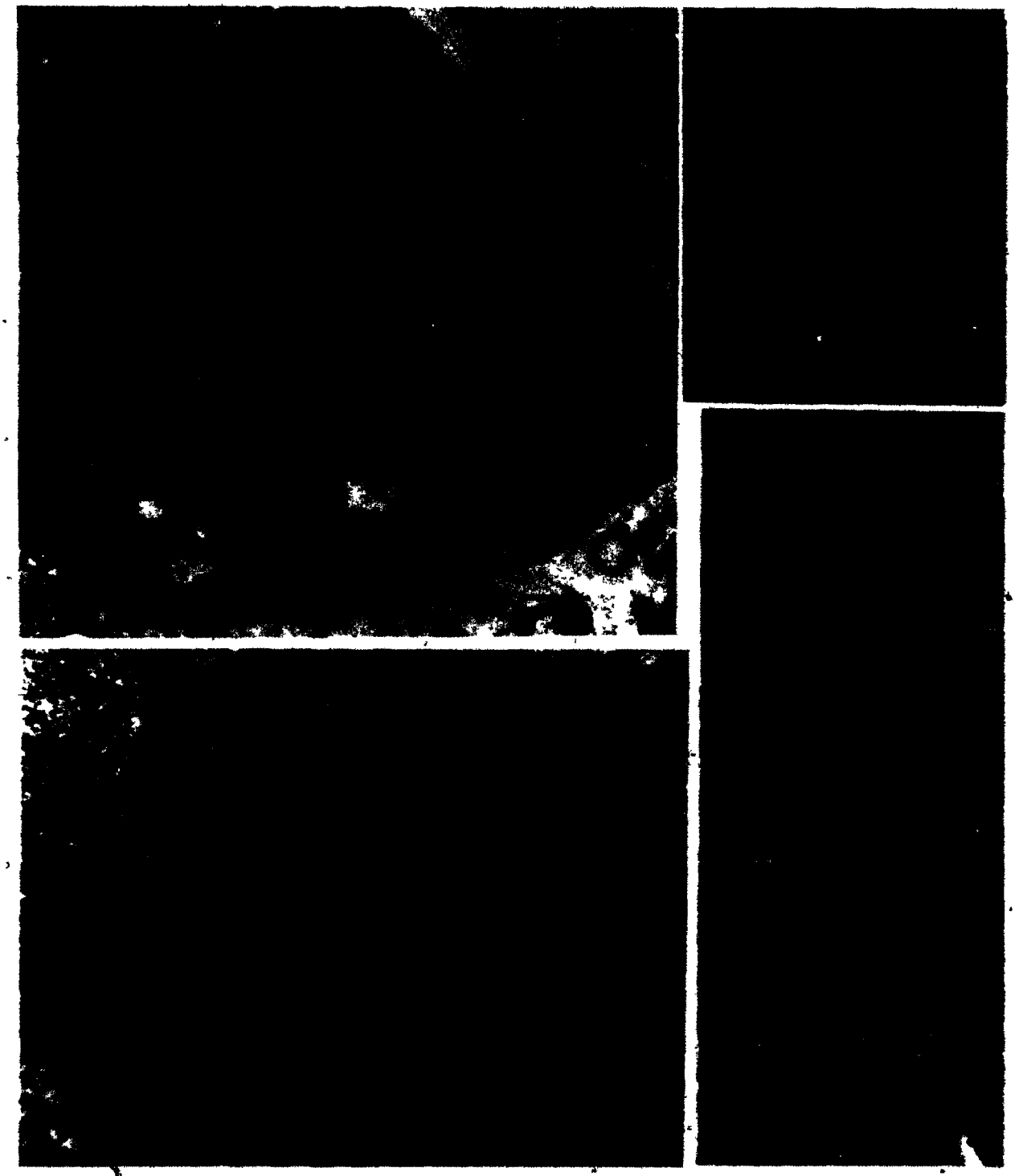
Figures 21-22. Lophophore regression: Portions of figure 17, showing the karyorrhexic cluster (fig 21) and a small island of basal lamina (fig 22). The vacuole at the top of figure 22 contains muscle.
21. x 16,600 (scale 1 /um).
22. x 25,700 (scale 0.5 /um).

Figure 23. Lophophore regression: Some of the ciliary shafts that are shed are picked up into heterophagosomes along with other debris by macrophagic activity of some of the regressing lophophore cells.
x 16,000 (scale 1 /um).

incorporated by heterophagy (figs 17, 22). At some time during condensation of the lophophore the 60 nm nodular periodicity of the collagen is lost and infraperiods of 13-14 nm become apparent (fig 26).

Epithelial cells of the tentacles which seem to behave as amoebocytes can die and become necrotic themselves. Some of the cell debris which occurs in the center of the regressing mass appears to be derived from ruptured necrotic cells, but much of the debris becomes incorporated into cells by heterophagy. The free epithelial cells contain, besides identifying rootlets, naked axonemes (figs 13, 14, 20, 24, 25, 27). There is no vacuolar membrane either. In addition, therefore, to being sloughed off from the cell surface shafts may be withdrawn, as though the axoneme were being injected into the cell (fig 27). This phenomenon is known in reorganizing ciliated cells where axonemes detach from the basal bodies and are withdrawn into the cell (Paulin, 1973). Inside the cells of Cryptosula the axonemes break apart giving random microtubular profiles in the sections. As well as nuclear pyknosis, homogenization of nucleoplasm and karyorrhexis occur. Myelin figures are often abundant in necrotic cells, although it should be noted that as small myelin figures occur in otherwise healthy cells of the living polypide, they are hardly characteristic of necrosis.

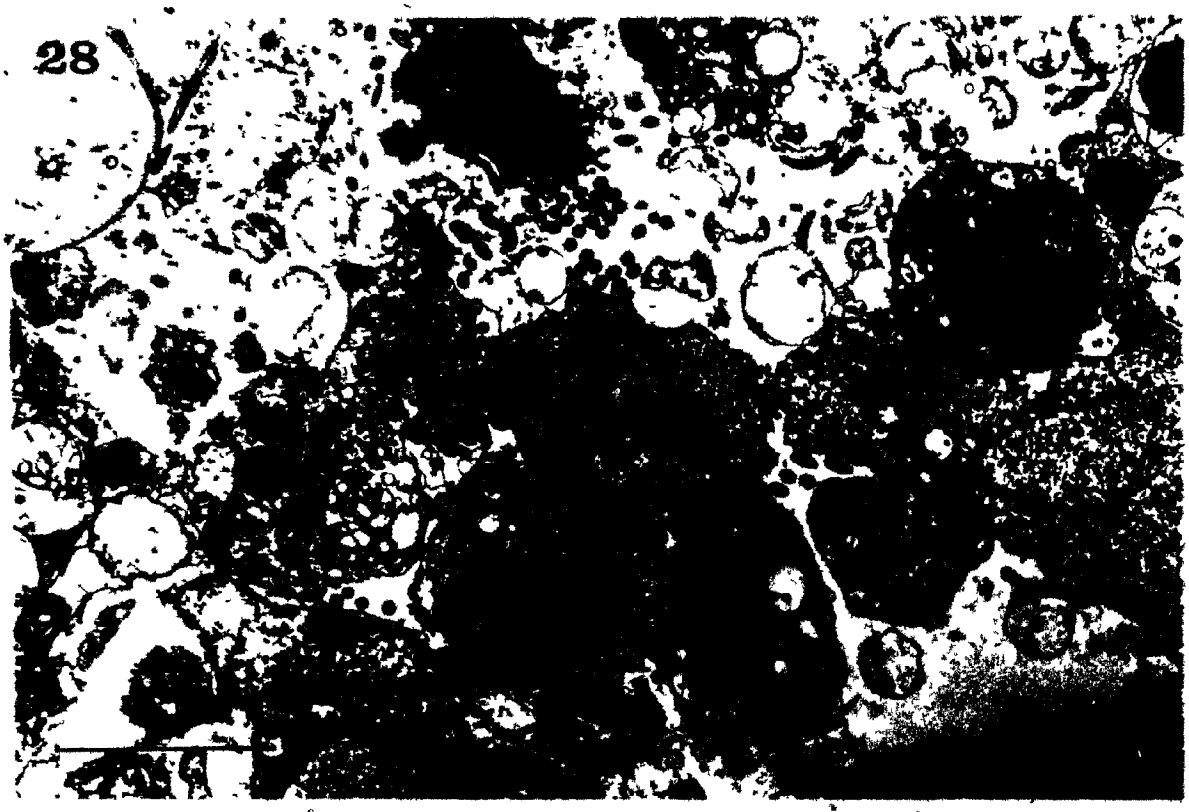
Muscle cells in the tentacles and lophophore base presumably shorten, as in sections of the regressing mass they are seen to be balled-up with filaments lying in all directions. The balled-up cells may then be incorporated into others by



Figures 24-25. Lophophore regression: Liberated and loose cells of former ciliated epithelium containing resorbed cilia and fragmented nuclei (arrows) (the karyorrhexic condition).
24. x 25,700 (scale 0.5 /um).
25. x 22,600 (scale 0.5 /um).

Figure 26. Lophophore regression: Collagen fibrils show a different periodicity when regressing, of 13-14 nm (arrows). x 208,000 (scale 50 nm).

Figure 27. Lophophore regression: Cilia in the process of being resorbed into a parent epithelial cell. x 33,300 (scale 0.5 /um).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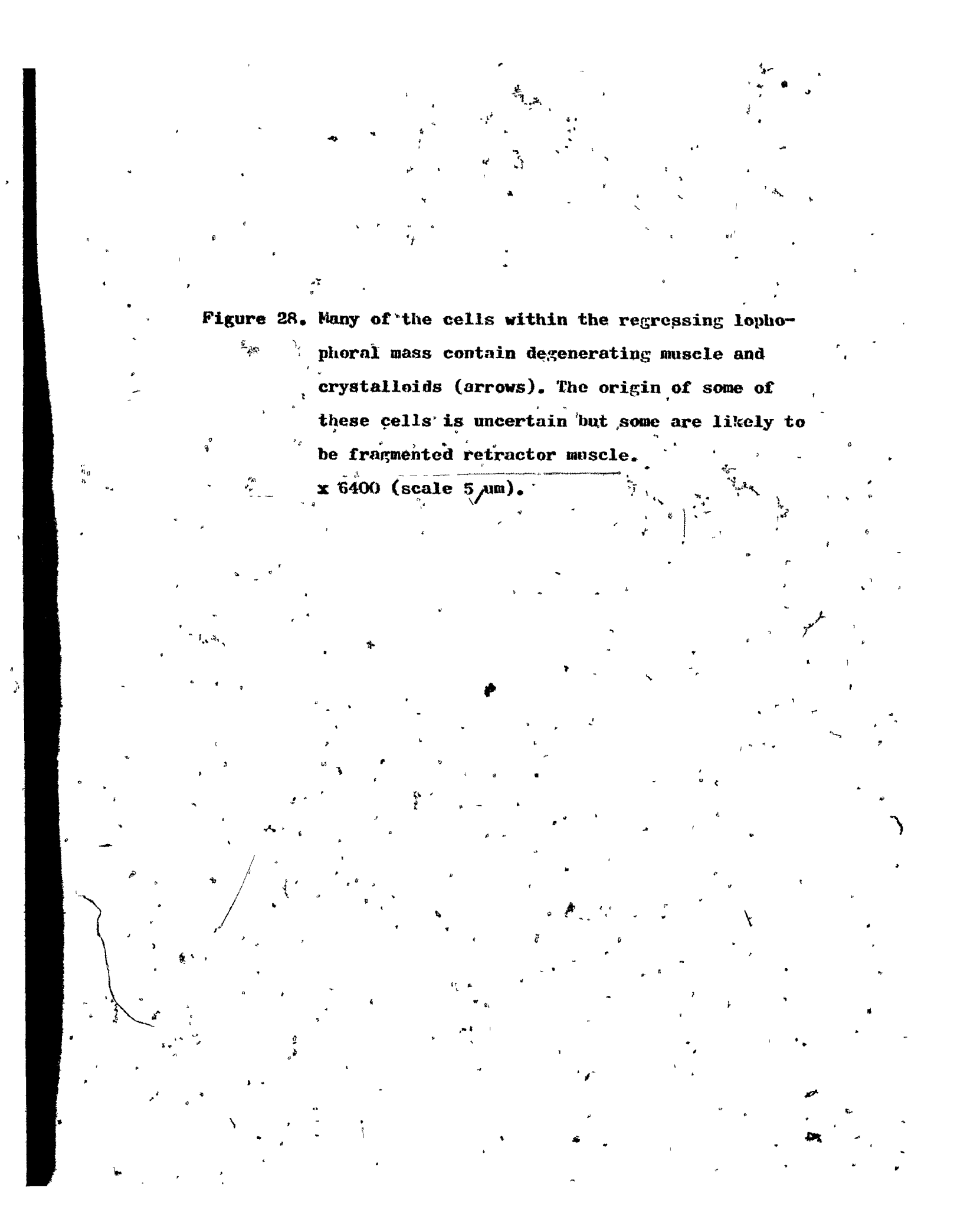


Figure 28. Many of the cells within the regressing lophophoral mass contain degenerating muscle and crystalloids (arrows). The origin of some of these cells is uncertain but some are likely to be fragmented retractor muscle.
x 6400 (scale 5 µm).

heterophagy. In the circular muscles of the mouth lipid droplets appear and also vacuoles containing membrane lamellae (fig 29) comparable to some described by David (1970:113,149) in other organisms. Filaments cease to lie in ordered arrays.

In addition, there are some small cells (the sarcolytes of Bobin & Prenant, 1957?) which contain predominantly degenerating muscle (fig 28). Fraying retractor muscle appears to be taken care of in this way. Within some of these cells muscle filaments undergo transformation into crystalloidal material consisting of overlapping arrays of helical filaments (figs 30, 31). More compact hexagonal crystalloids of apparently related structure were encountered in the parietal muscle cells which run from the floor of the compensation sac to the basal wall of the cystid. It is not certain if parietal muscles regress in Cryptosula or not. The crystalloids were encountered only during polypide regression and at this time necrosis of epithelial and mesenchyme cells was occurring around the ends of the muscle cells which themselves showed some pathological changes (oedema of mitochondria, ground plasm of free ribosomes). The diameter of the filaments in the macrophages and in the parietal muscles is the same (5-9 nm) and it seems likely that the images seen in the macrophages represent longitudinal profiles of incipient crystalloids, while those in the parietal muscle are transverse profiles of more fully formed crystalloids. In transverse sections the rows of filaments are set at angles of 59-64^{0} with respect to one another (fig 31). The tilt

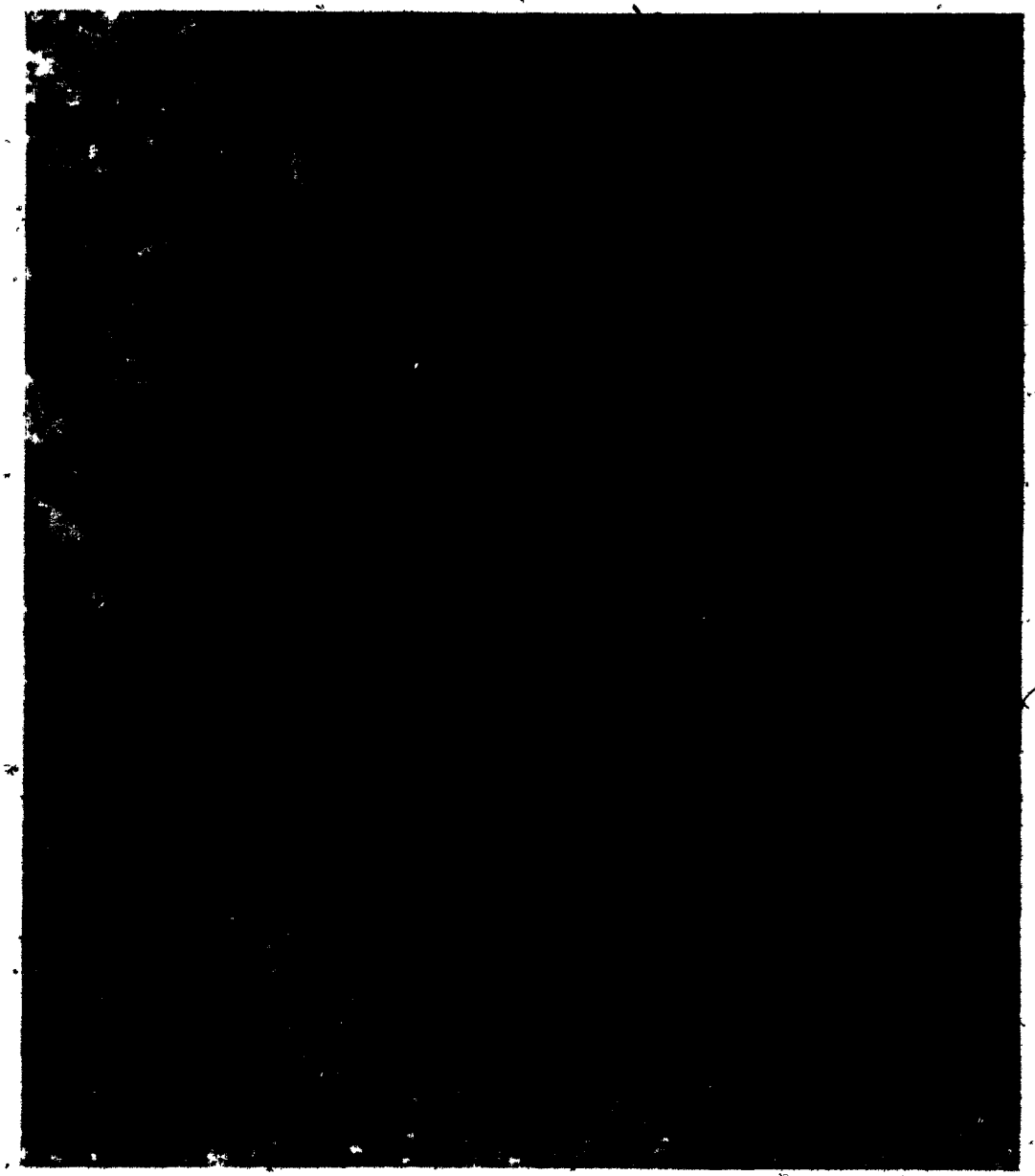


Figure 29. A regressing buccal circular muscle cell: Fibrils are no longer arrayed in regular sarcomeres, and the cytoplasm becomes homogeneous as endoplasmic membranes disappear. Large lipid droplets l_{A-C} appear as well as membranous whorls and dense bodies. $l_{A,B}$ occur in the muscle cell. l_C is in a neuron under the basal lamina.
x 27,400 (scale 1 µm).

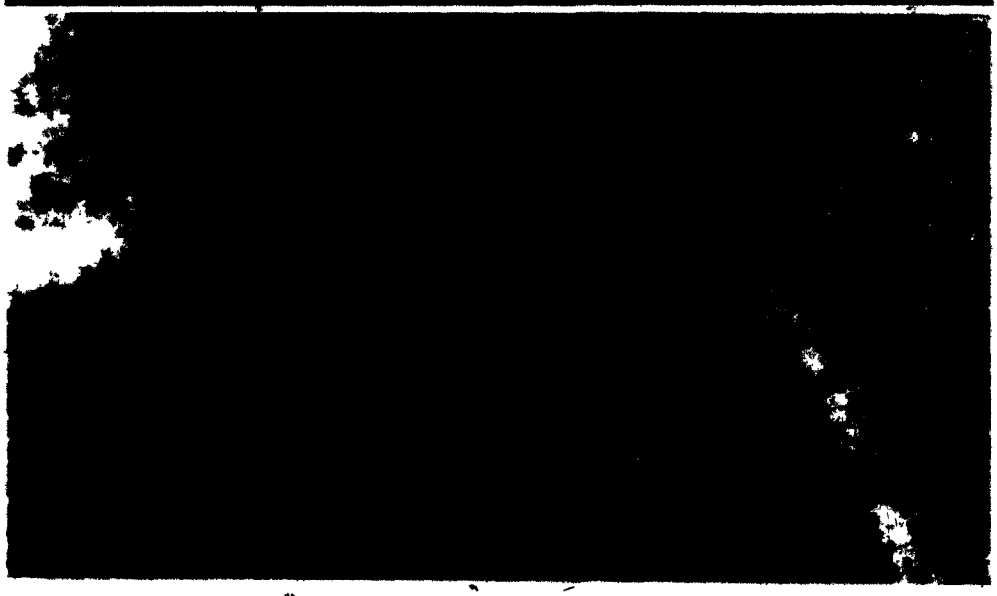
angle of the helices in longitudinal section is 34-36°. The chief features of lophophore regression are summarized in figure 41.

The regressing lophophoral mass is characteristically an orange-red colour which progressively deepens as condensation occurs. The origin of the colour is not clear. While there are a number of pigment droplets not surrounded by membranes it is unlikely that these could account for the strong colouration because they are so small (0.5-1.25 µm diam). Since the isolated coloured cells or cell clusters scattered in the mesenchyme are also red and contain heterophagic vacuoles and chromatin densities from nuclear condensation it seems that these latter structures are a contributing factor. Again, however, it should be pointed out that such colouration is absent from regressing polypides whose colonies lack natural pigmentation to the same extent.

4.42 The regressing gut

Differential rates of regression occur in different parts of the gut. The rectum and pharynx regress concurrently with the lophophore, while the stomach, albeit full of secondary lysosomes and residual bodies, maintains its integrity for a longer period.

Early regression of the pharynx is marked by increasing density of vacuolar sap due to the appearance of small granules and membranous structures (fig 32). There is minor blebbing of the perinuclear space, endoplasmic cisternae become converted to small vacuoles with concomitant liberation of ri-



Figures 30-31. Muscle filaments are transformed into paracrystalline arrays of filaments with a helical disposition, aligned longitudinally in figure 30 (from the regressing lophophore) and transversely in figure 31 (from a parietal muscle cell). Two nuclei in figure 30 appear as a dense aggregation of chromatin and a larger homogeneous structure which were probably derived originally from the same nucleus. Nuclear fragmentation with chromatin condensation (karyorrhexis) is common in necrotic cells.

30. x 33,500 (scale 0.5 μm).

31. x 104,000 (scale 200 nm).

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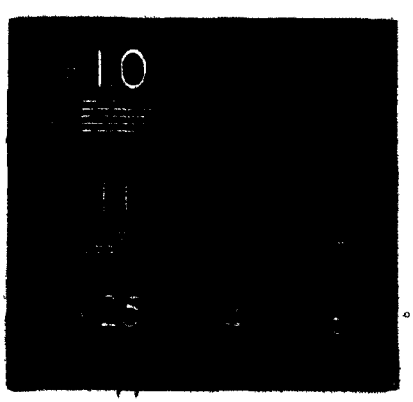


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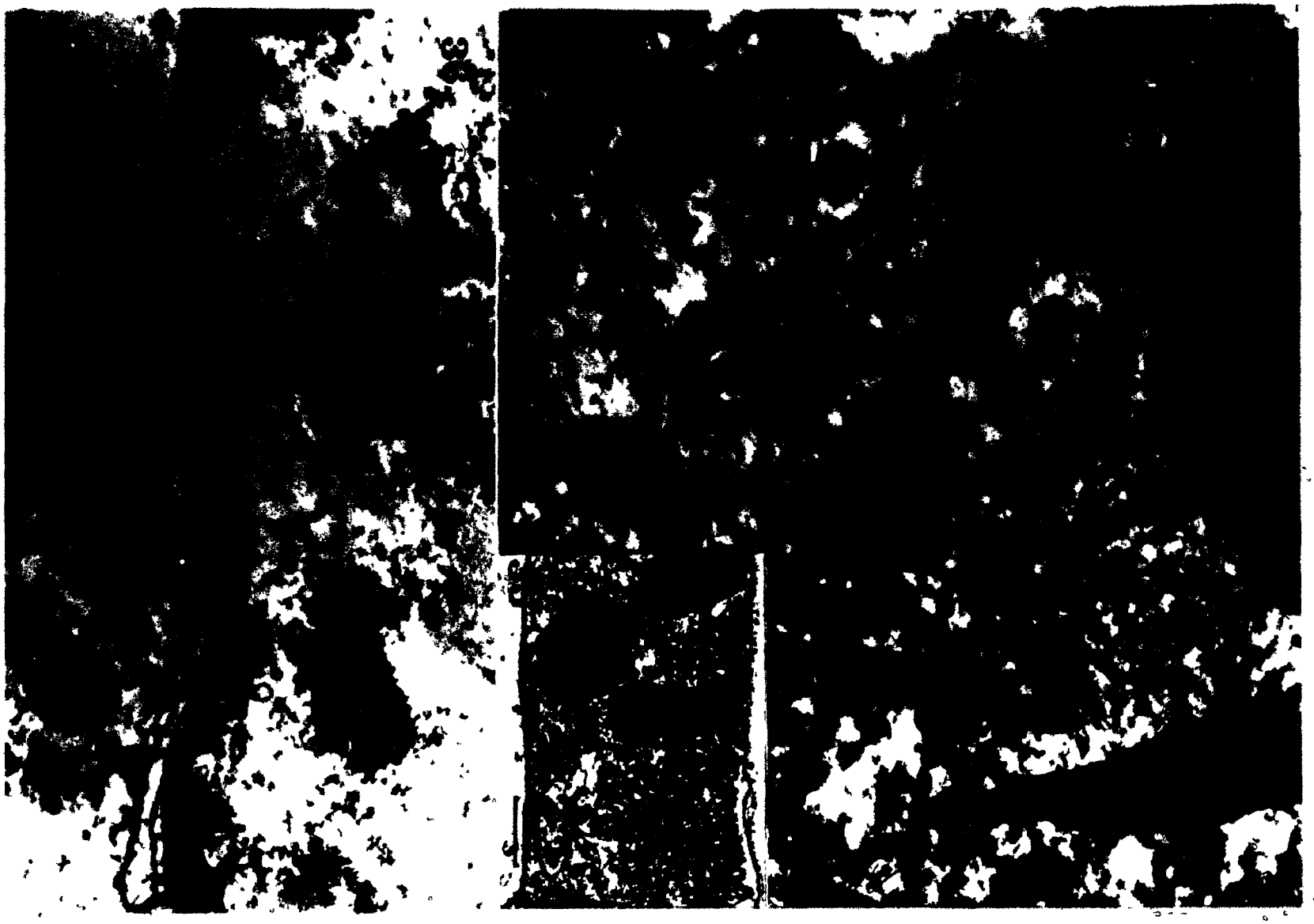
Figures 32-33. Pharynx regression: The fluid in the two vacuoles in figure 32 is quite dense and full of particulate matter. Endoplasmic cisternae are transformed into small vacuoles (v) and there is minor blebbing of the perinuclear space (arrow). Muscle filaments become dissociated.
32. x 29,500 (scale 0.5 um).
33. x 32,400 (scale 0.5 um).

bosomes, and muscle fibrils become disorganized (fig 33).

In the rectum products of absorption that accumulate during the life of the polypide are a prominent feature (figs 34, 35). These comprise vacuoles containing homogeneous contents which may be polysaccharide in nature. Renieri (1970) demonstrated the polysaccharide nature of the matrix between the microvilli of the caecum of Bugula neritina, and in Cryptosula the matrix is not apparently different from caecum to rectum. Other vacuoles (secondary lysosomes) contain membranous lamellae, dense bodies and granular material. Lipid droplets occur but are not abundant. Regular cytoplasmic changes seen in other regressing cells occur in the rectum. Necrosis leads to practically the total loss of all membranes including those around the residual bodies (fig 36). Both the rectum and pharynx become part of the regressing lophophore mass.

The whole stomach from cardiac to pyloric sphincter regresses as a unit. The orange-brown inclusions that have accumulated and enlarged during the feeding life of the polypide come to constitute the major part of the brown body proper and it is these that, through further condensation and digestion, give the dark brown colouration to this body. Even prior to regression myelin figures are commonly encountered at the base of cardiac cells (fig 39). These may give rise to at least some of the residual bodies that resemble lipofuscin seen in early regression near the cell apex (figs 37-38). Lipid droplets are also encountered. Endoplasmic cisternae are converted into small vacuoles and mitochondria lose their



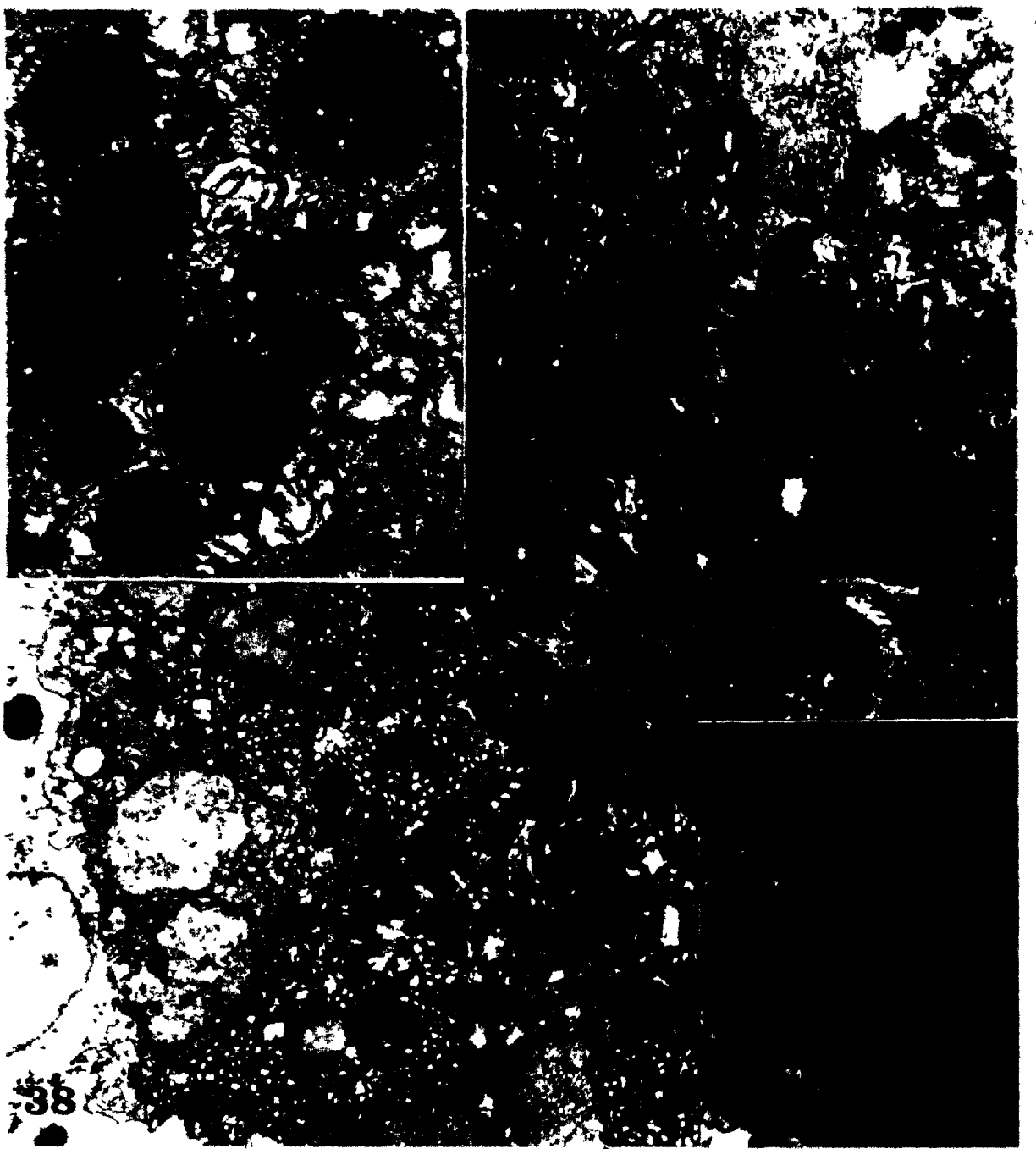
Figures 34-35. Rectal regression: The membrane and particle containing granules that contribute to rectal autofluorescence in feeding animals are very large in regressing cells (fig 34), often occupying a considerable volume of the cell (fig 35) and are probably derived from autophagy, the denser granules (fig 35) being derived from digestion of absorbed material.

34. x 32,000 (scale 0.5 /um).

35. x 6300 (scale 2 /um).

Figure 36. Rectal regression: In late regression the rectum comprises large masses of residual material in a homogeneous matrix, with bands of more regular (though denser) cytoplasm.

x 31,500 (scale 0.5 /um).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Figures 37-39. Cardiac regression: In very old and regressing cells of the cardia lipofuscin-like granules are characteristic (figs 37,38) and lipid droplets (arrows) (fig 38). Lyelin figures (fig 39) are found in feeding animals prior to regression (as shown here) and contribute to formation of denser granules.

37. x 24,900 (scale 0.5 um)

38. x 6990 (scale 2 um)

39. x 39,900 (scale 0.5 um).


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Figure 40: Caecal regression: The wall of the stomach caecum shows large lytic vacuoles, lipid droplets (l) and some autophagic activity (a). The caecum has this appearance while the lophophore has condensed considerably. Nuclei in this micrograph barely show signs of chromatin condensation (arrows). x 8000 (scale 2 µm).

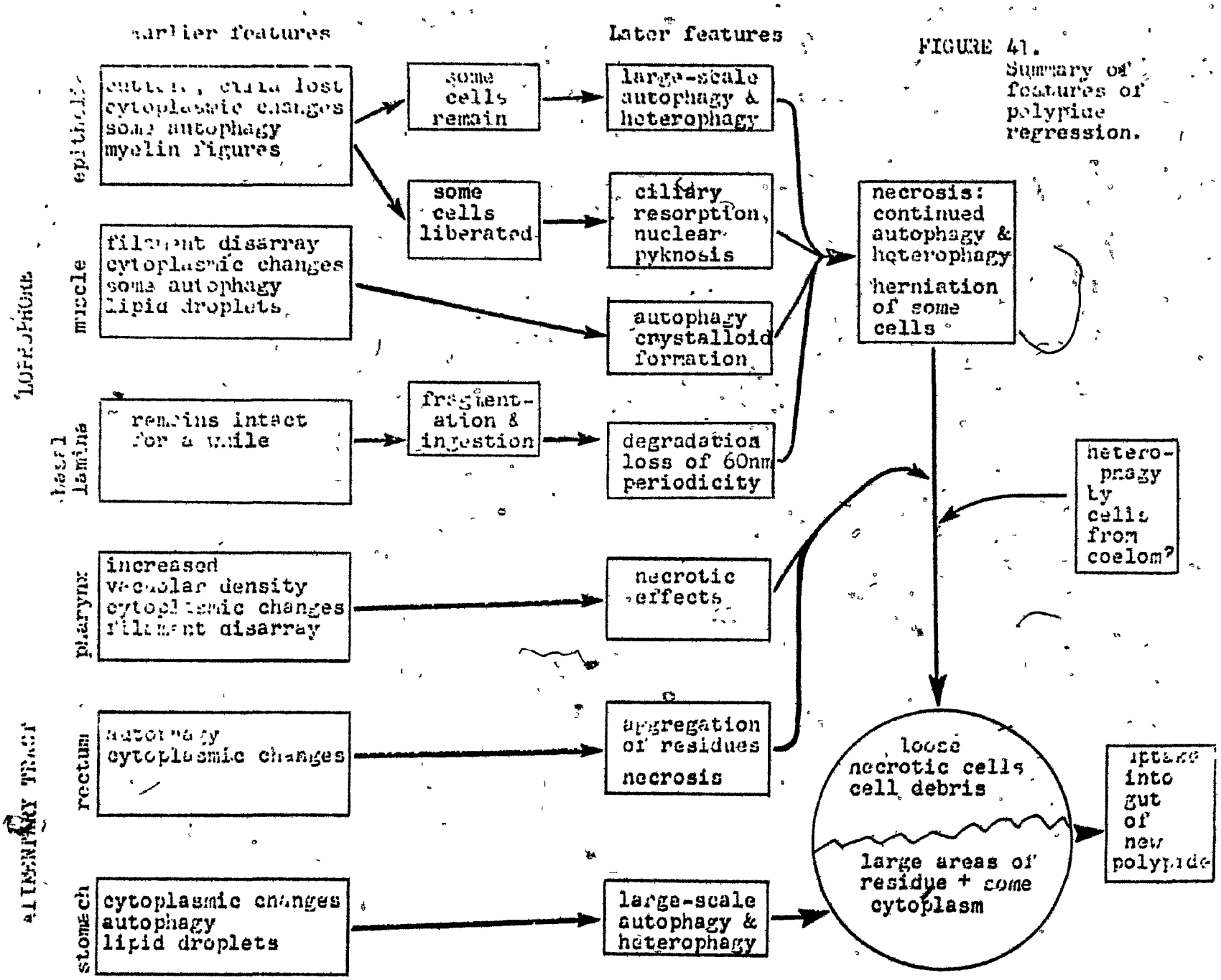

FIGURE 41.
Summary of features of polypide regression.

smooth contours.

Lipid also occurs in regressing caecal cells (fig 40). Here the secondary lysosomes that developed during feeding are huge, up to 8 µm diam and twice the size of the nucleus. It is clear from the large numbers of pyknotic and karyorrhexic nuclei seen in heterophagosomes that condensation of the stomach occurs through heterophagy of some cells by neighbours. Autophagy also takes place. Actively secreting Golgi bodies are seen in regressing cells and these are mostly those that produce both small clear vesicles and macrovesicles containing fibrous elements but whose function is unknown. Mitochondria are irregular or small and rounded with few slender cristae. As noticed in all regressing tissues so far, ribosomes become liberated as endoplasmic membranes are lost. Depending on stomach contents at the time regression was entered into, food fragments like diatom frustules and unidentifiable skeletal fragments may be encountered, being derived from either intracellular material in the caecum or the ergatula in the pylorus. Ciliary shafts from the surfaces of the ciliated cells are incorporated into the stomach cells as in the epithelial cells of the tentacles. The chief features of gut regression are summarized in fig 41.

The muscle cells and peritoneum of the stomach regress but the basal lamina remains intact, not as in the regressing lophophore, and forms a kind of limiting membrane around the developing brown body proper.

During regression glycogen may accumulate, sometimes in massive amounts, in cells of the mesenchyme and in the epithelium under the floor of the compensation sac (fig 42), but its

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Figure 42. Under the floor of the compensation sac during regression large amounts of alpha glycogen rosettes are deposited. (The compensation sac comprises a fibrous cuticle with a cuticulin-like surface (arrow)). x 31,400 (scale 0.5 um).

deposition has not always been encountered in sections of regressing polypides. This may be due to the tearing-off of mesenchyme cells when a polypide is dissected from a zooid prior to embedment. Some mesenchyme cells always adhere but these do not always contain glycogen. Epon sections of undecalcified zooids were stained with Schiff's reagent in order to locate sites of glycogen deposition in situ in the zooid. PAS-positive staining sites are found throughout the mesenchyme, both proximally and distally in the zooid and under the floor of the compensation sac. Glycogen typically occurs in the form of alpha rosettes. While glycogen occurs in mesenchyme cells, these cells may themselves regress. Some lipid can occur with the glycogen.

4.5 The brown body

Continued condensation by autophagy and heterophagy of the regressing polypide results in one or two brown bodies depending on whether the regressed lophophore, pharynx and rectum detach from the regressed stomach. In essence, the brown body represents a huge residual body. Actually, it is made up of large cells, each comprising a thin halo of nucleated cytoplasm surrounding an enormous residual body. The long dimensions of these cells is comparable to that of normal stomach cells (c.15-20 µm) but they are up to three times as wide due to heterophagy of some cells by neighbours, and they sometimes take on a polygonal shape. The cytoplasm comprises a little RER, mitochondria, Golgi bodies of two types and nuclei (figs 43-44). The residual body comprises the remains of

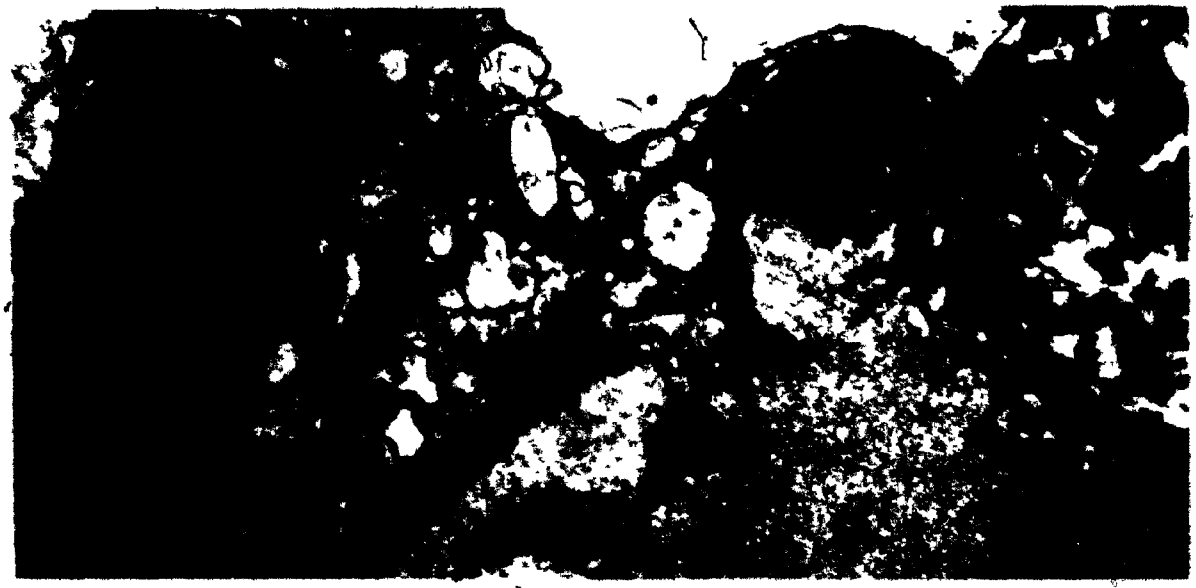
several smaller residual bodies (of membranous elements, opaque granules, myelin figures, and amorphous aggregates), no longer membrane-bounded, in a more or less homogeneous matrix of fine fibrous material.

Even after the formation of a definitive brown body degeneration continues. What little cytoplasm remains disappears entirely, evidently by autophagy. The Golgi body with the fibrous macrovesicles, mitochondria and nuclei persist right to the end. Fusion of cells can result in at least a binucleate condition (fig 44). Eventually all membranes tend to degrade and the matrix and residual material become progressively less structured (fig 46). The part of the brown body derived from the lophophore, pharynx and rectum is in a most degenerate state. Cells with intact membranes contain residual bodies, myelin figures, whorls, lipid and bizarre membranous structures quite unlike any in the remainder of the brown body derived from the stomach.

None of the cells around the fully-formed brown body were ever seen to contain glycogen. Glycogen deposition therefore seems to occur mainly during early regression and then cease, the deposits being utilized for other aspects of metabolism. One possible reason for the disappearance of the glycogen will be discussed later.

4.6 The fate of the brown body

After formation of the brown body one of two things may happen to it in bryozoans in general - it may remain in the zooid or it disappears. In Cryptosula it follows the latter course. Between 4-15 days after regression begins a polypide



Figures 43-44. Brown bodies: Portions of brown bodies with areas of regular cytoplasm (nuclei, RER and Golgi bodies) around large areas of residue and homogeneous matrix. In figure 44 a binucleate cell is shown (arrows indicate two nuclei). The Golgi body (g) is the kind found in the stomach, which produces macrovesicles of fibrous material.

43. x 31,500 (scale 0.5 µm)

44. x 31,900 (scale 0.5 µm).

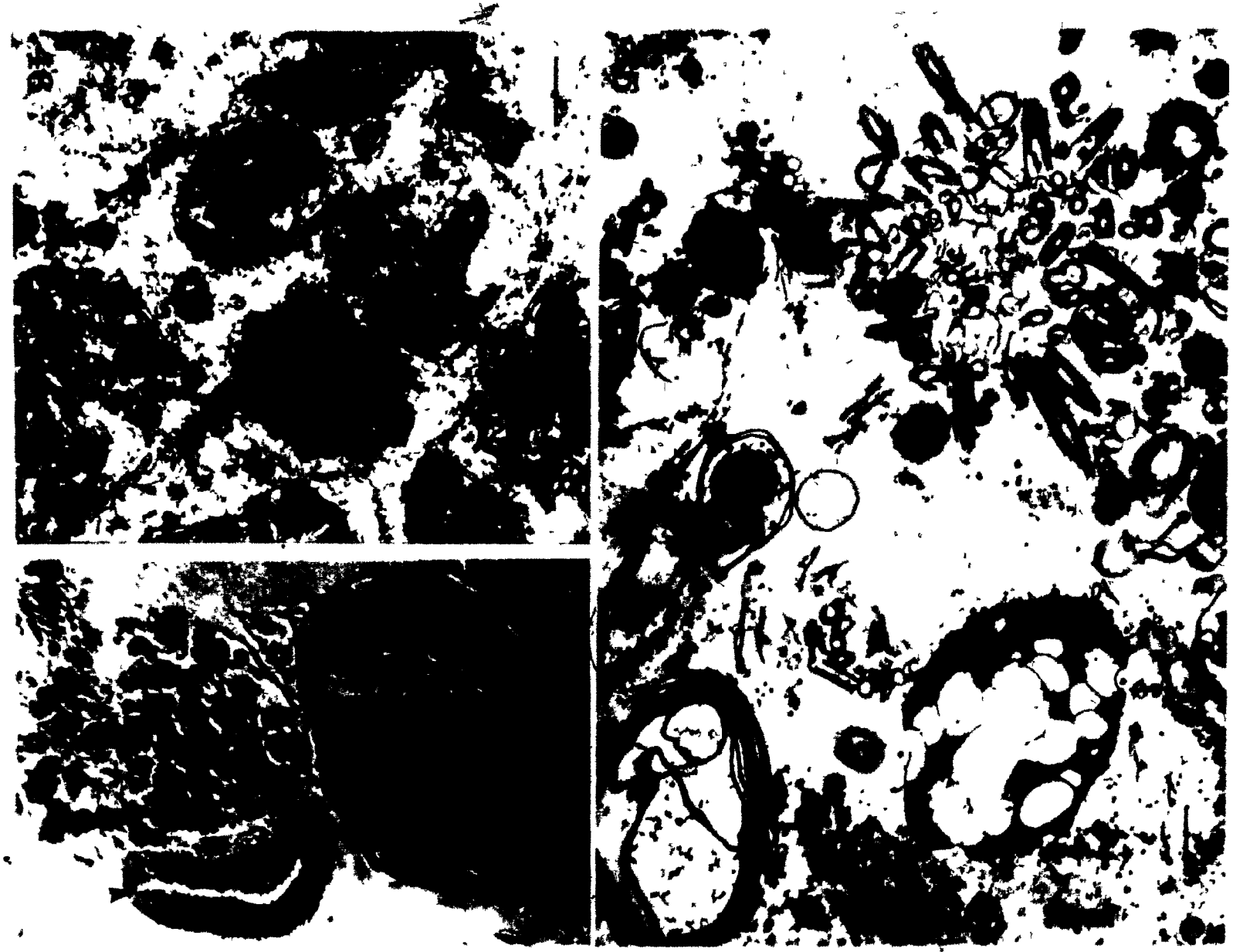


Figure 45. Outside the brown body are seen bizarre formations derived from herniated regressed lophophore and peritoneal cells. x 26,000 (scale 0.5 /um)

Figure 46. Brown body: Residue comprises membrane lamellae, dense granules, amorphous aggregates and a fine fibrous material. x 25,000 (scale 0.5 /um).

Figure 47. Fate of the brown body: The gut anlage (arrow) of a new polypide is seen beginning to grow alongside the brown body. Above the brown body the lophophore is differentiating. (Interference micrograph of a paraffin section). x 520 (scale 20 /um).

primordium is seen developing backwards from the distal body wall. The time of the appearance of the new polypide is related to the rate of regression. If regression is rapid the primordium is seen after a few days. If regression is slow it appears after a week or longer, such that by the time a brown body is fully formed the backwardly growing primordium encounters the brown body and the gut anlage develops (fig 47). The caecum of the new gut caps over the brown body, enveloping it by growing over it, and incorporating it into its lumen. Whether or not the brown body contains digestible material serving as an energy source prior to the polypide's first evagination, the remains of the brown body can be clearly seen in the caecum, pylorus and rectum (fig 6). When the polypide evaginates to feed for the first time the remains of the old polypide are defaecated.

If a new polypide does not grow the brown body remains in the zooid for some months or until all tissues having disintegrated, the operculum falls off, and ciliates, nematodes and other organisms enter the dead zooid (zooecium) and dislodge the brown body.

4.7 Discussion

4.71 Longevity

Data on rates of development, longevity and regression are few. Colony longevity is known to range from a few months in Bugula simplex (Grave, 1930) to twelve years in Flustra foliacea (Stebbing, 1971). Longevity of individual zooids of a colony is difficult to determine however, for the duration of life of a zooid can be known only by observation

Species	Polypide longevity	Duration of regression	Authority
Flustrellidra hispida	21-28[1]	6-7[2]	1 Rey, 1927 2 Joliet, 1877
Cryptosula pallasiana	15-72	6-17	this study
Electra pilosa	6-33	3-8	this study
Eurystomella foraminigera	20-60	5-15	Gordon, 1968
Fenestrulina malusii var. *thyreophora*	35-42	8-10	Gordon, 1968
Bugula flabellata	-	7-9	Joliet, 1877
Bugula neritina	-	2	Harmer, 1891
Carbasea papyrea	-	12	Harmer, 1891

Table 1. Polypide longevity and duration of regression in some bryozoans - time in days.

of individual zooids over periods of months or even years because zooidal longevity is a function of that of its components - the polypide (alimentary tract and lophophore) and cystid (body wall). It is theoretically possible for small coelomic spaces derived from the metacoel or the hypostegal coelom to remain alive, albeit dormant, for long periods of time providing they are in organic continuity with the remainder of a colony; and the body wall remains alive, of course, during polypide regression.

The duration of life (feeding time) of polypides is easy to determine but published data are few (Table 1). The figures in the table are absolute figures which need to be correlated with environmental parameters, but they are indicative of the limits that may be attained by polypides.

4.72 Lophophore regression

In Cryptosula the lophophore (and pharynx and rectum) undergoes regression earlier than the stomach, even though the stomach itself is laden with secondary lysosomes and residual bodies. This appears to be general in bryozoans. In Walkeria cuscuta, Bowerbankia imbricata, Alcyonidium polyoum, Membranipora membranacea and 'Lepralia' granifera the lophophore regresses first (Joliet, 1877). Bobin & Prenant (1957) observed that in Alcyonidium gelatinosum the tentacles sometimes may be ingested by the pharynx in early regression, and Matricon (1960) observed in A. polyoum a similar circumstance, where the distal part of the tentacles are introduced into the regressing rectum, while in A. hirsutum they penetrate as far as the caecum.

It seems to be agreed that the brown body is not derived from the whole polypide. Ehlers (1876) records separation of the tentacles from the gut during histolysis in Alcyonidium; Calvet (1900) indicates regression of the pharynx and lophophore apart from the stomach and rectum. Calvet also observed separate regression of the tentacles and Gerwerzhagen (1913) of the tentacles and foregut. According to Bobin & Prenant (1957) the cardia regresses apart from the rest of the stomach while the rectum may be incorporated into the brown body. Matricon (1960) observed that the sphincters of the stomach delimit those areas that will comprise the brown body proper and those which will not i.e. lophophore, pharynx and rectum regress as a unit in A. polyoum just as they do in Cryptosula. Rey (1927), on the other hand, recorded that degenera-

tion starts in the caecum, spreading to other parts of the gut and then the tentacles, and that the whole polypide excluding the muscles in Flustrellidra hispida contribute to the brown body.

4.73 Muscle regression and phagocytosis

In Alcyonidium polyoum muscle and basal lamina persist for some time while the tentacles are regressing (Matricon, 1960). According to Marcus (1926) occlusor and parietal muscles remain intact in Electra pilosa during regression but are replaced when a new polypide is regenerated, and the lophophore retractors share the fate of the lophophore. In Watersipora cucullata the parietals do not degenerate (Mawatari, 1952). Calvet (1900), Rey (1927) and Matricon (1960) agree that regressed muscle does not become part of the brown body proper, but is digested by cells called sarcolytes. According to Matricon sarcolytes are found only in old zooids, are derived from mesencyme and are responsible for phagocytosing musculature. They in turn may fuse and become necrotic.

It is not at all certain that sarcolytes are involved in Cryptosula. Many of the cells that seem to behave as macrophages during regression appear to be derived from lophophore cells, where, according to varying states of regression of these cells mutual ingestion can take place. It is not unlikely that coelomic cells could be found among the regressing cells but it would be difficult to identify them as such as nobody has characterized them fine-structurally, and they themselves can undergo regression and necrosis (Matricon, 1960; Bobin & Prenant, 1972). The latter authors have described five types of coelomic

cells as seen by light microscopy - vacuolated amoeboid cells, cells with refringent granules, cells with peripheral inclusions, ampoule cells, and stellate clusters of glycogen cells. These types were said not to be common to all bryozoans. Sarcolytes fall in the category of amoeboid cells, and only amoeboid cells are thought to remain after polypide regression. Some of the cells in the regressing mass in Cryptosula that contain muscle remains are not unequivocally derived from tentacle cells and might be sarcolytes.

The fate of parietal muscle was not followed completely in Cryptosula, but micrographs indicate that regression sets in well after condensation of the lophophore. The crystalloidal material found in the parietal muscle cells is of some interest. Identical crystalloids were found in entoproct socket muscle (Barentsia gracilis) (Reger, 1969). In Barentsia the filaments comprising the crystalloids were 3-7 nm diam, with the rows in transverse section set at angles of 58° with respect to each other and with a helical tilt angle of 35-45° (compare 5-9 nm, 59-64° and 34-36° respectively in Cryptosula). Such crystalloids are known elsewhere. Reger cites amphibian oocytes and embryonic cells and human muscle tumour cells. Entoprocts are known to exhibit cyclic degeneration and regeneration of the calyx (Harmer, 1887; Prouho, 1892) and Reger hypothesized that the crystalloids represent a form of storage prior to its deposition into filaments in young or regenerating animals. He did not know the age of his material and his micrographs (figs 6 & 7 in his paper) indicate early degenerative changes. I incline to the view that such crystalloids are

more likely to be a feature of regressing or pathological cells than regenerating ones.

Phagocytosis has been said to play an important role in the dissolution of the lophophore, rectum and pharynx. According to Römer (1906) phagocytosis takes care of those parts not entering into the brown body and Calvet (1900) thought the phagocytes had an excretory function, an idea further supported by Harmer (1931) who observed that certain coelomic cells take up dyes added to seawater. Matricon (1960) states that the tentacle sheath plays a phagocytic role as it contains sarcolytes. Marcus (1926), Rey (1927) and Cori (1941) state that phagocytes laden with residues 'disappear' or 'degenerate'. According to Rey phagocytosis of muscle only, by sarcolytes, occurs. Irrespective of which cells are involved, phagocytosis is important in Cryptosula in the condensation of the lophophore, and indeed, the rest of the polypide, but as previously stated, this might not involve special coelomic cells. Cells containing pigmented residue come to appear throughout the mesenchyme of the zooid but some time after a new polypide has been regenerated they are less apparent.

4.74 Stomach regression and the brown body

As the stomach regresses, it had been noted that histological structure disappears (Calvet, 1900), the lumen becomes a receptacle for cytoplasmic debris, and cytoplasm becomes vacuolated (Matricon, 1960). Some of these features are not seen by electron microscopy. In situ autolysis and heterophagy results in condensation of the stomach and as cell

contacts weaken cells invade the stomach lumen in Cryptosula. Vacuolation of cytoplasm is not marked but there is minor oedema of endoplasmic cisternae in cells which are probably destined to be engulfed by neighbours. Regressing stomach cells do not rupture.

It has long been recognized that the brown body is chiefly composed of the brownish granules that accumulate in the stomach walls (Joliet, 1877; Harmer, 1891; Prouho, 1892; Marcus, 1926; Rey, 1927; Cori, 1941; Soule, 1954; Matricon, 1960). Food residues may also occur in the brown body e.g. foraminiferan, radiolarian and diatom skeletons (Nitsche, 1871; Joliet, 1877; Rey, 1927) as in Cryptosula, and Nitsche (1871) and Rogick (1945) saw gizzard sconces in brown bodies of some stoloniferous ctenostomes.

The membrane around the brown body which was noted by Hincks (1880), Römer (1906) and Gerwerzhagen (1913) is seen in Cryptosula to be the former basal lamina of the stomach. In addition, peritoneum or mesenchyme cells may surround the brown body (Joliet, 1877; Calvet, 1900; Bobin & Prenant, 1957; this study). There may be some variation in the nature of the limiting boundary of a brown body according to whether it is destined to remain in a zooid or be eliminated via the digestive tract of a new polypide. Dr W.C. Banta (American University, Washington D.C.) has sent me photographs of presumed brown bodies from two Schizoporella species which possess a rind-like structure which thickens with age (figs 48, 49). According to Joliet (1877) a brown body membrane becomes chitinized. Since this would necessitate an enveloping secretory

48
49
50

Figures 48-49. Brown body; Epon sections (light microscope) of two apparent brown bodies with thickened 'rinds' from Schizoporella spp. (Photos courtesy of Dr J.C. Banta, American University, Washington, D.C.). approx. x 550 (scale 20 µm).

Figure 50. A brown body or more probably a funicular body from a Magellanic cellularioid. Under the thickened 'membrane' is residual material and presumed prokaryotic cells. x 36,000 (0.5 µm).

epithelium such an observation is doubtful, but it is clear that EM investigation of the thickened rinds that Dr Banta has encountered would be instructive.

It is possible that structures reported to be brown bodies in some species may not be so. My attention having been drawn to the variable nature of brown body 'membranes' by Dr Banta, I prepared for electron microscopy some presumed brown bodies with thick 'membranes' from a Magellanic cellularioid. These bodies were ovoid with a greenish-yellow to brown colour. This material from the Hudson '70 expedition was preserved in 70% ethanol but in spite of this clearly recognizable features are seen in fig 50. The limiting membrane is a finely fibrous cuticle-like structure 0.5-0.8 µm thick, thicker than a basal lamina would be expected to be. Inside is a deposit of organic residue and bacterial cells. The rest of the interior is empty. Grave (1930) described from Woods Hole Bugula simplex apparent brown bodies that had the appearance of an 'oval greenish-yellow or brown cyst', that occurred, one in each zooid, in winter. The presence of only one brown body was surprising (q.v. Harmer, 1931) as Bugula spp were reported to accumulate brown bodies in the zooid after repeated degenerations. Assuming, however, total degeneration of even brown bodies, the structures that Grave saw might be funicular bodies. Lutaud (1969) described in cellularioid bryozoans including Bugula, cyst-like structures that contained apparent bacteria. Such cysts have never been characterized ultrastructurally but I suggest that structures assumed to be brown bodies in some cellularioids may be the remains of funicular bodies.

4.75 Some ultrastructural aspects of regression

Regression of polypide cells is comparable to that of leucocytes described by Bessis (1964). During the phase of death agony polypide cells exhibit incipient vacuolation of the perinuclear space and vesiculation of endoplasmic cisternae. Mitochondrial membranes frequently become irregular. Loose cells develop short, blunt pseudopodia. These features tend to be enhanced during necrosis of dead cells, as well as nuclear pyknosis and karyorrhexis and formation of myelin figures.

Manasek (1969) has reported comparable changes during death and necrosis of myocardial cells, including cells breaking free from neighbours, liberation of ribosomes, occasional occurrence of glycogen, enlargement of perinuclear cisterns and nuclear pyknosis, all of which were encountered in Cryptosula. Some of these features of regression and residual body formation seem to be universal in ageing or pathological tissues and have been reported by numerous authors from a variety of organisms under different circumstances e.g. in regressing Campanularia hydranths (Brock, 1970), cockroach prothoracic gland during metamorphosis (Scharrer, 1966), amphibian intestine during metamorphosis (Hourdry, 1971), anuran exocrine pancreas during hibernation (Geuze, 1970), ageing plant cells (Berjak & Villiers, 1970) and crab androgenous gland (Payen, 1972). Mitochondrial changes in Cryptosula were variable - either mitochondria maintain a 'normal'-looking appearance for some time after regression or show slight roundedness and decreased matrix density. Mitochondrial oedema has been cited

as a feature of cell regression by Bessis (1964), David (1970) and Payen (1972) although Bonneville (1963) reported no marked change during regression of gut cells during bullfrog metamorphosis. Enhancement of oedema can be induced in Cryptosula by imprecise fixation, so one must be careful in interpreting regressing cells. Oedema of endoplasmic cisternae is common in regressing or malfunctioning tissues and has been reported by numerous authors (David, 1970:79) and by Geuze (1970) in involuting anuran pancreatic cells.

The behaviour of tentacle epithelial cells as amoebocytes in Cryptosula during regression is comparable to Campanularia hydranths where epithelio-muscular cells may engulf others (Brock, 1970:fig 14- Brock's interpretation is different, however). Cell destruction in animal tissues is generally self-contained within parent cell membranes or those of neighbouring cells should the former be phagocytosed. This is the case in Cryptosula, particularly in the stomach and, together with the persistence of the basal lamina, accounts for the compactness of the regressing stomach. The lophophore tissues, on the other hand, appear more disorganized and while some of the debris in the center of the regressing mass may be derived from herniated cells, some of this is phagocytosed by others.

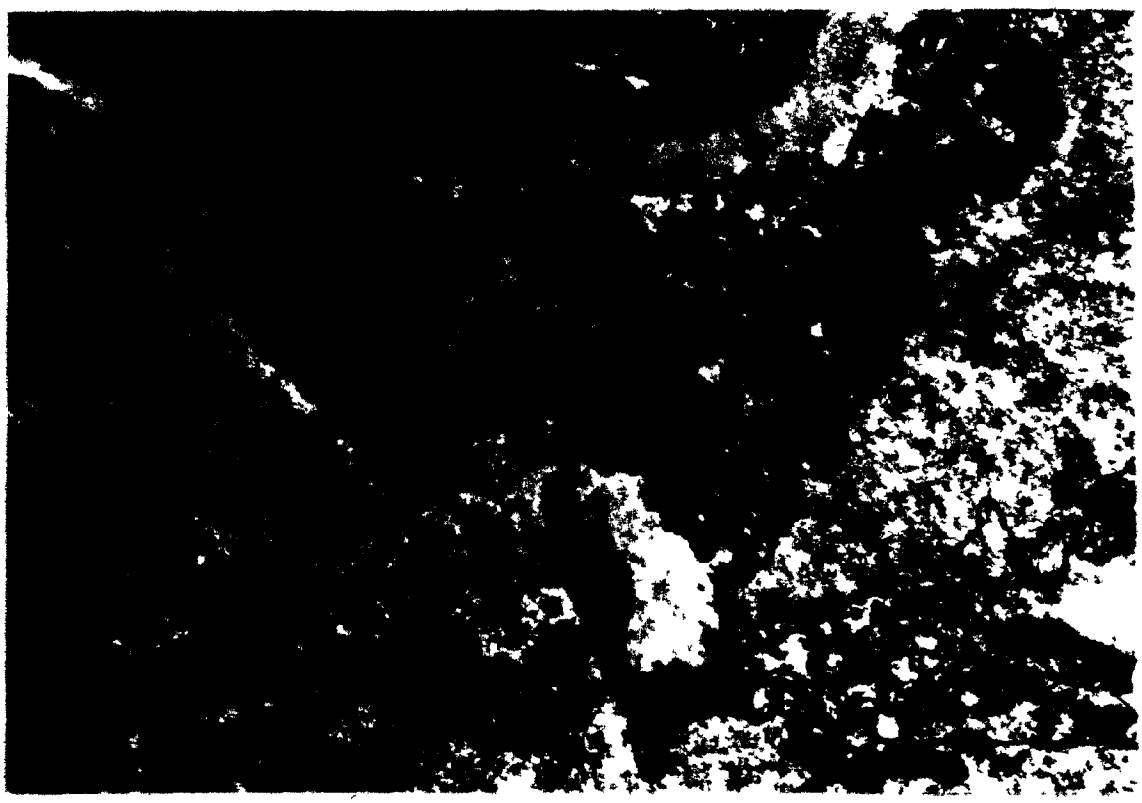
4.76 The fate of the brown body

The historical discoveries of the fate of the brown body have been covered in the introduction to this section. Smitt (1864) first illustrated the uptake of the brown body in Cryptosula into the caecum of a new polypide but he interpreted the brown body as a germ capsule from which the

new polypide arose. Next, Repiachoff (1876) described this process again in Cryptosula, correctly interpreting what he saw. As I mentioned previously, early workers speculated on whether the residue of the old polypide could contribute to the development of the new. In a previous section it was shown that the pigmented inclusions which accumulate in the stomach wall are classifiable as lipofuscin. As these inclusions accumulate in the old polypide their stainability and fluorescence diminish and disappear. Residual bodies are considered to be totally inert (Cohn & Fedorko, 1969) and it is unlikely that they are further digested in the gut of the new polypide. As seen in figure 51, however, there may still be some membranous structures remaining in the brown body at the time of incorporation and it is feasible that these and some of the cells from the regressed lophophore are digestible. Revolution of the brown body in the pylorus fragments the residues allowing greater possibility for absorption of digestible substances.

The new polypide bud in Cryptosula does not become evident until some time during advanced regression, but the primordium of the gut has started proliferating by time regression is more or less complete such that envelopment of the brown body by the developing caecum can take place. Marcus (1926) also observed that the polypide bud appears in Electra pilosa before regression is complete, while Gerwerzhagen (1913) for Carbasea carbasea and Matricon (1960) for Alcyonidium polyoum observed that the new bud is evident from the start of polypide regression.

The interaction between dead and living tissues is not



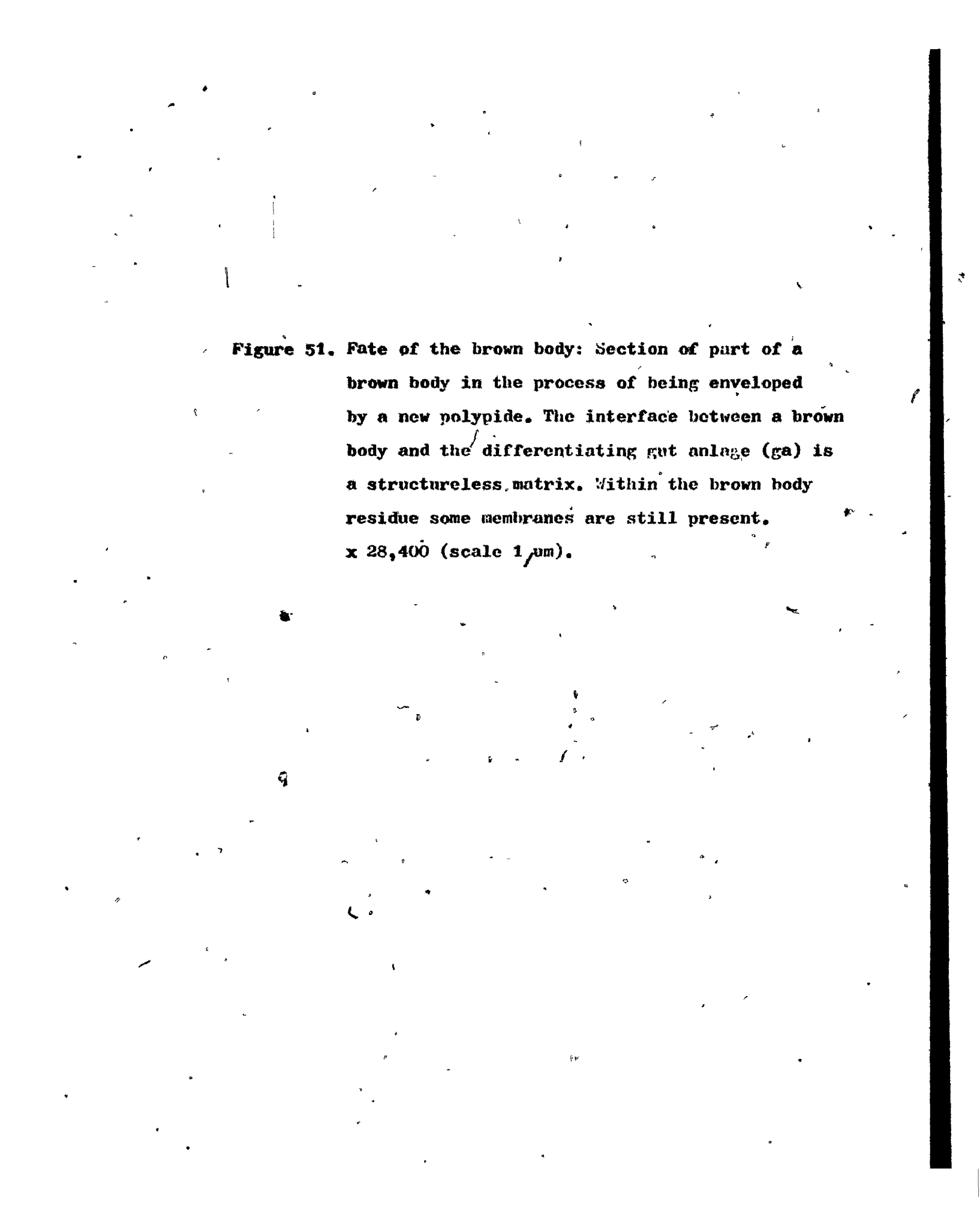


Figure 51. Fate of the brown body: Section of part of a brown body in the process of being enveloped by a new polypide. The interface between a brown body and the differentiating gut anlage (ga) is a structureless matrix. Within the brown body residue some membranes are still present. x 28,400 (scale 1 µm).

clear (Majno, 1964) but in bryozoans it seems that the envelopment of the brown body by the developing caecum is induced by its merely 'being in the way' as it were. Bronstein (1938) determined that it is the absence of a polypide from the body cavity that initiates the formation of a new one, rather than a necrohormone from the residue, suggesting that the polypide exerts an inhibitory effect (hormonal?) on the proliferating tissues of the cystid.

During regression, massive amounts of glycogen may occur in mesenchymal cells, sometimes around the regressing polypide or at distances from it. Glycogen is known to one of the storage products of metabolism in bryozoans (Ries, 1936; Bobin & Prenant, 1972). Bobin & Prenant described anastomosing clusters of 'glycogen cells' which are common at budding sites and can occur during the life of the polypide but are rare or absent in degenerated zooids. In Cryptosula the massive loads of glycogen that may be found during regression do not seem to persist and there was never any associated with the fully-formed brown body. The glycogen deposits should predictably serve as an energy supply for a simultaneously developing polypide.

4.77 Distribution of cyclic regression and regeneration

This section has been concerned with the regression phenomenon as it occurs in bryozoans of the class Gymnolaemata. Regression occurs in the two remaining classes Stenolaemata (Ellis, 1755; Harmer, 1898) and Phylactolaemata (Marcus, 1934) although brown bodies are definitely known to occur only in the former (but see Harmer, 1931:147). Presumed brown bodies have also been reported from fossil stenolaemates of the

order Trepostomata (Boardman, 1971).

Among entoprocts the calyx is periodically shed and renewed and Harmer (1887) saw in this a parallel to regression and regeneration in bryozoans. Prouho (1892), however, argued cogently against this point of view, with the facts that the entoproct calyx is not equivalent to a bryozoan zooid, guts are not replaced, and entoprocts possess protonephridia (if lack of excretory organs is a reason for regression). Furthermore, the entoproct larva does not typically lose its gut during metamorphosis (see also Nielsen, 1971) whereas cyphonautes' gut regresses. The residues of the larval gut in bryozoans is taken into the gut of the ancestrular polypide, foreshadowing later events (see also Repiachoff, 1875). Prouho is correct in being wary of carrying homologies too far. The great similarities in cyclic regression and regeneration that exist in different phyla is almost certainly related to the colonial way of life, if for no other reason. In some bryozoans whole zooids may drop off after polypide regression e.g. Triticella (Sars, 1874) and Mimosella (Banta, 1968). Similarly, Campanularia hydranths are periodically shed and regenerated (Crowell, 1953). 'Brown bodies' have also been described from the hemichordate Rhabdopleura (Stebbing, 1970) in which polypide degeneration occurs in response to embryogenesis and suboptimal water conditions. In some ascidians e.g. Perophora, zooids regress into a stolon from which a new zooid subsequently arises (Barth & Barth, 1966). The only common feature of all these animals is their small, sessile, colonial nature.

4.78 Cause and effect of brown body formation

What induces brown body formation? As seen already in the introduction, it is induced by a range of adverse environmental factors and sometimes embryogenesis, but in the absence of these factors it seems inescapable that the accumulation of residual material in the stomach wall is the prime cause. In a previous section I have related the form and function of the bryozoan caecum to the molluscan digestive gland and arthropod hepatopancreas. In the former, digestive and secretory cells accumulate residual material as in Cryptosula and regress. The important difference, however, is that as digestive gland cells are lost, others replace them. There is no such cell replacement in Cryptosula. Thus in bryozoans, when the stomach cells become so full of residual material they are no longer capable of absorption (e.g. of stains, Harmer, 1931), the whole stomach degenerates. It is interesting that actual regression should start in the lophophore (Marcus, 1934) whose cells are not full of residual bodies.

Beginning with Ostroumoff (1866) brown body formation was thought to be an excretory phenomenon associated with the lack of excretory organs. Marcus (1926) thought the brown body was toxic and comparable to 'uric deposits' in cyclopoids. Cori (1941) attributed degeneration to 'toxic nitrogenous substances' that accumulate in the gut. However, there is not, in Cryptosula any deposition of uric acid or guanine such as is said to occur in the 'hepatic' part of the stomach in entoprocts (Becker, 1938), and as I have shown, the cumulative substances are

residual bodies whose contents (in any tissue) are said to be inert (Cohn & Fedorko, 1969) and therefore non-toxic. Furthermore, as Calvet (1900) observed, since brown bodies are not always eliminated from a zooid, excretion cannot be the prime function of the regression-regeneration cycles.

Harmer's (1891) experiments with various biological stains, which were said to indicate an excretory function of the gut walls, leucocytes and funiculus, involved suspensions of dyes in seawater. Since incorporation of particles or molecules into the stomach cells would involve an endocytotic action similar to normal absorption of food material, it is not necessary to attribute this to excretion. The uptake of dyes by 'leucocytes' and funicular cells could be regarded as 'excretory' perhaps, or a microimmunological response to foreign molecules.

While lack of cell replacement in the stomach may be one of the reasons for the demise of the polypide it is worth considering that we are dealing with a colonial animal. Polypide regression and renewal may be a manifestation of growth in a colonial unit which cannot increase in overall dimensions. Since there is a maximum size imposed on zooids, it would be unfavourable to the life of the colony as a whole if, the stomach having accumulated residual material, the polypide were to die with no subsequent regeneration. This would lead, in the case of incrusting colonies, to a huge central dead area with a continually thinning band of live zooids at the periphery. It is therefore in the interest of the colony to replace polypides after regression.

In this regard, a consideration of regression cycles in other organisms is of some relevance. Crowell (1953), in discussing such cycles in Campanularia hydranths wondered if this was analogous to brown body formation in bryozoans. Campanularia hydranths live for a mean life-span of seven days. Products of regression are removed into the rest of the colony and the hydrotheca drops off. A new hydranth grows from the old pedicel. The feeding members of a colony are always youthful, and this phenomenon was therefore thought to be rejuvenatory. As in bryozoans, regression could be induced by suboptimal physical conditions but in their absence the occurrence of regression is nonetheless fixed. In more recent work on Campanularia Toth (1969) determined that repeated damage to, and regeneration of, the pedicel of isolated hydranths extended their life-span to twenty days, and concluded that tissue damage and reorganization function to maintain the hydranth. Data such as these carried over to Bryozoa would seem to strengthen the idea that repeated regression and regeneration are rejuvenatory for the zooid as a whole, implying that the lifespan of a zooid is likely to be extended further through these cycles, than if stomach cells were replaced and the polypide were merely capable of living longer. The accumulation of residue in the stomach should serve as a trigger for regression in the absence of provocative external stimuli or the onset of embryogenesis. I conclude that brown body formation is not an excretory phenomenon but is rejuvenatory, serving to extend the life-span of a zooid and therefore the colony.

4.8 Summary

The ultrastructural features of regressing polypides and brown bodies in a bryozoan are described for the first time. These features are as follows.

a. Cells of the lophophore move apart from each other and some of the ciliated epithelial cells appear to behave as amoebocytes. Cilia are either sloughed off or completely resorbed.

b. The regressing lophophore condenses by autophagy, heterophagy and concomitant cell movement.

c. Necrosis of lophophore cells is marked by liberation of ribosomes as endoplasmic cisternae vacuolate, nuclei become homogeneous in content or there is clumping of chromatin resulting in pyknosis and karyorrhexis. Some cells herniate.

d. Muscle becomes balled-up and is ingested by macrophages of unknown origin. Within the macrophages muscle filaments are often transformed into paracrystalline arrays such as have been described for entoprocts.

e. The stomach regresses as a unit and remains bounded by the basal lamina while condensing by autophagy and heterophagy.

f. Brown bodies are formed predominantly from the regressed stomach and comprise large cells containing residue in a homogeneous matrix.

g. Glycogen accumulates under the floor of the compensation sac and in the mesenchyme during regression but is absent from around the fully-formed brown body.

h. Reproductive activity has no effect on the state of the polypide in Cryptosula, being in no way a contributing factor to regression.

1. Brown body formation in Cryptosula is not an excretory phenomenon. Its functional significance seems to be that of rejuvenation, serving to extend the lifespan of each zooid and thereby the whole colony.

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