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CONTENTS

THE BODY WALL AND MUSCULATURE OF THE MARINE TRICLAD *PALOMBIELLA STEPHENSONI* (PALOMBI, 1938)

PART 2 : FURTHER MORPHOLOGICAL OBSERVATIONS

By

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ABSTRACT

A cell dissociation technique and observation of 0.25μ and 0.5μ araldite sections are employed in a further morphological study of the body wall and muscles of the marine triclad *Palombiella stephensoni*. Basiphil secretions in the epidermis previously described as products of unicellular epidermal glands are determined to be intraepidermal portions of sub-epidermal basiphil glands. Cavities are present between the bases of the epidermal cells. The "basement membrane" is composed of a thin layer beneath epidermal cells, and a thicker layer of "connective tissue". The muscle cell body and nucleus may lie some distance away from the contractile part of the muscle cell. There is no conclusive evidence for the presence of connective tissue fibres in the parenchyma. Cells which are likely to be the eosinophil gland cells in early secretion stages show a well developed ergastoplasm. Pigment granules occur in pigment cells which ramify between muscle cells.

INTRODUCTION

Palombiella stephensoni (Palombi, 1938) is a marine triclad (Phylum Platyhelminthes, Class Turbellaria, Order Tricladida) of the family Bdellouridae. The morphology of the body wall and musculature of this animal as seen by light microscopy of histological preparations has been described (Wineera, 1969). In the present study the methods of investigation employed allowed a much better characterisation of certain of the body wall constituents. The tissues of flatworms are notoriously difficult subjects for study, and the methods of "conventional" histology, such as the examination of stained paraffin sections frequently give poor results. This is particularly true for the parenchyma. In this tissue nuclei can be seen, but cell boundaries are indistinct or invisible. Extraction of fats during tissue processing, and distortion of tissues causes the appearance of irregular vacuoles which may be mistaken for intercellular spaces (Pedersen, 1961a). The walls of such vacuoles may appear as connective tissue fibres. Moreover, as Pedersen further states, none of the classical

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staining methods gives meaningful histological pictures, and the need for thin sections precludes the use of celloidin or frozen sections. These factors contributed to the parenchyma being called syncytial.

The methods of tissue processing employed for electron microscopy have recently (Pedersen, 1961a; Richardson, Jarett and Finke, 1960) been found to be excellent for the study of thin (0.25 to 3μ) sections by light microscopy. One of these methods is employed in this study, and promises to be an important tool in future cytological and histological work.

MATERIAL AND METHODS

The collection of animals and their maintenance in the laboratory has been described (Wineera, 1969). Worms were fixed in buffered 4% formaldehyde (Pease, 1964, p.52) for 18 hr. Some of these worms were then postfixed in 1% osmium tetroxide in distilled water for 4 hrs. at room temperature. Specimens fixed in these two ways were embedded in araldite according to the method of Richardson, Jarett and Finke, (1960). Propylene oxide (two changes of 15 mins. each) was used between dehydration and resin infiltration stages. Polymerisation was carried out at 60°C for 8-12 hrs. Blocks were cut on a LKB ultramicrotome using a glass knife. Section thickness of approximately 0.25μ and 0.5μ was obtained by advancing the block manually after each cut by means of the "microfeed" control. The sections produced were placed on microslides. Unstained sections were examined by phase contrast microscopy, and sections were subjected to the following staining procedures: (i) Methylene blue-borax/Azure II (Richardson et al, 1960)*; (ii) The Falg technique (Gurr, 1965).

Remarks: Most conventional staining methods give negative results with tissues fixed by osmium, and tissue constituents which are usually distinguished by a characteristic staining reaction may prove difficult to identify. However, the alkaline methylene blue/Azure II staining method is designed for use with osmium fixed material, with which it gives excellent results, even in extremely thin (0.25μ) araldite sections. Results with thin formaldehyde fixed material are poor, but by comparing (i) formalin fixed sections stained with methylene blue/Azure II with those stained by more conventional methods such as the Falg technique and (ii) osmium and formaldehyde fixed sections stained with methylene blue/Azure II it is possible to identify in osmium fixed material the "eosinophil" and "basiphil" substances of conventional staining methods.

Some worms fixed in buffered formaldehyde were embedded in 20% gelatin and sectioned longitudinally at 10μ on a freezing microtome. The sections produced were placed on slides, mounted in glycerogel and were examined by phase contrast microscopy.

For observation of lving cells, a modification of the disassociation procedure of Pedersen (1961a) was used. Worms were placed on cavity slides in a few drops of 0.5% trypsin in sea water. The worms were then cut into small pieces using fine needles and the preparations were covered

*Hereafter the letters "MBAII" will represent the words "Methylene blue-borax/Azure II".

by a petri dish and left at room temperature for 3-4 hrs. After this time the small pieces of worm were placed on microslides in a drop of the trypsin/sea water solution and were gently squashed beneath a coverslip. The preparations were ringed with petroleum jelly and were examined by phase contrast and interference contrast microscopy. This technique resulted in fairly good cellular dissociation and the cells remained alive (as judged by contraction of muscle cells and the beating of cilia on epithelial cells and in flame cells) for 4-5 hrs.

RESULTS

The Epidermis and "Basement Membrane": The epidermal "channels" (Wineera, 1969) are well shown by phase contrast microscopy of frozen sections (Pl. 1 Fig. 1.EC). They are seen to extend, in many cases, to the bases of epidermal cells, and often appear continuous with small "cavities" situated between the bases of epidermal cells (Pl. 1, Fig. 1, arrow). These cavities are more clearly seen in thin (0.5μ) osmium fixed sections stained with MBAII (Pl. 1 Fig. 2, C). The cells through which subepidermal eosinophil glands open to the surface are devoid of cilia (Pl. 1, Fig. 2, A), but this is not the case in the cells through which subepidermal basiphil glands open.

IN MBAII stained sections, the bases of epidermal cells can be traced by a thin dense line (Pl. 1, Fig. 2, arrow). Below this line is material of the "basement membrane". Small vacuoles, or spaces are common within this latter structure, as are sections of the ducts of subepidermal glands.

The Muscles: The longitudinal muscle layers differ in size at the dorsal and ventral surfaces. At the dorsal surface the muscle fibres constitute a layer from 5-7μ thick. At the ventral surface this layer is thicker often approaching 25μ. Both in section (Pl. 2, Fig. 1, C) and as seen by dissociation preparations (Pl. 2, Figs. 2 and 3, C) the cell body lies up to 7μ away from the contracticle part of the muscle cell, and is joined to it by a thin cytoplasmic connection. In osmium fixed MBAII stained sections the muscle cell body colours a purplish blue while the contractile part colours a light blue grey. The nucleus is often irregularly shaped and contains a characteristic pattern of densely staining chromatin. This pattern is made up of relatively large chromatin clumps situated at regular intervals around the periphery of the nucleus, together with chromatin clumps of similar size in the interior of the nucleus. From circular muscle fibres seen in transverse section, and from dorso-ventral muscle fibres which approach the "basement membrane", fine processes (Pl. 1, Fig. 3, arrow; Pl. 1, Fig. 4, arrow), appear to connect with the "basement membrane" and with other muscle fibres. Muscle cells up to 200μ in length have been seen. Fine processes can sometimes be observed along the length of the muscle cells (Pl. 2, Fig. 2, arrows).

The Parenchyma: In the parenchyma the cells fit very closely together. In 0.25μ thick sections many vacuoles of varying size are present. None of these vacuoles can definitely be identified as being extracellular. Also, connective tissue fibres cannot be seen between cells in these thin sections. As well as containing neoblast cells (Pedersen, 1959, 1961a; Wineera, 1969) the parenchyma contains largish cells 10-12μ in diameter. In osmium fixed sections stained with MBAII these cells have cytoplasm

which is partly or wholly composed of moderately dense cytoplasmic strands. The strands are parallel to each other, and are arranged in curvilinear patterns (Pl. 3, Fig. 1, arrows). Other cells of similar size and staining reaction contain numerous vacuoles or pale granules (Pl. 3, Fig. 1, G) and yet others are seen in which both vacuoles and stranded cytoplasm are present. Pale staining large nuclei are present in the parenchyma but it is not possible to identify the limits of the cells to which they belong. Neoblast cells are visible in dissociation preparations, but fixed parenchyma cells (Pedersen, 1961a) have not been positively identified.

The Subepidermal Glands: Basiphil subepidermal glands are easily seen in osmium fixed MBAII stained sections. The cytoplasm is dark blue to purplish blue in colour. These glands open to the surface through the epithelial cells. In the cephalic and caudal regions of the animal the basiphil secretion granules often occur as aggregates in sac like structures within the epidermis.

Eosinophil subepidermal glands are either not stained by the above technique, or are stained a very pale greyish colour. Sometimes they colour with a faint yellowish tinge. In 0.5μ sections their secretion is seen to be made of discrete closely packed granules approximately 1.0μ in diameter. In the epidermis the granules colour a little more deeply, but do not approach the staining intensity of basiphil glands.

Pigment: In all dissociation preparations pigment granules are located in large rounded cells (Pl. 2, Figs. 4A & B,P). They may be few or many in number and may be scattered irregularly throughout the cytoplasm of these cells but often occur in groups several of which may be present in any one pigment cell. In incompletely dissociated cell masses from the dorsal region of the animal, pigment granules occur densely between muscle cells (Pl. 2, Fig 5). In these conditions pigment cell boundaries can not be discerned, but groups of pigment granules may be seen (Pl. 2, Fig 5, arrow).

DISCUSSION

The Epidermis: In a previous paper (Wineera, 1969) it was stated that unicellular basiphil glands occur scattered throughout the epidermis. From the results of the present study it appears, however, that the basiphil secretions which were described in the epidermis are in reality the thin gland cell "necks" described by other workers (Hyman, 1959; Pedersen, 1963 as belonging to the subepidermal basiphil glands.

The epidermal "channels", and the cavities, or spaces between the bases of epidermal cells are interesting features. However, the relationship between these structures cannot be decided at present. Török & Röhlich (1959) state that planarian epidermal cells are attached to each other only superficially, and are surrounded by intercellular spaces of varying size, and Skaer (1961) and Pedersen (1961a) have described fluid filled cavities between the "basement membrane" and the bases of epidermal cells in fresh water triclads. Skaer states that the cavities appear to form a continuous system ramifying above the basement membrane, and suggests that their function may be hydrostatic or associated with the extrusion of granular secretion products through the epidermal cells.

The present study supports the view of Wineera (1969) that the "basement membrane" as described in triclads is not a true basement membrane. That is, it is not a basal lamina (Fawcett, 1966). Plate 1, Figures 2-4 show two components in the "basement membrane": One is the thin dense line immediately beneath the epidermis, and the other is the thicker, less dense layer beneath the thinner one. It seems probable that the former represents the true basement membrane (basal lamina) of the epidermis or at least approaches it more closely than any other structures visible with the light microscope, while the thicker layer is the "connective tissue layer" or "connective layer" as previously described (Wineera, 1969).

The Muscles and Parenchyma: The muscle fibres of *P. stephensoni* appear to resemble closely those described by Skaer (1961) for a fresh water triclad. In both instances the fibres give off fine processes along their length, and the nuclei may be situated some distance from the contractile part of the cell. According to Skaer the fine processes consist of aggregates of mitochondria bounded by the plasma membrane of the cells. It is probable that these processes account for some of the fibres described as connective tissue fibres (Wineera, 1969).

Three recent studies on turbellarians give slightly different pictures regarding the parenchyma and the occurrence within this tissue of connective tissue fibres. Two of these studies were carried out on triclads: In one (Skaer, 1961) no mention is made of the occurrence of fibres in the parenchyma; in the other (Pedersen, 1961a) it is stated that the cells of the parenchyma are closely packed together leaving a gap only a few hundred angstroms wide between two opposing cell membranes. Occasionally this gap is widened, especially around muscle cells, and filamentous material is often found in this narrow intercellular space. The third paper is a study of a marine polyclad turbellarian (MacRae, 1965), in which is shown the occurrence between muscle fibres and around muscle fibre bundles of extracellular fibrillae, presumed by MacRae to be an early form of collagen. Measurements of the regions of these fibrillae from MacRae's electron micrographs show them to be from 800-2,800 A in diameter. These larger regions possibly would be visible with the light microscope as thin fibres. It is possible that the connective tissue fibres associated with muscles as described by Wineera (1969) may be regions of extracellular fibrillae similar to those described by MacRae. Pedersen (1961a, p.597) suggests that the filaments (extracellular fibrillae of MacRae) occurring between muscle cells might be identical to those occurring in the basement membrane (Pedersen's "basement membrane" is equivalent to the basement membrane plus the connective layer of the present study) and says that " . . . there often seems to be a continuity between the subepidermal basement membrane and the intercellular space around the outer circular muscle cells." This same feature is recorded by Wineera (1969), and is seen in the present study. However, the present study has shown that the terminology used by Pedersen to describe this feature should be modified: It is not the basement membrane but the connective layer which projects between the muscles (Pl. 1, Fig. 4, arrow).

None of these papers mentions the origin or insertion of these muscle fibres or fibre bundles. It is axiomatic that muscle fibres or fibre bundles have to be attached in at least two places some distance apart in order to effect movement of body parts. In higher animals it is connective tissue which provides anchorage. MacRae (1965) suggests that a parallel exists between the filamentous material surrounding muscle fibres in the planarians and the endomysium round the muscle fibres of higher animals; and the material round planarian muscle fibre bundles and perimysium in higher animals. In the present study fine processes arising from muscle fibres are seen to join the connective layer (i.e. the basement membrane of other workers) (Pl. 1, Figs. 3, 4, arrows). It is suggested that these processes are analagous to tendons and similar structures of higher animals serving as attachments to the "connective tissue skeleton" supplied by the thick connective layer ("basement membrane").

The cells in the parenchyma which show curvilinear patterns in their cytoplasm are particularly interesting. At first sight these cells bring to mind the picture of a cell with a highly developed granular endoplasmic reticulum in which the cisternae and their attached ribosomes are arranged in parallel arrays. This specific arrangement of granular reticulum is characteristic of cells elaborating protein rich secretions for export from the cell (Mercer, 1961; Fawcett, 1966). Garnier (1897, 1899; quoted in Haguenau, 1958) noted the occurrence of basiphil "filaments" or "rods" in the basal cytoplasm of sumaxillary salivary gland cells. For these structures he coined the term "ergastoplasm" (Greek "ergazomai" —to elaborate and transform) for he believed in their importance in the secretory activity of the cells. The electron microscope reveals that "ergastoplasm" consists of the particular arrangement of cisternae and ribsomes described above. Bloom and Fawcett (1966, p.10) show an electron micrograph of pancreas at a magnification of 2,700 x. The appearance in this micrograph of the granular reticulum is very similar to that of the strands of basiphil cytoplasm of these parenchyma cells of *P. stephensoni*. Moreover, measurements of the cisternae plus attached ribsomes from Bloom & Fawcett's micrograph show them to be in excess of 0.2μ wide. This would make them visible (theoretically) in the light microscope, providing the sections were thin enough and the ribsomes were present in sufficient quantity to confer the needed basiphilia on the cytoplasm around them. It is seen from the micrograph of Bloom & Fawcett that at this magnification the lumen of each cistern is obliterated, so that each cistern plus its attached ribsomes appears as a single dense line. It is possible, then, that the "strands" seen in these cells in *P. stephensoni* may be dense lines representing cisternae plus ribsomes of a very well developed and regularly arranged granular endoplasmic reticulum. However, for the present the term "ergastoplasm" must be used to describe these structures since this term refers to cytoplasmic features visible with the light microscope. Pedersen (1963) shows that a highly developed granular reticulum occurs in subepidermal eosinophil gland cells in fresh water triclads in the early stages of the secretion cycle. Measurements from an electron micrograph published by Pedersen of such a cell show that the cisternae plus ribsomes of the reticulum range from 0.1 to 0.35μ wide. It is, then, possible that in the present study the cells in which the ergastoplasm occurs are eosinophil gland cells at an early

stage of secretion. The cells of similar size and staining reaction which contain pale granules would then possibly be eosinophil gland cells at a later stage of the secretion cycle containing partly formed secretion granules. It is noticeable that, as has already been mentioned, the eosinophil secretion granules stain very faintly or not at all with MBAII.

Two other possible explanations of these cells must be noted. The first explanation is that the strands may represent an extremely well developed Golgi apparatus. However, the extent of the strands in relation to the size of the cells tends to negate this suggestion; also it is not understood what would bind the stain if these strands were part of a Golgi apparatus. A second "explanation" is that the "strands" are manifestations of some other cellular constituent, such as microtubules, or are arrangements of particular substances. This is a question which probably could be solved with the electron microscope.

Pigment: In a previous study (Wineera, 1969) it was concluded that the pigment granules of *P. stephensoni* were distributed at the sites of connective tissue fibres particularly where these fibres course between the muscle fibres of the dorsal body wall. This view can no longer be held.

It is now clear from this present study that pigment is present in distinct pigment cells which are located in the parenchyma. Pigment cells "in vitro" are rounded in shape (Pl. 2, Fig. 4A, 4B). However, the shape of the cells "in vivo" probably is more stellate, and they probably ramify between muscle cells in order to appear as they do in sections. Support for this view is given by Needham (1965, p.210) who states that pigment in *Polycelis nigra* (a fresh water triclad) occurs in "fairly orthodox asteroid cells". However, Skaer (1961) states that *Polycelis* pigment occurs within muscle cells and nerve cells.

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LEGEND TO THE PLATES

Plate 1—Fig. 1: Sagittal frozen section, dorsal body wall. Phase contrast photomicrograph x 700.
B, "basement membrane"; EC, epidermal channels; P, pigment; arrow, one of the "cavities".

Fig. 2: Sagittal 0.5μ section, dorsal body wall. MBAII stain, phase contrast photomicrograph x 1700.
A, adhesive gland secretion in an epidermal cell; B, "basment membrane); C, "cavities"; CM, circular muscles; E, epidermis; arrow, thin dense line at base of epidermis.

Figs. 3 & 4: Saggital 0.5μ sections, dorsal body wall. MBAII stain, phase contrast photomicrographs x 1700.
B, "basement membrane"; C, circular muscles; D, dorsoventral muscles; E, epidermis; L, longitudinal muscles; arrows, connections between "basement membrane" and muscles.

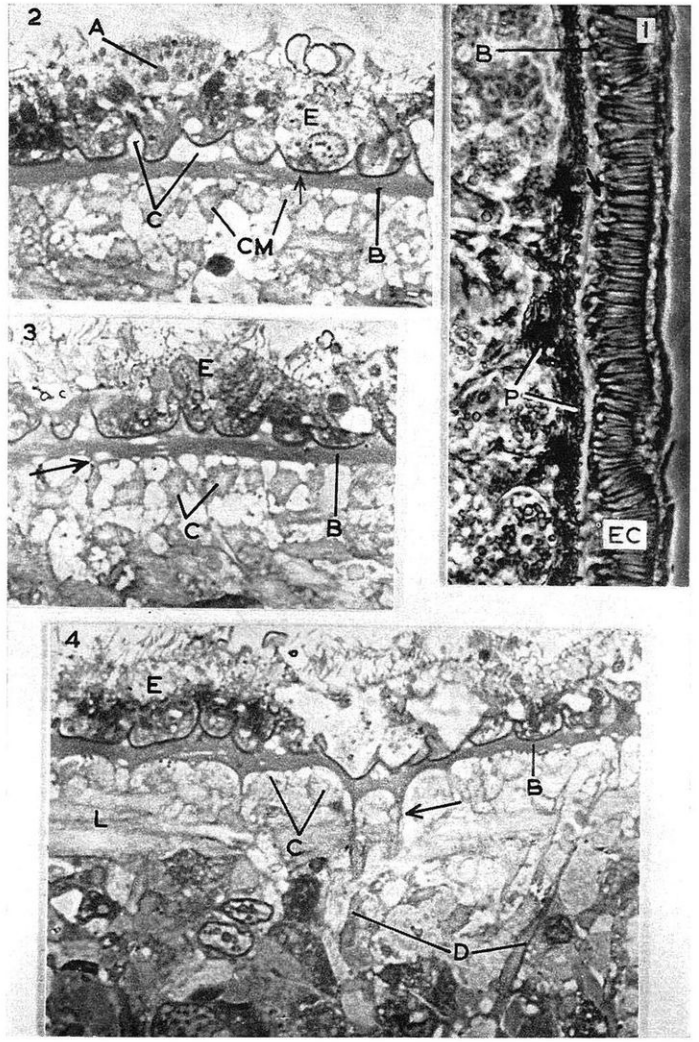


Plate 2—Fig. 1: Longitudinal section of muscle cell. MBAII stain, retouched phase contrast photomicrograph x 2,000.
C, cell body; F, contractile fibre; N, nucleus; P, parenchyma.

Figs. 2 & 3: Dissociated muscle cells. Phase contrast phtomicrographs. Fig. 2 x 1,200, Fig 3 x 1,700.
C, cell body plus nucleus; F, contractile fibre; arrows, fine processes.

Fig. 4: Dissociated pigment cells. Fig. 4A photographed by bright field microscopy; Fig. 4B photographed by Nomarski interference mciroscopy. Both x 1,200.
P, pigment cell; PC, pigment granule clusters.

Fig. 5: Incomplete cell dissociation showing occurrence of pigment granules and clusters of granules (black, some arrowed), between muscle cells. Nomarski interference photomicrograph x 1,200.
M, muscle cells.

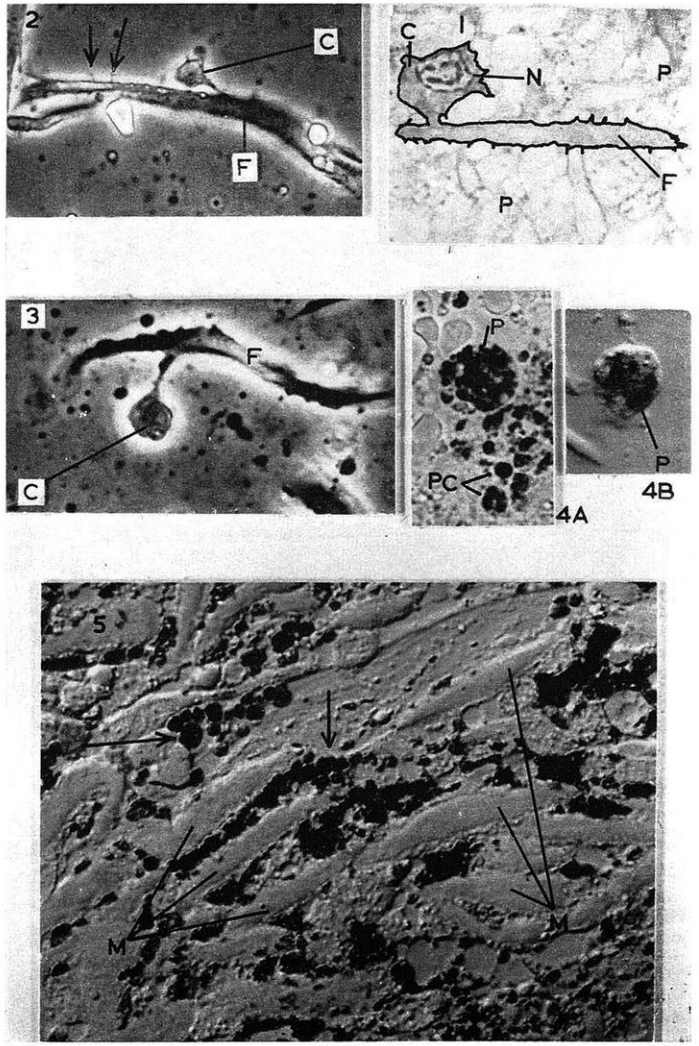
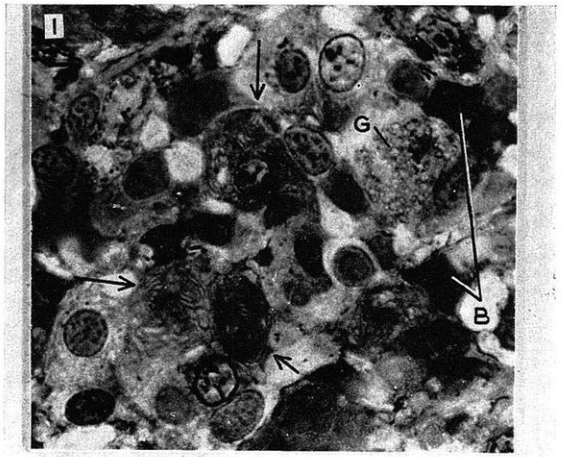
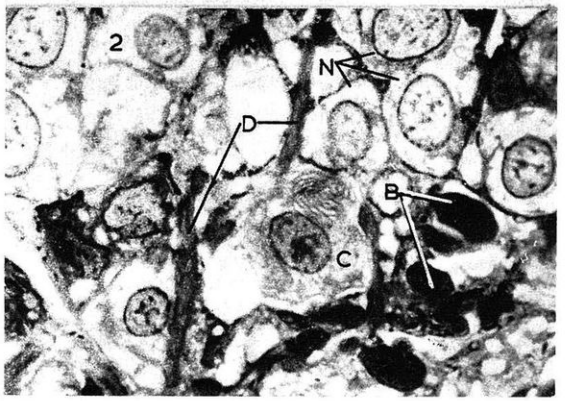


Plate 3—Fig. 1: Sagittal 0.5μ section caudal parenchyma. MBAII stain, phase contrast photomicrograph x 1,750. Cells present include those with stranded cytoplasm (arrows) and pale granules (G).
B, basiphil secretion.

Fig. 2: Sagittal 0.5μ section cephalic parenchyma, at base of brain. MBAII stain, phase contrast photomicrograph x 2,200.
B, basiphil secretion; C, cell with stranded cytoplasm; D, dorso-ventral muscle fibres; N, nerve cells.





THE BODY WALL AND MUSCULATURE OF THE MARINE TRICLAD *PALOMBIELLA STEPHENSONI* (PALOMBI, 1938)

PART 3 : HISTOCHEMICAL OBSERVATIONS

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ABSTRACT

The histochemistry of the body wall and musculature of the marine triclad *Palombiella stephensoni* (Palombi, 1938) is investigated. The secretions of the subepidermal basiphil glands contain neutral mucopolysaccharide, and in addition the intraepidermal portion of these glands contains protein. It is suggested that this protein is a product of the epidermal cells. The subepidermal eosinophil glands contain protein. There are two types present; one is associated with the reproductive apertures, and the other is the adhesive type which assists in adhering the animal to the substrate and has been previously described. The subepidermal pigment may be a melanin.

INTRODUCTION

Palombiella stephensoni (Palombi, 1938) is a marine triclad (Phylum Platyhelminthes, Class Turbellaria, Order Tricladida) of the family Bdellouridae. The morphology of the body wall and musculature of this animal as seen by light mocroscopy of histological preparations has been described (Wineera, 1969; 1971). In the present study histochemical methods are employed in an effort to characterize further the various body wall constituents described earlier.

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MATERIALS AND METHODS

The preparation of material for paraffin embedding (collection of animals and their maintenance in the laboratory; anaesthetization; dehydration and embedding) has been described elsewhere (Wineera, 1969). For paraffin embedding in the present study animals were fixed in Lillie's alcohol-acetic acid-formalin (Pearse, 1960, p.788), and in buffered 4% formaldehyde (Pease, 1964, p.52) for 18 hrs. Sagittal, transverse and frontal sections were cut at 5μ on a rotary microtome. For the study of lipids, some worms were fixed in Bakers formol-calcium (Pearse, 1960, p.787), embedded in 20% gelatin and cut at 8μ and 5μ on a freezing microtome. The following histochemical tests were applied:

Tests for Protein:

1. The mercury/bromphenol blue test (Pearse, 1960). Staining times of ½ hr. and 2 hrs. were employed.

2. The Ninhydrin/Schiff test for protein bound NH_2 (Pearse, 1960).

3. The Sakaguchi reaction for arginine; Baker's 1947 modification (Pearse, 1960).

4. The Millon reaction for tyrosine (Casselman, 1959). The Millon reaction as given by Pearse (1960, p.791) differs from that given by Casselman, although both authors claim it to be the Baker modification of this test. Both authors give the same instructions for the preparation of 200 ml. mercuric sulphate reagent. Pearse then instructs that 0.5 ml. 0.25% sodium nitrate is to be added to the mercuric sulphate reagent, while Casselman says to add 0.5 ml. 0.25% sodium nitrite to 5 ml. of the mercuric sulphate reagent for use. In the present study a positive reaction for tyrosine in tissue sections was obtained with Casselman's method but not with Pearse's.

5. The DMAB-nitrate reaction for tryptophan (Adams, 1957, cited in Pearse, 1960).

Tests for Lipid:

1. Staining with Sudan black B. The stain was used as a saturated solution in 60% isopropyl alcohol (Casselman, 1959). The position of Sudan staining tissue components in some sections were recorded by means of a microscope stage micrometer and the sections were then decoloured by soaking (1 hr. to 16 hrs.) in 60% isopropyl alcohol. They were then restained with Sudan black to test whether tissue components which took the stain initially exhibited "true sudanophilia" (Casselman, 1959, p.74).

2. Some section were extracted with pyridine at 60°C for 24 hrs. and then stained in Sudan black.

3. Unstained frozen sections were examined by phase contrast microscopy.

Tests for Carbohydrates:

1. The periodic acid-Schiff reaction (Casselman, 1959). Oxidation was for 9 min. at room temperature in 0.5% periodic acid. The Schiff's reagent used was a variant of Lillie's, cited in Casselman (p.36). Some sections were treated with malt diastase (1% in distilled water at 37°C for ½ hr.) prior to the PAS test, and others were treated with Schiff's reagent without periodic acid oxidation. Nuclear counterstains used were Delafield's haematoxylin, and 0.1% Azure A in 30% ethanol.

2. Metachromasia. Sections were stained for 5 min. in 0.01% Azure A in 30% ethanol (Kramer and Windrum, 1953) or in 0.1% toluidine blue in 30% ethanol for 1 min. All sections were examined in distilled water before being dehydrated, cleared and mounted in DPX.

3. Sulphation/metachromasia. The low temperature sulphation of Moore & Schoenberg (1957) was employed. Sections were then stained and examined as in 2 above.

4. Mowry's colloidal iron method for acid mucopolysaccharides (Mowry, 1958).

5. Alcian blue staining for acid mucopolysaccharides (Wagner and Shapiro, 1957).

6. A combination of sulphation/Mowry's colloidal iron technique.

7. Sulphation followed by Alcian blue staining.

Tests for Nucleic Acids:

1. The Feulgen reaction (Pearse, 1960) for deoxyribosenucleic acid. Hydrolysis was for 10 minutes in N.HCl at 60°C; the "Schiff" reagent used was Azure A-Schiff prepared according to Himes and Moriber (1956).

2. The methyl green/pyronin Y method of Kurnick (cited in Pearse, 1960) for deoxyribosenucleic acid and ribosenucleic acid. The proportions of methyl green to pyronin Y, and the staining time were modified. Serial sections were treated with RNase (Sigma(R)), 1 mg./ml. in glass distilled water for 1½ hrs. and 3hrs. at 37°C prior to staining. Controls were placed in distilled water at 37°C for corresponding times.

Combination Methods:

1. The Allochrome procedure (Lillie, 1951). Although not strictly a histochemical technique, this test successfully distinguished between certain tissue constituents. The picromethyl blue solution employed was 40 mg. methyl blue per 100 ml. saturated aqueous picric acid. The staining time in this solution was 6 min.

2. Feulgen-PAS technique for demonstration of DNA and polysaccharide in the same section.

3. The Himes & Moriber (1956) method for DNA, protein and polysaccharide. This method is a Feulgen-PAS technique followed by staining in naphthol yellow S, a dye which Deitch (1955) has shown is specific for basic protein within a wide range of dye concentrations and staining times.

4. Sudan black B/PAS. Sections were stained in Sudan black as described above (Tests for Lipid, 1). The positions of sudanophil structures were recorded using a microscope stage micrometer, and the sections were then decoloured. The PAS test was then applied, and the positions

of the previous sudanophilia were examined. Some sections were placed in Schiff's reagent without periodic acid oxidation, as controls.

5. Sudan black B/Naphthol yellow S. As for 4 above, but the PAS routine was replaced by staining in naphthol yellow S, 0.02% in 1% acetic acid for 5 min.

6. Mowry colloidal iron method/PAS, for demonstration of acidic and neutral polysaccharides in the same section (Mowry, 1958).

Additional Methods:

1. Bleaching of pigment. Sections were placed in 10% hydrogen peroxide, and in peracetic acid (Pearse, 1960, p.860) until the pigment was bleached.

2. Whole worms were placed in dilute (5%) hydrochloric acid in an attempt to extract pigment.

3. The ultraviolet/fluorescence method of MacRae (1961) for demonstrating the presence of porphyrins in planarians.

4. Falg technique (Gurr, 1965).

RESULTS

Tables 1 and 2 summarise the results. Additional features are given below.

TABLE 1 - Reactions for Carbohydrate

Tissue Constituent \ Method	PAS	Metachromasia	Sulphation/ Metachromasia	Colloidal Iron	Alcian blue	Sulphation/Colloidal Iron
Epidermis	+ to ++		-	-	-	
Basement membrane	- to +	-	-	- to +	-	-
Muscles	-	-	-	-	-	-
Adhesive glands	-	-	-	+	-	-
Caudal glands	-	-	-	-	-	-
Basiphil glands A	+++	- to +	++	-	-	++ to +++
Basiphil glands B	++ to +++	- to +	++	-	-	++ to +++
Parenchyma	-	-	-	-	- to +	-

- no reaction; + weak positive reaction; ++ moderately strong positive reaction; +++ strong positive reaction; A, intraepidermal portion; B, subepidermal portion

TABLE 2 - Reactions for Protein, Lipid, and RNA

Tissue Constituent \ Method	Mercury/ Bromphenol blue	Ninhydrin/ Schiff	Naphthol Yellow S	Sakaguchi	Millon	DMAB/ Nitrite	Sudan Black	Pyronin Y
Epidermis	-	-	-	-	-	-	-	- to ++
Basement membrane	+ to ++	+	+	-	-	-	-	-
Muscles	++	- to +	+ to ++	-	-	-	-	-
Adhesive glands	+++	++	+++	++	-	-	+	- to +++
Caudal glands	++	-	++ to +++	-	++	++	+	±
Basiphil glands A	++	+	±	-	-	-	++	-
Basiphil glands B	-	-	-	-	-	-	+	-
Parenchyma	-	-	-	-	-	-	+ to ++	-

- no reaction; + weak positive reaction; ++ moderately strong positive reaction; +++ strong positive reaction; ± doubtful reaction; A intraepidermal portion; B subepidermal portion.

The Epidermis: The Feulgen reaction coloured nuclei only. With the methyl green/pyronin test the cytoplasm adjacent to the nucleus often coloured with pyronin (Pl. 1, Fig 1,C). Digestion of sections with RNase for 1½ hrs. reduced pyronin staining, and digestion for 3 hrs. elminated this staining reaction.

The "Basement Membrane": The Allochrome procedure coloured the membrane deep blue.

The Muscles: A small area of cytoplasm around muscle cell nuclei was coloured with pyronin in the methyl green/pyronin test. This colour was removed by digestion of the section with RNase.

The Parenchyma: The parenchyma exhibited an overall dark greyish colour after staining with Sudan black B. Many very small refractile bodies are present throughout the parenchyma, but it is difficult, because of their small size and refractility, to tell whether or not they stain with Sudan black.

A small group of cells situated caudally in the parenchyma immediately behind the penis gave a strong positive result with the Mowry colloidal iron method. These same cells were PAS negative.

The Feulgen reaction demonstrated many nuclei in the parenchyma.

Subepidermal Glands: Two major types of subepidermal gland have been described (Wineera, 1969) for this animal. These are the eosinophil glands and the basiphil glands.

The Eosinophil Glands: Two types are present in *P. stephensoni.* One type (hereafter termed adhesive glands) was described earlier (Wineera, 1969). The other type (hereafter termed caudal glands) are

located in an area of parenchyma just above the ventral body wall in the caudal region of the worm. The spermathecae and the genital opening also occur in this region of the animal. The glands are intensely eosinophil (Pl. 1, Fig. 6, black material). They contain closely packed granules approximately 1.2μ in diameter, and clusters are found crossing the basement membrane and within the epidermis. With pyronin staining the adhesive gland cells varied from colourless to bright red. The staining could be eliminated by prior treatment of sections with RNase for 3 hrs. Some cells contained colourless secretion granules surrounded by pyronin staining cytoplasm, and others possessed pyroninophil cytoplasmic strands.

The Basiphil Glands: On one occasion basiphil glands displayed metachromasia after staining with toluidine blue and mounting in DPX. The sulphation/Azure A and sulphation/toluidine blue techniques demonstrated metachromasia when the sections were viewed in water. After dehydration and mounting in DPX the colour changed from red to purple. With the allochrome procedure subepidermal portions of the basiphil glands coloured a purplish colour, while the intraepidermal parts coloured red.

Pigment: In paraffin sections the pigment granules are bleached by 10% hydrogen peroxide after 6 days. With 40% peracetic acid made according to Pearse (1960, p.860) the bleaching time was 17 hrs. The colour of the dorsal surface of whole worms was not lessened by extracting them with 5% hydrochloric acid for 2 weeks. The ultraviolet/fluorescence method of MacRae (1961) for demonstrating the presence of porphyrins in planarians gave negative results. The same test applied to a fresh water triclad gave a positive result.

DISCUSSION

The "Basement Membrane": It was suggested in an earlier paper by the present author (Wineera, 1969) that the "basement membrane" probably is collagenous. The results of the present study do not contradict this suggestion, but nor do they give it unequivocal support. The "basement membrane" exhibits a variable PAS reaction, and weak positive staining for protein, which could be expected of collagen (Pearse, 1960, p.162). It showed no metachromasia after staining with toluidine blue or azure A either before or after sulphation, suggesting that acidic carbohydrates are not present in this structure. There is the possibility that some are present but that the reactive groups which give rise to the metachromatic phenomenon are not present in sufficient quantity or are not placed at suitable intervals along the polysaccharide molecule. The weak positive staining with Mowry's colloidal iron reagent is best considered as non specific staining by the reagent and not indicative of the presence of acidic muco-polysaccharides. In the allochrome procedure the "basement membrane" coloured deep blue, which Lillie (1951) considers a characteristic of collagen when subjected to this procedure. Skaer (1961) describes in a fresh water triclad a thick basement membrane which he concludes contains collagen.

Parenchyma: The parenchyma of turbellarians continues to be an extremely difficult subject for study. The elements clearly distinguished in the present study were the neoblast cells (Pedersen, 1959) and the

gland cells. As found by Pedersen for neoblast cells in a fresh water triclad, these cells in *P. stephensoni* contain large amounts of cytoplasmic RNA. The present study sheds no light on the problem of the origin of the "basement membrane", or the fibres continuous with it (Wineera, 1969). The extent to which lipids occur in the parenchyma, and their distribution, cannot be decided until further work involving electron microscopy is undertaken. The cells in the parenchyma which gave a strong positive reaction with the colloidal iron reagent are considered to be glands associated with the penis.

The Eosinophil Glands (Pl. 1, Fig. 3; Pl. 1, Fig. 7,S): These are clearly protein in nature. The adhesive glands are shown to contain arginine, and the caudal glands tyrosine and tryptophan. They contain no polysaccharide material. The weak staining of the adhesive glands in the Mowry colloidal iron technique is considered non specific staining by the iron reagent. This view is supported by the fact that the glands do not colour with Mowry's reagent after sulphation, and do not exhibit metachromasia with azure A or toluidine blue. The Mowry colloidal iron method is based on the demonstration of bound ferric iron as prussian blue (Mowry, 1958). Wagner & Shapiro (1957) indicate that some proteins bind ferric iron and could give positive results in tests which demonstrate this ferric iron, as for example, the colloidal iron test for acidic polysaccharides. The staining of both types of gland by Sudan black is not considered "true sudanophilia" due to lipids, since staining was not diminished by hot pyridine extraction. The variable staining of the adhesive cells with pyronin indicates variable amounts of RNA in these cells. This can be correlated with the protein nature of the adhesive gland secretion. The variable nature of the reaction may indicate different stages of the secretion cycle in different cells. The cells showing "stranded" pyroninophilic cytoplasm probably represent those described by Wineera (1971) as possessing ergastoplasm. Pedersen (1959, 1963) records the presence of eosinophil adhesive glands in fresh water triclads. The distribution of the glands described by Pedersen is similar to the distribution of the eosinophil glands in *P. stephensoni*. Pedersen also describes the glands as being protein in nature, but states that they contain, in addition, a phospholipid component. The caudal glands referred to in this study are considered to be associated with the reproductive organs since they are found only in the region of the genital openings.

The Basiphil Glands: The reactions of these gland cells indicate that they contain neutral mucopolysaccharides: They exhibit diastase resistant PAS positivity, and are negative in tests for protein, lipid, and nucleic acid. Also they do not stain in the tests for acidic polysaccharides except after sulphation (Pl. 1, Fig. 5) which is a recognized procedure (Pearse, 1960; Moore and Schoenberg, 1957; Kramer and Windrum, 1954) for forming acidic polysaccharides from neutral ones present in tissue sections. The observed case of metachromatic staining of the basiphil glands with toluidine blue before sulphation seems anomalous in the light of the previous discussion, for it should indicate the presence of acidic mucopolysaccharides (Pearse, 1960; Moore and Schoenberg, 1957). Nucleic acids can display metachromasia under certain conditions (Pearse, 1960; Bergeron and Singer, 1958) but in the present case the gland cells gave

negative results in tests for nucleic acids. It would appear best at this time to attach no significance to this case of metachromatic staining because (i) the staining was weak and (ii) the staining was an isolated case and could not be repeated in several subsequent attempts.

The nature of the basiphil secretion appears to change both in morphology and in staining reaction from the subepidermal parts of the glands to those in the epidermis: The subepidermal parts of these glands are granular and, as has already been mentioned, contain neutral mucopolysaccharides. The intraepidermal portions often appear homogenous, and stain in tests for protein (e.g. mercury/bromphenol blue test) (Pl. 1, Fig. 4, S) as well as in the PAS test. In the allochrome method the distinction between these two parts is seen clearly. The significance of these differences within different parts of the basiphil glands is not known. It is possibly that the protein part of the secretion is a product of the epidermal cells themselves, since these were found to contain varying amounts of RNA adjacent to their nuclear region.

The basiphil glands stain with Sudan black B, but this does not indicate the presence of lipids, as extraction of sections with hot pyridine does not lessen the staining reaction. Presumably the Sudan black acts, in this case, as a weak basic dye (as Casselman (1959) shows it is able to do), and not as a non-ionic fat soluble colourant.

In his studies of fresh water triclads, Pedersen (1959, 1963) describes three types of basiphil subepidermal gland. One type (called type 3) owes its basiphilia to RNA granules in its cytoplasm and is found immediately in front of the pharynx. This type appears to have no counterpart in *P. stephensoni*. The other two types, taken together, are similar in morphology, distribution, and staining reaction to the basiphil glands in *P. stephensoni*. In the present study slight differences in the basiphil gland cells occurred. For example the size and degree of packing of secretion granules varied slightly from some gland cells to others. But all basiphil glands exhibited the same staining reactions, and the variations observed were not thought sufficient to justify the division of the basiphil glands into two groups. However, it is possible as Skaer (1961) suggests, that the basiphil glands include more than one histochemically distinct type.

Pigment: The pigment in *P. stephensoni* may be a melanin. It is slowly bleached by hydrogen peroxide, and is bleached by 40% peracetic acid in a time which is very close to that given by Pearse (1960) for the bleaching of melanin in tissue sections by this substance. Skaer (1961) concludes that the pigment in the triclad *Polycelis nigra* is a melanin, but Needham (1965) states that *P. nigra* pigment can be extracted by dilute (4%) hydrochloric acid, which is not characteristic of melanin. In the present study the pigment granules were insoluble in 5% HCl.

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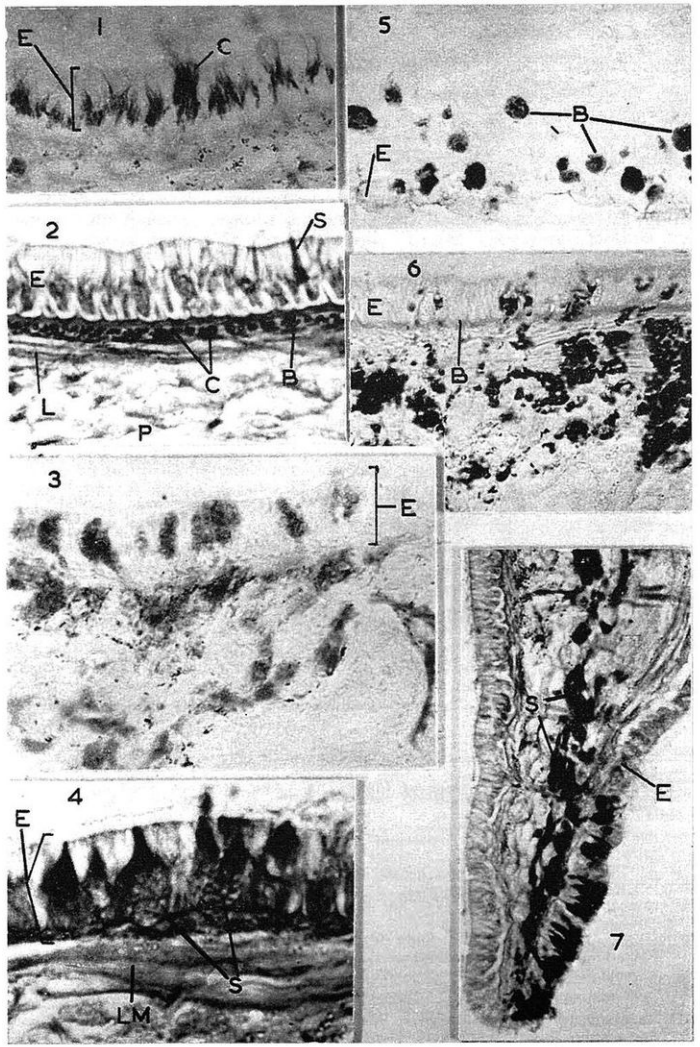

LEGEND TO THE PLATE

Plate 1—Fig. 1: Sagittal section of dorsal body wall in head region. Methyl green/pyronin stain, x 700.
C, cytoplasm of an epidermal cell; E, epidermis.

Fig. 2: Sagittal section of dorsal body wall. Mercury/bromphenol blue stain, x 1,000.
B, basement membrane; C, circular muscles; E, epidermis; L, longitudinal muscles; P, parenchyma; S, intraepidermal basiphil secretion.

Fig. 3: Sagittal section of caudal region showing caudal glands. Mercury/bromphenol blue stain, x 850.
E, ventral epidermis.

Fig. 4: Sagittal section of ventral body wall to show intraepidermal portions of subepidermal basiphil glands. Mercury/bromphenol blue stain, x 1,500. Note increasing intensity of staining towards epidermal surface.
E, epidermis; LM, longitudinal muscle; S, "sacs" of basiphil secretion.

Fig. 5: Sagittal section of ventral body wall in cephalic region. Sulphation/mowry colloidal iron method, x 800.
B, basiphil glands; E, epidermis.

Fig. 6: Sagittal section of ventral body wall in caudal region showing strongly fuchsinophil caudal glands (black). Falg stain, x 500.
B, basement membrane; E, epidermis.

Fig. 7: Sagittal section of cephalic region of animal showing adhesive glands opening through ventral epidermis. Mercury/bromphenol blue stain, x 400.
E, ventral epidermis; P, parenchyma; S, adhesive gland secretion.

THE BODY WALL OF THE SEA ANEMONE *ISACTINIA OLIVACEA* (HUTTON, 1878)

PART 1 : HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS

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ABSTRACT

The histology and histochemistry of the column body wall of the sea anemone *Isactinia olivacea* (Hutton, 1878) are described. The ectodermal supporting cells bear non-motile projections at the free surface. These projections may assist in the shedding of unwanted secretions from the surface of the animal. Unicellular ectodermal glands show a wide variation of morphology and type of secretion. Both proteinaceous and polysaccharide containing glands are of wide occurrence. Mesogloeal fibres have staining properties characteristic of collagen. Cells are abundant in the mesogloea, and contain protein, polysaccharide and lipid granules; their role in the mesogloea is discussed. The endoderm is composed mostly of flagellated epitheliomuscular cells; it is regarded as being trilaminar: the epithelial portions of the cells form one layer, and the circular muscle fibres form another; in between these two is a fluid filled layer which it is suggested has a hydrostatic function. Proteinaceous and polysaccharide containing gland cells are present between the epitheliomuscular cells.

INTRODUCTION

The coelenterates have long been a favoured group for study by invertebrate marine zoologists. One reason for this is that these animals are abundant and usually easily obtainable. Another reason is that they are generally regarded as being the most primitive, in an evolutionary sense, of the metazoan animals, and it is believed that study of this group might reveal useful information regarding evolution of the lower metazoa. The coelenterates may be regarded (depending on one's opinion as to the correct evolutionary position of the group) as the first animals to show tissue specialization. Although sponges possess, for example, muscle cells (Hyman, 1940), these are not organised into muscle tissue or muscles such as one finds in coelenterates. Connective tissue in some coelenterates is also well developed (Chapman, 1953; 1966) and often has the appearance and properties of some vertebrate connective tissues. The coelenterates as a group thus often appeal to histologists as worthy of study, for many tissues seen in vertebrates make their first appearance here, and it could be expected that study of these tissues in coelenterates may lead to a better understanding of tissue structure and function in higher animals.

The present paper deals with structure and function of the column body wall of a sea anemone, *Isactinia olivacea* Hutton. It is one of a

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series of papers which study the body wall of various coelenterates and flatworms (phylum Platyhelminthes), and it is hoped that these studies may reveal information useful in a consideration of the phylogenetic relationship between these groups.

I. olivacea is found in the lower littoral zone of the sea shore attached to rocks, particularly in surge channels. An average sized specimen measures 1.5 cm. long with an oral disc diameter of 1 cm. The colour varies from olive green to brown.

MATERIALS AND METHODS

COLLECTION OF ANIMALS: The animals were collected from Houghton Bay, Wellington, N.Z. by removing them from rocks at low water. A cold chisel and hammer were used to fracture the rock on which the animals were resting, in order to obtain the specimens attached to small pieces of rock. The animals were then placed in quart jars filled with sea water and were transported immediately to the laboratory. Here they were set in aquaria (10 animals to a tank measuring 27 x 17 x 12 cms.). The time lapse between removal from the sea and placement in aquaria was about 1½ hrs. The water in aquaria was replaced after 12 hrs., and thereafter every 2 weeks. No aeration of tanks was necessary. The animals were fed chopped mussel *(Mytilus)* every two weeks, 12 hrs. before the water was changed.

ANAESTHETIZATION: The animals proved difficult to anaesthetize in an extended condition. The best narcotics of the several used were menthol, chloral hydrate-menthol, and isotonic magnesium chloride in sea water. The chloral hydrate-menthol mixture consisted of 4 g. menthol 6 g. chloral hydrate and sufficient water to make a thick paste. This mixture was added drop by drop, over a period of 2-3 hrs. to a beaker of sea water containing an expanded anemone (Le Thi, unpublished thesis). After this time the tentacles no longer responded to touch, and the beaker water was pumped into the coelenteron with a rubber bulb pipette. This served to complete anaesthetization, and to expand the animal slightly. The menthol method of narcotization used was that of Lee (1924), and the method using magnesium chloride in sea water was applied according to Batham, Pantin, and Robson (1960).

It must be noted that none of the methods used was entirely satisfactory with *I. Olivacea,* and resulted, generally, in the oral disc and tentacles being somewhat retracted inside a slightly overinflated column. The results with any one method also varied with different individuals.

FIXATION: When anaesthetized, the animals were either removed to buffered 4% formaldehyde (Pease, 1964), or formalin was added to a beaker of fresh sea water containing the animal to make a concentration of 10% formalin. In both cases fixative was repeatedly pippetted down the throat of the animals for several minutes to ensure adequate fixation of internal parts. Fixatives were allowed to act for at least 12 hrs.

HISTOLOGICAL METHODS: For study of sections, fixed animals were embedded in paraffin and sectioned longitudinally and transversally at 5-6μ. Some animals were embedded in 20% gelatin and sectioned on a freeze microtome. The following histological staining methods were employed: 1. Heidenhain's iron haematoxylin-orange G; 2. Delafield's haematoxylin-eosin-fast green FCF (Wineera, 1968); 3. Mallory's triple stain (Gray, 1954); 4. Mallory/Azan method (Gurr, 1962); 5. The Falg technique (Gurr, 1962); 6. Gordon and Sweets' method for reticulin (Pearse, 1960); 7. Fullmer and Lillie's method for elastic fibres (Fullmer and Lillie, 1956); 8. The Allochrome procedure (Lillie, 1951).

Histochemical methods used were:

A. *Polysaccharides:* 1. PAS reaction with and without diastase digestion and pyridine extraction (Pearse, 1960). The Schiff solution was prepared according to Coleman's method (cited in Gray, 1954); 2. Mowry's method (1958) for acidic polysaccharides; 3. Toluidine blue staining (5 min. in 0.1% toluidine blue in 30% ethanol). Sections were examined in water prior to dehydration and mounting; 4. Low temperature sulphation (Moore and Schoenberg, 1957) followed by toluidine blue staining as in (3) above.

B. *Proteins:* 1. Mercury/bromphenol blue method (Pearse, 1960); 2. Naphthol yellow S staining (Deitch, 1955); 3. The Sakaguchi reaction for arginine (Pearse, 1960); 4. The Millon reaction for tyrosine (Casselman, 1959); 5. The DMAB-nitrate method for tryptophan (Pearse, 1960).

C. *Nucleic Acids:* 1. The Feulgen reaction (Pearse, 1960); 2. Methyl green-pyronin staining. Chloroform washed methyl green and pyronin Y made up according to Kurnick (cited in Pearse, 1960) were used in the proportions 10 parts of methyl green solution to 8 parts of pyronin Y solution to 27 parts of distilled water. The staining time was 6 min. Ribonuclease digestion (the enzyme was from Sigma) by a solution of glass distilled water containing 1 mg. enzyme / ml. water was performed for 3 hrs. on some slides before staining.

D. *Lipids:* 1. Staining with oil red O in 60% isopropyl alcohol (Casselman, 1959) (frozen gelatin sections used). Some slides were treated with pyridine at 60°C for 24 hrs. before staining. One slide was stained with oil red O and the positions of positive staining sites were recorded using a microscope stage micrometer. The slide was then extracted in hot pyridine (as stated above), restained in oil red O and examined.

E. *Cambination Methods:* 1. Himes and Moriber's triple stain (1956) for DNA, protein, and polysaccharide; 2. Mowry colloidal iron-PAS; 3. Mowry colloidal iron-PAS-naphthol yellow S (Mowry/PAS/NYS) with and without prior treatment in pepsin (2 mg./ml. in 0.02 N HCl-acetate buffer at 37°C for 2 hrs.). Slides not treated with pepsin were placed in buffer solution at 37°C for a similar time.

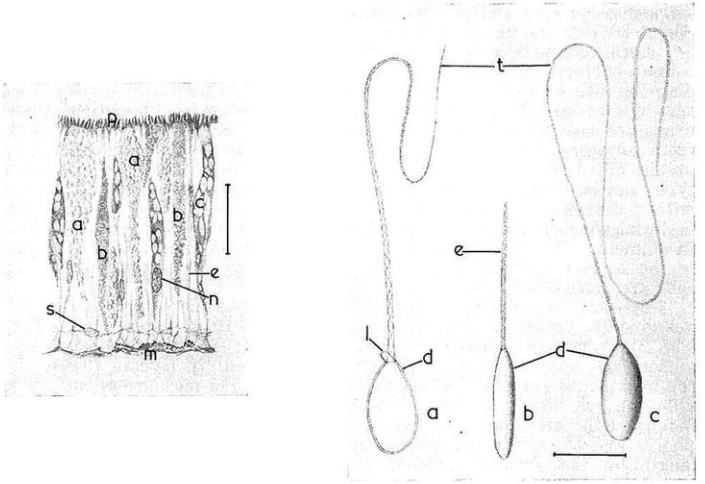
Additional Methods: 1. Small pieces of body wall were post fixed in 1% OsO_4 in distilled water, and embedded in araldite according to Richardson, Jarett, and Finke (1960). Sections were cut at 0.5 and 1.0μ on a LKB ultrotome, and placed on albuminized slides. Staining methods used were the methylene blue-Azure II procedure of Richardson et al (1960), and the PAS test. 2. Dissociation preparations of the body wall

were prepared using the maceration technique of Goodrich (1942) and from trypsin digestions (the enzyme used at 0.5% in sea water at room temperature for 4 hrs.) on fixed and fresh tissue. "Milton" and "Janola" (commercial solutions of sodium hypochlorite) were also used as dissociating agents, both on fresh and fixed tissue. The cells obtained by these techniques were examined by phase contrast microscopy. Ectoderm and endoderm layers of the body wall were isolated after partial dissociation by scraping one or other from the surface. 3. Determination of ciliary beats. Particles of charcoal were placed on the column, oral disc, tentacles, and the pharynx surface with the aid of a binocular microscope. The animal was then studied to determine whether or not the particles were moved over these body surfaces. 4. Staining with reduced methylene blue for nervous elements of the body wall. Pieces of body wall were cut from well expanded anaesthetized animals and stained, vitally, according to the method of Batham, Pantin and Robson (1960). The reduced methylene blue was prepared according to Pantin (1948). Tissue was examined in sea water under a cover glass prior to fixing in 5% ammonium molybdate and the preparation of permanent whole mounts or paraffin sections.

RESULTS

A. HISTOLOGICAL OBSERVATIONS: The body wall of *I. olivacea* when seen in sections varies between 30-70μ thick depending on the state of contraction of the animal. It is made up of 3 layers; an outer epithelial layer (ectoderm), an inner epithelial layer (endoderm), and a middle layer (mesogloea) (Pl. 1, Fig. 1).

Ectoderm: The ectoderm is composed mainly of supporting cells and gland cells. The supporting cells (Pl. 1, Fig. 2; Text Fig. 1e) are tall and slender, with a small (4-5μ x 2-3μ) nucleus which usually is placed about half way up the cell but which often occurs near the free surface. Frequently these cells are so slender as to appear thread-like. In such cases the nucleus bulges out from the cell. At the free surface the cells are usually expanded slightly and bear a short (3μ), flattened, tongue-like projection. At the mesogloea the cells taper away, but may have 2 or 3 short strands, or root-like processes. These features were noted from dissociated ectodermal tissue. It was found to be impossible to note all of these features in sections of body wall, as the cells were too long and thin and were often obliterated by dense packing of heavily staining gland cells. Many small oval nuclei are visible in sections, but it is difficult to assign them to any particular cell. The surface of the ectoderm appears ciliated when seen in section (Pl. 1, Figs. 1, 3, 5). However experiments with charcoal particles revealed no ciliary or flagellar current over the surface of the column. In fact only on the pharynx surface were the particles moved. When placed on pharynx tissue the charcoal particles moved rapidly towards the gastric cavity. In trypsin dissociation preparations no ciliary or flagellar movement could be identified on ectodermal cells, while cells of the endoderm showed vigorous flagellar movement.



Text Fig. 1: Diagrammatic representation of the column ectoderm as seen in a transverse section stained by the Mallory/Azan technique.
(Left) Scale measures 20μ.
a, type A gland cells; b, type B gland cells; c, type C gland cells; e, supporting cell; m, mesogloea; n, nucleus; p, projection; s, nerve cell.

Text Fig. 2: Nematocysts found in the column ectoderm in **I. olivacea.**
Scale measures 10μ.
(Right) a, atrichous anisorhiza; b, microbasic amastigophore; c, holotrichous isorhiza; d, capsule; e, butt; l, lid; t, tube.

The *gland cells* in the ectoderm are numerous and often closely packed. In Mallory/Azan preparations three types of gland cell can clearly be distinguished on the column (Text Fig. 1). One type contains granules which stain orange red and are up to 1.5μ in diameter. These will be called type "A" cells. They are narrow (about 3μ wide) but often are wider (up to 7μ wide) at the surface, and they make up about 35% of gland cell numbers. A second type ("B" gland cells) contain granules which are about 0.7μ in diameter, and which stain a red-purple colour. The granules are very closely packed and stain so intensely that often individual granules are difficult to discern from their neighbours. These cells are 3-4μ wide with a tapering base, and account for about 40% of gland cell numbers. The third gland cell type (type "C") stains a pale blue colour. The cells are usually about 4-5μ wide throughout their length, and appear bulkier than the other two types. They contain pale staining oval granules about 2μ long (long axis). The periphery of the granules often colours more deeply than the centre, giving the cell contents the appearance of a coarse, irregular network. Near the mesogloea the net-

work may be very coarse, and the granules in this case are not visible. About 20% of the gland cells are of this type.

Localised modifications of this ectodermal pattern occur over the column surface. At various sites oval or circular areas of about 0.1 mm. diameter can be seen with a binocular microscope, which are paler than the olive green colouration of the rest of the column. In sections these areas are seen to be ectodermal invaginations which differ histologically from adjacent areas in that the previously described gland cells are absent (Pl. 1, Fig. 5). In Mallory/Azan preparations two new gland cell types can be distinguished. One type contains orange staining granules with a diameter of about 0.5μ; the other holds deep blue-grey coloured material which may appear homogenous or as ill-defined granules 0.5-1.0μ in diameter. The bases of both types of cell are 3-5μ wide, and they open to the surface through long, slender necks. The ectodermal supporting cells are more clearly seen in these areas.

Dissociation techniques and phase contrast microscopy show the *nematocysts* present in the column body wall to be of three types (Text Fig. 2) : microbasic amastigophores, holotrichous isorhizas, and atrichous anisorhizas (according to the classification of Weill, in Hyman 1940). In the unexploded state the microbasic amastigophores measure about 18μ x 2-3μ and are slightly curved. The holotrichous isorhizas measure 15μ x 3-4μ, and the atrichous anisorhizas 15μ x 7μ. The microbasic amastigophores and holotrichous isorhizas are the same width for most of their length, but the atrichous anisorhizas are ovoid.

Attempts at nerve staining with reduced methylene blue were unsuccessful. However, an ectodermal nerve net is visible in sections stained by Mallory/Azan (Pl. 1, Fig. 1N) and in iron haematoxylin preparations. An endodermal nerve net is also visible in these preparations. Both nerve nets are intraepithelial. Connections between the nerve nets through the mesogloea were not observed. These findings are in line with those of Bullock and Horridge (1965) concerning the nervous system of actinians.

Mesogloea: The middle body layer varies in thickness with the state of contraction of the animal, between about 10 and 30μ. It is reduced to about 3μ in thickness beneath the modified ectodermal areas described above. Both cells and fibres are present in this layer.

In section the cells (Pl. 1, Fig 1, arrow; Pl. 2, Fig. 3, arrow) are irregularly shaped, often elongated between bundles of fibres, and have round or elongate nuclei of about 3μ diameter or 6μ long respectively. The cytoplasm of the cells often closely invests the nucleus as a thin layer, but often is seen to be aggregated to one or other ends of the cell. Very fine granules (0.3μ diameter) which stain orange in the Mallory/Azan technique are sometimes seen in the cytoplasm. The cells frequently are located in spaces between bundles of fibres.

Staining with reduced methylene blue (Pantin, 1948) resulted in blue granules appearing in the cytoplasm of the mesogloea cells, often in a paranuclear position. Other similar sized granules appear to lie free between mesogloea fibres.

The fibres of the mesogloea stain intensely with the aniline dyes aniline blue (in the Mallory and Mallory/Azan technique, Pl. 1, Fig. 1) and fast green FCF (in the Wineera triple stain). In a well extended animal the fibres are arranged as bundles which appear to run mostly as

alternate circular and longitudinal layers. In a contracted specimen this orderly arrangement is not so apparent. Fibres from different layers anastomose frequently. In transverse sections the layers of fibres immediately beneath the endoderm appear to be the thickest and most orderly in arrangement (Pl. 1, Fig. 6). In the outer region of mesogloea the fibres form a more or less loose network. Immediately beneath the ectoderm is a thin, dense line which stains blue in Mallory/Azan preparations. Between the mesogloeal fibres are spaces of varying size.

Endoderm: The inner epithelial layer is composed of epitheliomuscular cells (Pl. 1, Fig. 4) and gland cells. The former type are the most numerous, making up about 90% of the cells present.

The *epitheliomuscular cells* vary greatly in length and breadth according to the state of contraction of the animal. In a contracted animal the epithelial part of a cell may reach 100μ in length and in this case appears thread-like. This lengthening of the epithelial portion of the cell is complementary to and is caused by, a shortening of the basal muscle fibre. Conversely in a relaxed animal the epithelial part of the cell is about 30μ long and is more columnar; the base of the cell tapers for a short distance before fanning out (in one plane) to enclose the extended muscle fibre. The nucleus of these cells is ovoid in sections, measures about 6 x 3μ and is placed in the half of the cell closest to the coelenteron. This region of the cells is expanded slightly and bears a single flagellum at the end. The basal muscle fibres are ribbon-like structures which run in a cirmcumferential direction in the body wall of the column, making up the "circular muscles" of the animal. Once again, many of these features could be noted only by examination of dissociation preparations.

Large numbers of greenish algae are present in the epitheliomuscular cells.

The gland cells present in the endoderm colour differentially with the Mallory/Azan technique. One type, which contains closely packed bright red granules 0.7-1.0μ in diameter accounts for about 95% of the gland cells present. The other 5% of gland cells is made up by cells which stain blue. Indistinct granules 1.0μ in diameter are sometimes apparent in these cells, but most frequently the contents have a turbid appearance, similar to that of the Type C ectodermal cell.

B. HISTOCHEMICAL OBSERVATIONS: These results are summarised by tables 1 and 2.

Ectoderm: Histochemical results for the ectodermal supporting cells were gained by examining the areas of modified ectoderm described earlier, in which the supporting cells are especially numerous. In other areas of the column it is impossible to study the supporting cells because of the abundance of gland cells. The classification of gland cells based on the uptake of dyes in various histological techniques is found to be too simplified when histochemical methods are applied: The type "A", "B",

and "C" cells retain their individuality, but several other types are recognisable also. Firstly, one type of cell which contains granules of similar size to those of cell type "B". These cells, which will be called "B2" cells, are much more slender than the type "B" cells, and are clearly distinguished by their histochemical reactions (see Tables 1 and 2). A second new type of gland cell (type "D") is composed of very fine (0.5μ)

TABLE 1 — REACTIONS FOR CARBOHYDRATE

	HISTOCHEMICAL TEST / TISSUE CONSTITUENT	PAS	MOWRY COLLOIDAL IRON	METACHROMASIA WITH TOLUIDINE BLUE: BEFORE SULPHATION	METACHROMASIA WITH TOLUIDINE BLUE: AFTER SULPHATION	MOWRY/PAS/NYS: TEST SECTIONS	MOWRY/PAS/NYS: PEPSIN TREATED CONTROL SECTIONS
ECTODERM	FRINGE	++ to +++	-	-	++	+++PAS	++PAS
	SUPPORTING CELLS	+	-	-	-	+NYS	+NYS
	GLAND CELL A	-	- to +	-	-	+++NYS	++NYS +MOWRY
	GLAND CELL B	-	-	-	-	+++NYS	+++NYS
	GLAND CELL B2	+++	-	-	?	+++PAS	+++PAS
	GLAND CELL C	+++	++	++	++	+++PAS ++MOWRY	+++PAS ++MOWRY
	GLAND CELL D	ONE COMPONENT +++	ONE COMPONENT +++	?	?	(+++MOWRY) (+++PAS)	(+++MOWRY) (-PAS)
	GLAND CELL E	+++	+++	?	?	++PAS +++MOWRY	+PAS +++MOWRY
	MICROBASIC AMASTIGOPHORES	-	++	++	?	++MOWRY	++MOWRY
	HOLOTRICHOUS ISORHIZAS	-	+	-	-	+MOWRY	+MOWRY
	ATRICHOUS ANISORHIZAS	THREAD +	-	-	-	THREAD +PAS	THREAD +PAS
ENDODERM	EPITHELIO-MUSCULAR CELL	-	-	-	-	MYONEME +++NYS	MYONEME ++NYS
	GLAND CELL 1	-	-	-	-	+++NYS	++NYS +MOWRY
	GLAND CELL 2	+++	++	+	++	+++PAS ++MOWRY	+++PAS ++MOWRY
	GLAND CELL 3	-	+++	+	++	+++MOWRY	+++MOWRY
MESOGLOEA	CELLS	SOME ++	-	-	-	SOME ++NYS OTHERS ++PAS	SOME +NYS OTHERS ++PAS
	FIBRES	++ to +++	- to +	-	++	+MOWRY ++PAS	+MOWRY ++PAS

+ WEAK REACTION; ++ MODERATE REACTION; +++ STRONG REACTION; - NEGATIVE REACTION
? DOUBTFUL REACTION

Table 1: Reaction of the body wall constituents of **I. olivacea** to tests for carbohydrates.

TABLE 2 — REACTIONS FOR PROTEIN, LIPID, AND RNA

	TISSUE CONSTITUENT / TEST	PYRONIN STAINING: NO RNase	PYRONIN STAINING: RNase	OIL RED O NO PYRIDINE	OIL RED O STAINING AFTER PYRIDINE	MERCURY BROMPHENOL BLUE	NAPHTHOL YELLOW S	MILLON	SAKAGUCHI	DMAB-NITRITE	ELASTIN	RETICULIN
ECTODERM	FRINGE	-	-	-	-	- to +	++	-	-	-	-	-
	SUPPORTING CELLS	-	-	?	-	+	+	-	-	-	-	-
	GLAND CELL A	-	-	-	-	+++	+++	++	-	++	-	-
	GLAND CELL B	+++	++	-	-	+++	+++	++	-	-	-	-
	GLAND CELL B2	-	-	-	-	-	-	-	-	-	-	-
	GLAND CELL C	-	-	-	-	-	-	+ to ++	-	?	-	+++
	GLAND CELL D	-	-	-	-	?	?	?	?	?	-	?
	GLAND CELL E	-	-	-	-	-	-	?	-	-	-	+++
	MICROBASIC AMASTIGOPHORES	+++	++	-	-	-	-	-	-	-	-	-
	HOLOTRICHOUS ISORHIZAS	-	-	-	-	-	-	-	-	-	-	-
	ATRICHOUS ANISORHIZAS	-	-	-	-	-	-	-	-	-	-	-
ENDODERM	EPITHELIO-MUSCULAR CELL	MYONEME ++	MYONEME +	EPITHELIUM ++	-	MYONEME ++	MYONEME ++	MYONEME ++	-	-	-	-
	GLAND CELL 1	CYTOPLASM ++ GRANULES+	CYTOPLASM - GRANULES+	-	-	GRANULES +++	GRANULES +++	GRANULES ++	-	-	-	-
	GLAND CELL 2	-	-	-	-	-	-	-	-	-	-	-
	GLAND CELL 3	-	-	-	-	-	-	-	-	-	-	-
MESOGLOEA	CELLS	SOME ++	-	+++	-	-	SOME ++	?	-	SOME +	-	++
	FIBRES	-	-	-	-	+	++	-	-	-	-	++

+ WEAK REACTION; ++ MODERATE REACTION; +++ STRONG REACTION;
- NEGATIVE REACTION; ? DOUBTFUL REACTION

Table 2: Reactions of the body wall constituents of **I. olivacea** to tests for protein, lipid, and Nucleic acids.

granules which appear to be of two kinds, judging by their staining reactions. These cells are best seen at the base of the ectoderm. They open to the surface by very fine cell necks. A third type of cell (type "E") is present, and is distinguishable on histochemical grounds, although it resembles the type "C" cell, in morphology. This type of gland cell opens to the surface by a narrow duct. The histochemical reactions of the various gland cells are summarised in tables 1 and 2.

As mentioned earlier the patches of modified ectoderm scattered over the column surface possess two types of gland cells, one which stains orange red with the Mallory/Azan method and one which stains blue. These also show different and distinctive histochemical reactions. The former colour with naphthol yellow S, and the latter colour red in the PAS technique. When pepsin digestion precedes the Mowry colloidal iron-PAS-naphthol yellow S procedure, bright blue structures appear in these areas of ectoderm at the level of the nerve net. They are often stellate in appearance (Pl. 2, Fig. 4, arrows) and do not approach the size of the gland cells. They are not connected to the surface of the ectoderm.

Mesogloea: Many of the cells of the mesogloea contain diastase resistant PAS positive granules, and naphthol yellow S positive granules. The cytoplasm of many was also shown to colour moderately strongly with pyronin (Pl. 2, Fig. 3, arrows), and RNase treatment removed the stain. A strong positive reaction was obtained for lipid in these cells; the lipid is present as very small globules scattered throughout the cytoplasm. Pyridine extraction prevents the staining with oil red O.

The mesogloeal fibres gave a positive reaction in the PAS test, and also stained with naphthol yellow S. After sulphation and toluidine blue staining they coloured purple, and this colour persisted in DePeX mounted sections. The fibres immediately outside the muscles stained moderately strongly for reticulin (Pl. 2, Fig. 2) as did a very thin layer immediately beneath the ectoderm.

Endoderm: The gland cells containing granules which colour red with Mallory/Azan (type "1" cells) colour with naphthol yellow S (Pl. 3, Fig. 1, P). The ones which stain blue with the Mallory/Azan method are distinguishable histochemically into two types: One type (type "2" cells) stain both with the PAS procedure (Pl. 3, Fig. 1, arrows) and with the Mowry colloidal iron method, and when these methods are used in combination these cells appear deep purple. Another type of gland cell (type 3) stains intensely with the Mowry colloidal iron method but is PAS negative (Pl. 3, Fig. 2, arrows).

DISCUSSION

Ectoderm: Although some anemones possess longitudinal columnar muscle (in the form of epitheliomuscular cells), these are considered to be "primitive" in this respect (Stephenson, 1928) in comparison to those forms in which the longitudinal body musculature is restricted to the mesenteries. Le Thi (1968, unpublished M.Sc. Hons. Thesis) described epitheliomuscular cells from the ectoderm of the column of *I. olivacea*, but

in the present study these were not seen. Stephenson (1928) states that the muscle fibres are absent from the column ectoderm in the majority of forms.

According to Batham and Pantin (1951) the absence of ectodermal longitudinal muscles in the column in favour of the longitudinal endodermal parietal muscle is an advantage in large-disked forms since the muscles acting on the disc (the parietals and the retractors) are part of the same system. Also, they point out that grave mechanical difficulties would arise during contraction in diameter and length of the column if the longitudinal musculature was in the form of a continuous endodermal sheet, because the two muscle layers (circular and longitudinal) would be forced to buckle at right angles to each other.

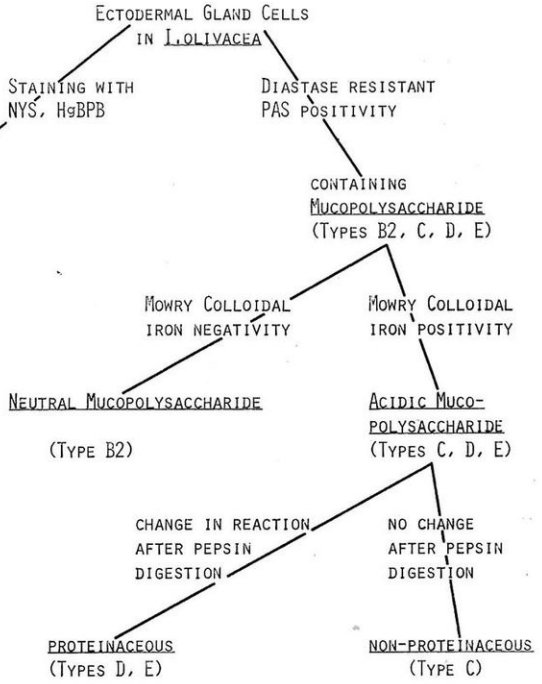
The supporting cells of the ectoderm do not appear to be the same as the typical ciliated cells first figured by the Hertwigs (1879) and since used as the "typical" anemone supporting cell (Hyman, 1940, p.374; Stephenson, 1928). While the appearance of sections could certainly lead to the conclusion that the ectoderm is ciliated, direct examination of the living animal, experiments with charcoal particles, and dissociation experiments do not support this view. An electron microscope study of the body wall of *I. olivacea* is being undertaken at present, and should reveal the fine structure of the projections. However, the present conclusion must be that the ectoderm is not ciliated.

Pantin (1942) describes the occurrence of numerous conical, refractile projections scattered over the tentacle ectoderm of most actinians. The appearance of these projections as figured by Pantin is similar to the appearance of the ectodermal projections in *I. olivacea*. Pantin supports the findings of earlier workers that the projections are compound structures of fused cilia and that they are probably sensory structures. No reference is made to the occurrence of these projections on other body surfaces. In *I. olivacea* the projections are present on the adhesive disc, column, and oral disc as well as the tentacles, and because of this it would seem best at present not to consider the projections as of the same "type" as those described by Pantin. If the projections in *I. olivacea* are in fact sensory structures, it is difficult to see why they should be present on the adhesive disc. The further question then arises as to the significance of the projections seen at the surface in *I. olivacea*. One explanation is that the tongue-like projections assist the animal to shed unwanted mucous coverings or surface debris. The numerous glands present in the ectoderm indicate that a great deal of secretion takes place onto the surface, perhaps forming a temporary "cuticle" of hardened slime (Stephenson, 1928, p. 23). The ectodermal projections would probably act as "spacers" between this "cuticle" and the ectodermal cells (Pl. 1, Fig. 3). The secreted layer would thus not be firmly applied to the ectodermal cells, and perhaps could be shed more easily when required.

The results of this study suggest that the usual classification of actinian gland cells as either "mucous" and "ganular" (Hyman, 1940) or "mucous" and "albumen' (Stephenson, 1928) is useful only in the broad sense. In the present study the terms "ganular" and albumen" would be applicable to protein secreting cells, while "mucous" would probably refer to polysaccharide secreting cells. It would seem best to eliminate as far as possible solely morphological terms such as "granular" when describing gland cells because granules in one cell which show similar morphology to

those of another cell may show different histochemical activity, indicating different composition and function. In *I. olivacea* for example, both protein staining and polysaccharide staining granular cells occur.

TABLE 3.



NYS = NAPHTHOL YELLOW S

HgBPB = MERCURY-BROMPHENOL BLUE

PAS = PERIODIC ACID - SCHIFF

Table 3: Classification of the ectodermal gland cells of **I. olivacea** according to their staining reaction.

Histochemical results (Tables 1 and 2) indicate that 6 different types of secretion product can be recognised in the ectoderm of *I. olivacea*. The proteinaceous secretions (types "A" and "B") can be distinguished by the DMAB-nitrate test for trptophan, and by enhanced affinity for colloidal iron of the type "A" secretion after pepsin digestion. Also, the "B" secretion is pyroninophilic and some of this pyroninophilia is due to RNA. The other secretion types are strongly PAS positive. Type "B2" is distinguished by its negative reaction with the Mowry colloidal iron test. Types "C" and "E" are best distinguished by the criterion of reduced PAS positivity of the type "E" secretion after pepsin digestion. Type "D" is distinguished by the small size of the granules and by the fact that the secretion has PAS positive as well as Mowry positive components which remain separate within the cell. It is evident that the gland cells can be classified broadly as being either proteinaceous (types "A" and "B") or mucopolysaccharide containing (types "B2", "C", "D" and "E"). This latter group can be subdivided according to their reactions (see Table 3).

It is possible that the 6 "secretion types" (types "A" - "E") may represent fewer than 6 gland cell types, for some of the secretions may be earlier or later stages in the secretion cycle of one particular cell type. Secretion types "C" and "E" for instance, are similar histochemically and sometimes morphologically. However, until the secretion cycle of the cells in question are known, it would seem best to accept the results as indicating 6 different gland cell types, while at the same time realizing that the classification may have to be altered in the future.

The local modifications to the ectoderm which have been described have been called verruco-cinclides (Le Thi, 1968 unpublished M.Sc. Hons. Thesis). They have the appearance of imperforate cinclides (Stephenson, 1928) in that each is an ectodermal concavity with only a thin layer of mesogloea beneath it. Stephenson states that these structures are usually formed by greater development of one cellular layer than of the other. In *I. olivacea* they would appear to have been formed by proliferation of ectodermal supporting cells together with a reduction in thickness of the mesogloea beneath the cells. According to Stephenson cinclides probably function as "safety valves", and always are associated with water currents in some way. The imperforate cinclides can rupture neatly when needed, allowing fine jets of water to escape from the coelenteron during rapid contraction of the animal.

Mesogloea: The few tests applied in this study indicate that the mesogloea has certain similarities with the fibrous connective tissue of higher animals. First, both cells and fibres are present in a matrix; second, the fibres show staining reactions characteristic of collagen. Indeed Hyman (1940, p.281) refers to this type of mesogloea as a fibrous connective tissue often containing layers of fibres coursing in different directions among which are scattered amoebocytes and connective tissue cells.

Chapman (1953) attaches some importance to the supporting tissues of coelenterates because of the possibility that they could shed some light on the origin and composition of connective tissues in general. He presents evidence to show that the bulk of the connective tissue protein material of coelenterates examined by him conforms in histological

appearance and physical and chemical properties to the collagen of vertebrates. The histological examination of an anemone *Calliactis parasitica* by Chapman yielded results very similar to those obtained for *I. olivacea* in the present study. In both cases the thick fibrous layer which constitutes the mesogloea stains with aniline blue in Azan and Mallory techniques, does not stain for elastin, does not stain metachromatically with toluidine blue (without sulphation) and contains no fat. *I. olivacea* mesogloea fibres gave a positive result for reticulin in sections, but it is not stated whether Chapman attempted to test for reticulin in *Calliactis*. The fibres of *I. olivacea* stain moderately strongly in the PAS test, indicating the presence of mucopolysaccharides. This result is frequently obtained with connective tissues from coelenterates (Chapman, 1966). It is shown by Chapman (1953) that in *Calliactis* the mesogloea fibres are arranged in sheets parallel to the surface and to fibres at 45° to the long axis of the animal. It is possible that fibres in the mesogloea of *I. olivacea* approximate this condition more closely than has been observed in this study. Most specimens used were not able to be fixed in as extended a state as is desirable for such mesogloeal studies. Chapman also shows, by the use of tangential sections, that in the inner and outer mesogloeal layers the fibres form a lattice structure similar to the pattern seen in a woven fabric. The suggestion is made that the fibres owe their particular orientation to the forces of muscles and environment which are exerted throughout the life of the anemone and not to any organized method of secretion by individual cells. Baitsell (1925) thought it possible that the orientation of developing fibres in chick connective tissue is due to stresses set up in the tissue by migrating mesodermal cells or other forces.

From a consideration of the action of muscles, and of the forces acting on the mesogloea during muscular contraction, Batham and Pantin (1951) argued that the part of the mesogloea in immediate contact with the muscle layer must have different properties to the rest of the mesogloea. They were proved correct by Grimstone et al (1958), who showed that the muscles are, in fact, attached to a specialized layer of mesogloea which they designated a basement membrane, and which is composed of amorphous material. The present study indicates a similar specialized region of mesogloea immediately beneath the ectoderm. It seems reasonable to expect that here also the electron microscope will show the presence of a basement membrane composed of similar amorphous material quite separate from the fibres of the bulk of the mesogloea. The ectoderm is, after all, an epithelium (as was the epitheliomuscular layer studied by Batham and Pantin, 1951; and Grimstone et al, 1958), and epithelia characteristically are attached to underlying connective tissues by a more or less typical basement membrane (Fawcett, 1966).

The origin of the fibres of the mesogloea is unknown (Hyman, 1940). In vertebrates it seems clear that the fibres of connective tissue arise in the ground substance, outside the cell (Baitsell, 1925; Wolbach, 1933). It has been shown that fibrous chemical compounds can be produced *in vitro* by mixing solutions of proteins with solutions of hexosamine sulphonic acids (Meyer, Palmer, and Smyth, 1937), and as Chapman suggests it is conceivable that the conversion of a homogenous proteinaceous matrix could be brought about by the secretion into it of a hexosamine sulphonic carbohydrate or similar carbohydrate by the cells

of the tissue (Chapman, 1953). In *I. olivacea* the mesogloeal cells are variable in shape, some being elongate and narrow, and others being rounded. They appear to be of the one type, but cannot be classified as amoeboid since they were not observed to move. Hyman (1940) does not state by what criterion the mesogloeal cells are classified into "amoeboid" and "connective tissue" types. These cells in *I. olivacea* do not give the appearance of secretory cells, although some contain PAS positive and naphthol yellow S staining granules, and they have been shown to contain RNA (Table 2; Pl. 2, Fig. 3, arrows). But the most characteristic component of these cells would seem to be lipid, which is present as small globules throughout the cytoplasm of most of them. Moreover, in some actinians, e.g. *Edwardsia callimorpha*, the mesogloea although fibrous is devoid of cells (Chapman, 1953) so that the presence of cells in the mesogloeal ground matrix cannot be essential for the secretion of the fibres. In those species containing both cells and fibres in the mesogloea Chapman found the cells to be in no way connected to the fibres, but Batham (1960) has published an electron micrograph showing an amoebocyte of the anemone *Metridium canum* in intimate contact with a banded mesogloeal fibre.

It has been suggested (Pantin, private communication to G. Chapman cited in Chapman, 1953) that the cells of the mesogloea are present in species which can absorb the mesogloea during starvation, and absent in those which cannot. This thought is restated by Robson (1957) who suggests that the passage of materials such as dissolved food and excretory products between endoderm and ectoderm, for example, and the reversible changes in the mesogloea which accompany growth, or regression during starvation, could perhaps be mediated by enzymes from these cells. She sees it possible that the mesogloeal cells form a physiological system throughout the body of the sea anemone, functioning in a continuous transport medium supplied by mesogloeal and subepithelial fluid.

The staining of "granules" in the mesogloeal cells with reduced methylene blue is interesting in the light of the above comments. Unfortunately it cannot be decided whether the staining is due to an affinity of pre-existing structures in the cells (such as granules, vacuoles, or cell organelles) for methylene blue, or whether it is due to other causes such as active engulfment of dye particles by mesogloeal cells. The former view seems probable as the property of "vital dyes" (especially netural red and methylene blue) in colouring cell valuoles and cisternae is well known (Baker, 1958). It is likely that the blue stained "granules" which appear to lie free between mesologeal fibres are in fact present in fine cytoplasmic extensions of mesogloeal cells. Although the origin of the mesogloea is not clear, there is little doubt as to its fundamental function. It is the base to which muscles are attached, and together with the muscles controls the deformation of the body wall (Batham and Pantin, 1951) by virtue of its visco-elastic properties. In addition Chapman (1966) states that it is tempting to look upon the mesogloea as a reserve of material on which the animal can draw in times of starvation.

Endoderm: The epitheliomuscular cells of cnidarian coelenterates are considered characteristic of this group. They were first described by Kleinenberg (1872, cited in Robson, 1957) in *Hydra* and have since been

found in other orders. As early as 1879 the Hertwig brothers showed that the muscle fibres in the endoderm of actinians were part of the endodermal cells themselves. The form of epitheliomusclar cells varies between the different orders in such characters as the number of myonemes present and the shape of the epithelial part of the cell, and their distribution throughout the body layers also varies. In *Hydra* for example, they are present in ectoderm and endoderm. The endoderm cells are phagocytic, and the ectodermal cells each have several muscle fibres and lack flagella (Goodrich, 1942). In *I. olivacea*, however, these cells are absent from ectoderm.

The endoderm of the column of *I. olivacea* is very similar to that from the mesentery of *Metridium senile* described by Robson (1957). In both cases the epithelial part of the cell is connected by a fine protoplasmic "foot" or "extension" to the muscle fibre part which lies on the mesogloea and forms (along with other muscle fibres) a muscle field (Batham and Pantin, 1951). As Robson points out, because the epithelial part of these cells is much wider than the part connecting with the muscle fibre, the connecting "stems' of these epitheliomuscular cells must traverse a cavity. In life this cavity, or space, would be filled with fluid, which thus would form a continuous thin layer between the epithelium and the muscle field, and probably would function in several ways. First, it would act as a hydrostatic layer when the muscle contracts, forcing the epithelial cell parts to become much longer and more slender (see section on endoderm under *Results)*. The epithelium thus follows closely any contraction of the muscle field. When the muscle fibres relax the epithelium returns smoothly to its original height. Relaxation is nearly always slower than contraction, and as Robson notes, is probably affected by viscosity of the protoplasm and mesogloea rather than by pressure changes in the subepithelial fluid. Second, Robson suggests that the subepithelial fluid is part of the general internal medium of sea anemone tissues, and must function to integrate local biochemical processes because diffusion could proceed readily in such a fluid layer. Later work (Batham, Pantin and Robson, 1960) has shown that the fluid provides the immediate environment for the neurites of nerve cells, as well as for all epithelial elements such as mucus cells and nematocysts (Robson, 1957).

Robson (1957) looks to the Turbellaria, Annelida, and vertebrates in search of an epithelial muscular structure which could show a functional parallel to the epitheliomuscular system of *Metridium*. She concludes that while true musculoepithelium is almost entirely confined to the cnidarian coelenterates, analogous tissues may occur in higher animals wherever a flexible epithelium covers unstriated muscle. Two examples can now be given from the Turbellaria which would seem to approach the situation described in *Metridium* by Robson, and in *I. olivacea*, more fully than the Turbellarian example used by Robson. These are the polyclad *Polycelis nigra* studied by Skaer (1961) and the triclad *Palombiella stephensoni* studied by Wineera (1971). In both of these flatworms no true epitheliomuscular cells are present, but sections of the body wall show that fluid filled spaces are present between the basal region of the cells of the ciliated epidermis. Skaer remarks that in *Polycelis* these spaces seem to form a ramifying system above the basement membrane. In both animals these spaces probably serve a hydrostatic function, in view of

the earlier study by Robson (1957), and taking into account the remarkable plasticity of the epidermis in these turbellarians. The morphology of these flatworms differs from that in *Metridium* and *I. olivacea* in that the muscles are not part of epithelial cells but are separated from them by a thick "basement membrane". However, a functional parallel does seem evident. Whether these fluid filled spaces in the flatworms also function to integrate local biochemical processes, as they may do in *Metridium* is not known, and elucidation of this problem will have to await more extensive studies on these animals.

The Hertwigs (1879) recognised that the condition as seen in the epitheliomuscular cells of actinians could be modified by stages in which the muscle fibre was developed at the expense of the rest of the cell. This is seen in the sphincter muscles of many actinians, and in, for example, the ectodermal tentacular muscles of *I. olivacea* (Pl. 2, Fig. 5), where the cell body is reduced to a small mass of cytoplasm surrounding the nucleus, and is connected by a narrow bridge of cytoplasm to the well developed muscle fibre. In these cases the cell body no longer performs an epithelial, or covering, function. The morphological similarity of a muscle cell taken from the tentacle of *I. olivacea* to that from the body wall of *Palombiella stephensoni* is striking (see Plate 2, Wineera, 1971). It is proposed to comment on this similarity at a later date.

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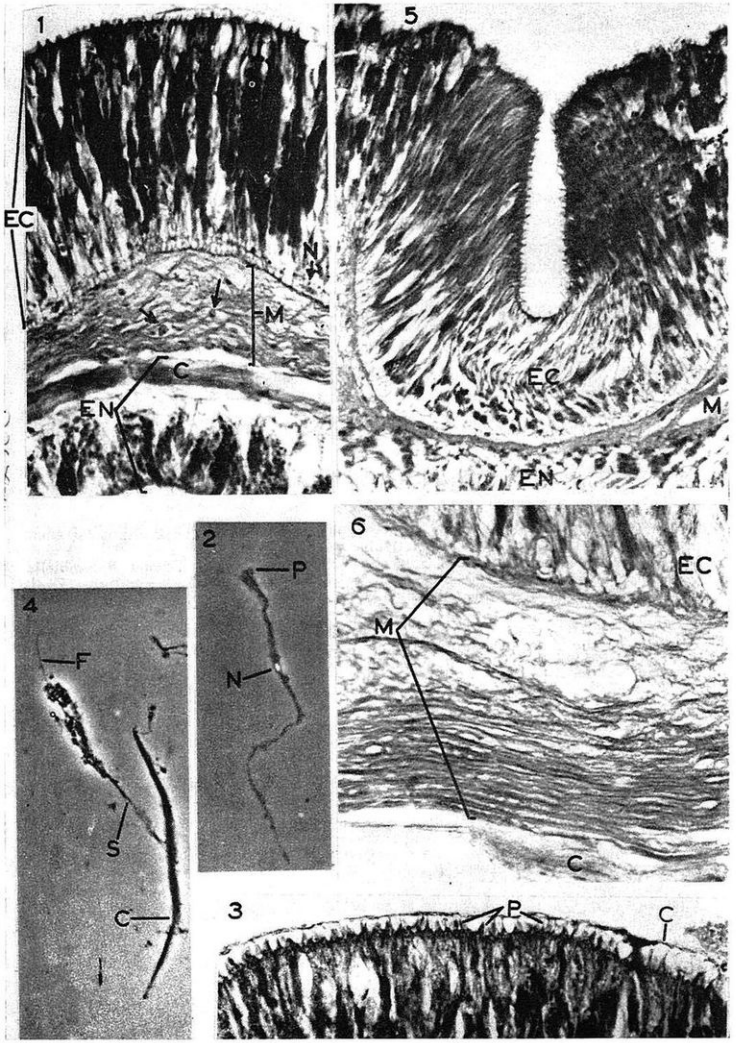

Plate 1—Fig. 1: The column body wall of **I. olivacea.** A transverse section stained by the Mallory/Azan technique. x 460.
C, muscle fibre; EC, ectoderm; EN, endoderm; M, mesogloea; N, ectodermal nerve net; arrows show mesogloeal cells.

Fig. 2: Supporting cell from the ectoderm of the column. Dissociation preparation photographed by phase contrast illumination. x 620.
N, nucleus; P, projection.

Fig. 3: Transverse section of the column showing the outer region of the ectoderm. Heidenhain's iron haematoxylin/orange G stain. x 930.
C, cuticle; P, projections.

Fig. 4: Epitheliomuscular cell from the endoderm of the column. Dissociation preparation photographed by phase contrast illumination. x 1,100.
C, muscle fibre; F, flagellum; S, cytoplasmic strand connecting muscle fibre to epithelial part of cell (Nucleus not showing).

Fig. 5: "Verruco-cinclide" from a transverse section of the column. Mallory/Azan preparation. x 490.
EC, ectoderm; EN, endoderm; M, mesogloea.

Fig. 6: Transverse section through column body wall showing orientation of mesogloeal fibres. Sulphation toluidine blue stain. x 740.
C, muscle fibre; EC, ectoderm; M, mesogloea.

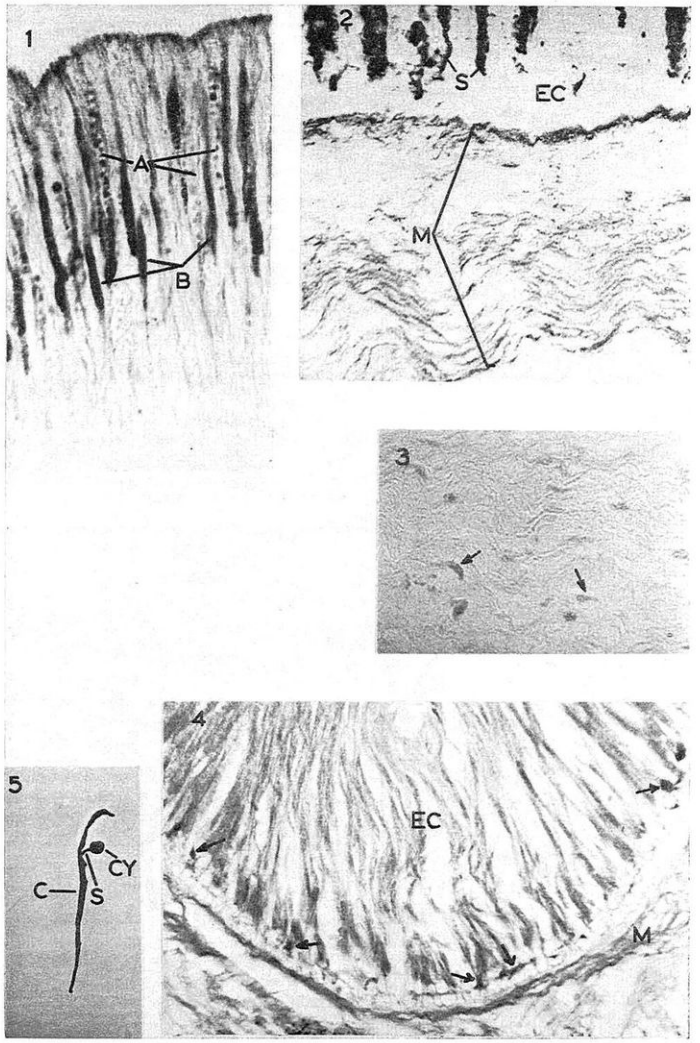

Plate 2—Fig. 1: Ectoderm of the column, stained with mercury/bromphenol blue. x 780.
A, glands cells of type A; B, glands cells of type B.

Fig. 2: Transverse section through column body wall to show mesogloeal fibres. Stained for reticulin by Gordon & Sweet's method. x 1000.
EC, ectoderm; M, mesogloea; S, secretion of gland cells of type C and E.

Fig. 3: Section of the mesogloea of the column body wall. Methyl green/pyronin stain; the cytoplasm of mesogloeal cells has stained with pyronin. Arrows show the location of some cells. x 900.

Fig. 4: Basal Portion of verruco-cinclide from section stained by Mowry/PAS/NYS after pepsin. Structures at the level of the nerve net (arrows) stain darkly with Mowry's reagent. x 760.
EC, ectoderm; M, mesogloea.

Fig. 5: Muscle cell from ectoderm of a tentacle of **I. olivacea**, drawn from a photograph of a dissociation preparation. x 760.
C, muscle fibre; CY, cytoplasm surrounding cell nucleus; S, cytoplasmic strand.

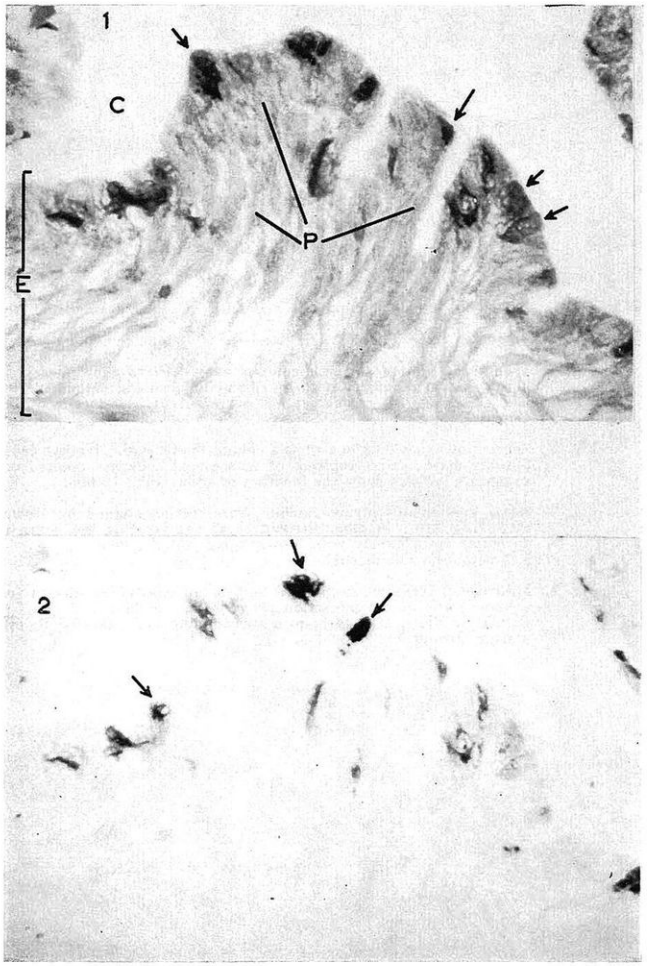

Plate 3—Fig. 1: Longitudinal section through endoderm of body wall, showing gland cells. Mowry colloidal iron/PAS/NYS stain. Photographed through a deep blue filter to emphazise the PAS staining of type "2" gland cells (arrowed), and the NYS positive type "1" gland cells (P) x 500. C, coelenteron; E, endoderm.

Fig. 2: The same section as Fig. 1, but photographed through a red filter to emphasize the Mowry colloidal iron positive type "3" gland cells (arrows). x 500.

THE BODY WALL OF THE HYDROID *SYNCORYNE TENELLA* (FARQUHAR, 1895)

PART 1: HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS

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ABSTRACT

The histology and histochemistry of the gymnoblastic hydroid polyp *Syncoryne tenella* (Farquhar, 1895) is described. The perisarc at the hydranth base is composed of an inner layer of a proteinaceous substance and an outer layer of acidic mucopolysaccharide. The hydranth is closely invested by a thin periderm of mucoprotein which is continuous with the inner perisarc layer of the hydrocaulus. The epitheliomuscular cells of the ectoderm of the hydranth contain mucoprotein secretory granules. The hydranth endoderm is differentiated into apical, middle, and basal regions. These are specialized for secretion of lubricating substances, for intracellular and extracellular digestion, and for intracellular digestion respectively. Gland cells present in the middle region are similar morphologically and histochemically, and probably functionally, to vertebrate pancreas acinar cells. Migration of cnidoblasts through the ectoderm of hydrocaulus and hydranth is proposed to explain their distribution in mature individuals. A new interpretation of the cells forming the core of solid tentacles is given. The mesogloea is commonly fibrous. It contains acidic mucopolysaccharide and mucoprotein.

INTRODUCTION

This paper is concerned with the structure and function of the body wall of a hydroid polyp, *Syncoryne tenella* (Farquhar, 1895). It is one of a series of papers which study the body wall of representative coelenterates, and flatworms (Phylum Platyhelminthes), with the ultimate goal being a consideration of the phylogenetic relationships between these groups.

Most hydroid research in the last 10-15 years has centred on the fresh water genus *Hydra*, and this animal is used extensively in the teaching of coelenterate and particularly hydroidean morphology and histology. It is, in fact, as Cowden (1965) has stated, difficult to obtain a general account of the histology or cytology of a hydroid other than a representative of the genus *Hydra*. Indeed, this was the reason which prompted Cowden to study another gymnoblastic hydroid *Pennaria tiarella* from the histological point of view. The present study on *Syncoryne tenella* provides further information on the structure of the hydroidean polyp as seen in more a typical member of the Order Hydroida than is *Hydra*. In addition the various histochemical tests applied give some idea of the

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functional relationship of the different components of the tissues present, and the results form the basis for comparison with *Pennaria tiarella*.

Syncoryne tenella is a colonial gymnoblastic (athecate) hydroid. Although the polyp is without a hydrotheca, the stolons forming the hydrorhiza, and the upright hydrocauli which bear the polyps, are composed of a well defined perisarc surrounding the coenosarc. The general morphology of *Syncoryne* hydranths has been described (Hyman, 1940). The feeding polyp is elongate, bearing scattered capitate tentacles (Pl. 1, Fig. 1). The base of the polyp is more or less a naked extension of the hydrocaulus and broadens steadily but smoothly into the body of the polyp. This is of uniform diameter throughout in an extended specimen, except at the hypostome which is often slightly swollen. Gonophores bud from the sides of hydranth, between the tentacles. The present paper is restricted to a study of the hydranth, and does not deal with gonophore development, histology or histochemistry.

MATERIAL AND METHODS

COLLECTION AND CULTURE OF ANIMALS: Specimens of *S. tenella* were obtained from Kau Bay, Wellington, N.Z. In this region the animals are found in association with *Obelia geniculata* colonies growing attached to fronds of the bladder kelp *Macrocystis pyrifera*. Fronds of the kelp bearing dense populations of *Obelia* were transported to the laboratory in buckets of sea water where they were examined for colonies of *Syncoryne*. The *Syncoryne* colonies were then carefully cut from the kelp fronds with sharp scissors. Care was taken that as little kelp as possible was left attached to the colonies, which were then placed in fingerbowls 4" in diameter x 1½" deep containing filtered sea water. The filtered sea water was beaten vigorously with a glass rod to stir air into it before it was used in the culture dishes. Aeration by a mechanical pump hindered attachment of colonies of *S. tenella* to the culture dishes. It was necessary to change the water in the dishes every day until the remaining pieces of kelp attached to the colonies had disintegrated. After this time the water was changed every two or three days.

The animals were fed twice a week on brine shrimp *(Artemia)* newly hatched from sea water. For the first week, when colonies were still attaching to the culture dishes, brine shrimp were presented to hydranths individually, on the end of a probe. When colonies had become attached however, the method of feeding was to pipette 20-30cc sea water containing a dense population of brine shrimp into the culture dishes. The dishes were then left for about half an hour, with occasional stirring of the water or rotation of the dishes to ensure an even distribution of brine shrimp to the colonies. During this time most hydranths had ingested 3-4 brine shrimp and some had ingested as many as 8 or 10. The number ingested was determined by counting the eyespots visible in the coelenteron. After this period of time each culture dish was emptied and any brine shrimp adhering to the bottom or sides of the dishes were removed by swilling fresh sea water around in the dishes, and filtered sea water was placed in them once more. The water was replaced again after 12-18 hours to remove digestive wastes expelled by the polyps.

Colonies of *S. tenella* kept in this way for three months remained healthy, and grew to a larger size than had been observed in their natural habitat. Usually hydranths grow from short, unbranched caulomes in their natural habitat but when the hydroid is cultured in bowls tall, branched hydrocauli are often produced. Gonophores were produced only rarely on cultured animals.

While culturing *S. tenella* in this way it was found that small pieces of *Obelia geniculata* colonies, if obtained free of bladder kelp, would survive a considerable time in the culture dishes. One small colony bearing 3 hydranths survived for one month. Little growth of this colony occurred, however, and the hydranths required feeding more often than those of *S. tenella*.

ANAESTHETIZATION & FIXATION. For the study of live individuals, hydranths were detached from the colony with a sharp probe, at the junction of hydrocaulus and stolon. The animals were transported from culture dishes to petri dishes or cavity slides with pipettes.

Fixatives employed were 10% formalin in sea water, Baker's formol/calcium (Pearse, 1960) and Lillie's FAA (Pearse, 1960). All fixatives were allowed to act overnight at room temperature. Specimens fixed in FAA were then removed to 70% ethanol for storage, but those fixed in the other fluids were stored in their respective fixatives.

Some animals were anaesthetized prior to fixation by placing them in sea water containing magnesium chloride in the proportions 5 sea water: 1 of 7% aqu. $MgCl_2$. $6H_2O$. After approximately 15 mins. the animals were rendered insensible in an extended position, and were placed into fixative. Some specimens were starved for one week prior to fixation, and others were fixed 12-18 hrs. following feeding.

HISTOLOGICAL & HISTOCHEMICAL METHODS: Most specimens were embedded in paraffin wax and sectioned at 5μ thickness on a Cambridge rocking microtome. Serial transverse and longitudinal sections were prepared.

Some of the formol/calcium fixed specimens were embedded in 15% gelatin and cut at 10μ thickness on a freezing microtome, and some of the FAA fixed animals were embedded in celloidin and sectioned at 15μ.

The staining methods employed have been described elsewhere (Wineera, 1971). An additional method used was periodic acid-Schiff followed by alcian blue staining followed by naphthol yellow S staining. This method demonstrates neutral mucopolysaccharides (and other PAS positive substances), acidic mucopolysaccharides, and protein in the one preparation. It is particularly useful because the naphthol yellow S stains nuclei as well as various protein aggregations. Hereafter it will be termed the PAS/AB/NYS technique. Periodic acid oxidation was for 5 min. in 0.5% HIO_4, and staining in Schiff's solution (prepared according to Coleman (cited in Gray, 1954)) was for 20 min. The alcian blue was used as a 0.1% solution in acetic acid at pH 2.6 for 20 min. and the naphthol yellow S was employed according to Deitch (1955). Control slides bearing serial sections were treated either in 0.02N HCl containing 2 mg. pepsin/ml for 3 hrs. at 37°C, or in 0.05 m.borate buffer containing 0.2 mg. trypsin/ml for 30 min. at 37°C prior to the staining schedule. Test slides were placed in 0.02N HCl or in 0.05 m.borate buffer without enzymes at 37°C for a similar time before staining.

Dissociated cells were obtained using the maceration techniques of Goodrich (1942) and the Hertwigs (1879, cited in Lee 1924). These were examined by phase contrast microscopy.

RESULTS

A. HISTOLOGICAL OBSERVATIONS: The body wall of *S. tenella* is made up of two epithelial layers, an outer ectoderm and an inner endoderm, which are separated by a thin layer of mesogloea (Pl. 1, Figs 3, 5). Three morphologically distinct regions can be recognized in the sagittally sectioned hydranth (Pl. 1, Fig. 1). These are a basal region, a middle region, and an apical region. The cells of the body wall layers have characteristic features in these different regions.

ECTODERM. The ectoderm of the hydranth varies from a pavement to a columnar epithelium. In an extended animal the tentacle ectoderm is a thin, pavement-like epithelium, while the ectoderm of the body of the hydranth is more cuboidal. In a contracted specimen the tentacle ectoderm is more cuboidal, and that of the body of the hydranth often becomes somewhat columnar. At the mouth there is a distinct ectodermal/endodermal junction, the cuboidal ectodermal cells giving way to the tall columnar cells of the endoderm.

When seen in section the ectoderm is covered by a thin (0.3μ) layer which grades into the perisarc at the hydranth base. This layer stains densely with iron haematoxylin, and with aniline blue in the Mallory/Azan technique (Pl. 2, Fig. 1, C). In a contracted animal it is extensively folded (Pl. 4, Fig. 4, C). Sometimes it is seen in a tangled mass which has been separated from the ectoderm (Pl. 1, Fig. 4, C). At the basal region of the hydranth this thin layer becomes covered by a thicker but less densely staining layer which often seems loosely applied to it. These layers then grade rapidly into the perisarc of the hydrocaulus which has two components: an inner layer which stains densely with aniline blue in the Mallory/Azan technique and which is continuous with the thin layer present over the hydranth, and an outer layer which stains lightly and is continuous with the thicker layer present in the basal hydranth region described above (Pl. 2, Fig. 5). These observations apply only to the annulated region of perisarc immediately beneath the hydranth.

The most numerous cell type in the ectoderm is the epitheliomuscular cell. Cells of this type vary in shape from pavement to columnar epithelial cells (as mentioned above) and each possesses a single muscle fibre in its basal portion. The muscle fibres run longitudinally, constituting the longitudinal musculature of the hydranth. They are best seen in iron haematoxylin and Mallory/Azan preparation (Pl. 3, Fig. 1A, MY). When seen in FAA fixed sections the cells possess a large (5μ diameter) circular nucleus situated centrally. A prominent densely staining nucleolus is present in each nucleus. At the hydranth base the ectoderm becomes thickened, and sometimes appears more than one cell thick. However on close examination the epitheliomuscular cells appear to retain their connection with the mesogloea. In this region the cells stain more heavily with basic dyes such as haematoxylin, and with azocarmine in the Mallory/Azan technique.

Nerve cells are also present in the ectoderm. In vital methylene blue preparations they are most numerous in the hydrocaulus, but also occur scattered over the hydranth. The nerve cells are mostly spindle shaped and bipolar, sometimes multipolar, and are situated at the base of the ectoderm just above the mesogloea. They are visible in iron haematoxylin and Mallory/Azan preparations by virtue of their basiphil cytoplasm, and their orientation at right angles to the ectodermal cells (Pl. 2, Fig. 1). They are most clearly seen in Formol/calcium fixed material. Sensory cells, which extend from the level of the nerve cells to the free surface of the ectoderm are also present, and possess fine cilia-like processes (Pl. 2, Fig. 2, S). Where these processes reach the surface the thin outer layer of the ectoderm is absent. Sensory processes also reach the ectodermal surface directly from the nerve cells. The nerve cell processes of the hydrocaulus run between the bases of the ectodermal cells. It was not possible to discover the position of nerve cell processes in relation to epitheliomuscular cells for the hydranth, nor was it possible to determine whether the sensory cells or the sensory processes of nerve cells reach the ectodermal surface by passing through or between ectodermal cells. Nerve cell processes are occasionally seen crossing the mesogloea to endodermal cells (Pl. 1, Fig. 7, N). Another cell type is visible in sections. These cells are small, approximately 5μ diameter, with cytoplasm evenly distributed around a nucleus of approximately 3μ diameter. A nucleolus is present. Cells of this type are found singly or in pairs between the bases of ectodermal cells (Pl. 1, Fig. 5). They are easily distinguished from nerve cells by their small size, and lack of polarity.

Nematocysts (all of which are ectodermal) occur predominantly in the swollen heads of the scattered capitate tentacles. However they also occur in the tentacle shaft, scattered over the hydranth body, and in the hydrocaulus. In this last region they are numerous (Pl. 3, Fig. 4). Three types of nematocyst have been positively identified in dissociation preparations. These are stenoteles, atrichous isorhizas (according to the classification of Weill, cited in Hyman (1940), and a third type (Pl. 4, Fig. 6 B) resembling a stenotele without stylets. A fourth type (Pl. 4, Fig. 6A) is less frequently seen. This resembles type 3, but does not show the same curved structures within the capsule. Three size ranges of the first type occur, in which the capsule length x width is 8μ x 5μ, 12μ x 9μ, and 16μ x 13μ respectively. The second type of nematocyst measures 5μ x 3μ, and the third and four types 16μ x 13μ. Developing nemotocysts are seen in the stolon, and on the hydranth body. They have not been seen on the tentacles.

MESOGLOEA. The mesogloea is acellular, and in animals anaesthetized before fixation it appears in sections as a thin line. It stains intensely with aniline blue in the Mallory/Azan technique. It is more easily studied in non-anaesthetized animals, for contraction of the animals during fixation thickens the mesoglocal layer. In such animals fibres are visible in the mesogloea (Pl. 3, Fig. 1A and 1B). These appear to be arranged longitudinally beneath the ectodermal muscle layer, and circularly beneath the endodermal muscle fibres. However the fibres could be followed for only a short distance, so it is possible that they are cut obliquely and have a different orientation. Between these coarse mesogloeal fibres there often appears to be a much finer fibre network. In

the longitudinal section of a contracted animal, the mesogloeal layer immediately beneath the endoderm is thrown into folds, along with the endodermal epithelium (Pl. 4, Fig. 5, M).

ENDODERM. This layer consists of epitheliomuscular cells, gland cells, and nerve cells. The former are columnar epithelial cells which possess a basal muscle fibre, and a single flagellum at their free surface (Pl. 1, Fig. 8). The muscle fibres are orientated circularly constituting the circular muscles of the hydranth body wall. In the basal and middle regions of the hydranth the epitheliomuscular cells are usually large (9μ x 20μ in sagittal section) and often subtend two ectodermal epithelial cells at their base. In sections of FAA fixed specimens the basal half of the cell appears empty (Pl. 1, Fig. 3; Pl. 4, Fig. 5), with the cytoplasm and nucleus placed in the apical (distal) half. The nuclei of these cells measure 4-5μ diameter and appear circular or near-circular in section. Each possesses a prominent nucleolus. In the apical region of the hydranth the epitheliomuscular cells become more slender.

If an animal is sectioned after a period of starvation, the apical region of the cells contains vacuoles in which are small dark bodies (Pl. 4, Fig. 5). Flagella are clearly visible. When an animal is sectioned 12-18 hrs. after feeding these cells are packed with eosinophil globules 0.5 - 2.0μ in diameter. Many of the globules are aggregated inside vacuoles. They are very well shown in preparations fixed in Baker's formol/calcium (Pl. 2, Fig. 1). Flagella are not seen, in this instance, as commonly as when the animal is fixed and sectioned before feeding. Vacuoles containing similar staining globules are also seen in the tentacle endodermal cells (Pl. 4, Fig. 3).

Scattered throughout the endoderm of the middle hydranth region are secretory cells (Pl. 3, Fig. 2, D). They are tall and slender (15 - 20μ x 8μ) in section, with a round nucleus 4μ in diameter which is centrally placed. A prominent nucleolus is present in FAA fixed preparations. The basal cytoplasmic regions stain intensely with basiphil dyes such as haematoxylin, and with azocarmine in Mallory/Azan preparations. The apical region of the cells contains eosinophil granules approximately 1.5μ in diameter. After a period of starvation most of these secretory cells are packed full with eosinophil granules and little basiphil cytoplasm is evident. However 12 - 18 hrs. after feeding fewer eosinophil granules are present in the cells, which now stain heavily with basic dyes.

Gland cells are numerous in the apical hydranth region (Pl. 1, Fig. 2, G). They are tall and slender (15μ x 8μ) often with a slightly swollen distal end. Two types are recognizable histologically: One type possesses moderately eosinophil cytoplasm which is finely granular. The other type stains with aniline blue in the Mallory/Azan technique. Cells of this second type also appear finely granular, but sometimes a network of densely staining material is present. In an animal fixed with FAA without prior anaesthetization, the endoderm is thrown into villi-like radial folds. These are very prominent in the apical region of the hydranth (Pl. 1, Fig. 2). They run longitudinally down the endoderm becoming less prominent in the middle region and unrecognizable in the basal region of the polyp. The glands of the apical region of the polyp are disposed around the perimeter of the folds. In animals anaesthetized before fixation the folds are much less prominent.

TABLE I. REACTIONS FOR CARBOHYDRATE

TISSUE CONSTITUENT / HISTOCHEMICAL TEST		PAS Before Diastase	PAS After Diastase	MOWRY COLLOIDAL IRON	METACHROMASIA WITH TOLUIDINE BLUE AT pH 4.0 In H_2O	METACHROMASIA WITH TOLUIDINE BLUE AT pH 4.0 In DePeX	METACHROMASIA WITH TOLUIDINE BLUE AFTER SULPHATION	ALCIAN BLUE	PAS / AB / NYS Before Pepsin	PAS / AB / NYS After Pepsin
ECTODERM	CUTICLE — Apical & Middle Hydranth Regions	+++	+++	-	++	-	-	-	+++ PAS ++ NYS	
	CUTICLE — Basal Hydranth Region	++	++	++	++	?	?	++	+ PAS ++ AB	++ AB
	PERISARC — Inner Layer	+	+	-	-	?	?	-	+ PAS +++ NYS	-
	PERISARC — Outer Layer	-	-	+++	++	?	?	+++	+++ AB	+++ AB
	EPITHELIO-MUSCULAR CELL — Hyonome	-	-	-	-	-	-	-	+++ NYS	-
	EPITHELIO-MUSCULAR CELL — Cell Body	+++ Inc. +++ P.G.	- Inc. +++ P.G.	-	-	-	-	-	+++ PAS INC. +++ PAS P.G.	+++ PAS INC. - P.G.
MESOGLOEA		+++	+++	++ In Parts	++	-	++	++ In Parts	+++ PAS +++AB In Parts	++ PAS InParts +++ AB
ENDODERM	APICAL GLAND CELLS — No. 1	+++	+++	-	-	-	?	-	+++ PAS ++ NYS	-
	APICAL GLAND CELLS — No. 2	++	++	+++	+++	-	+++	+++	+++ AB	+++ AB
	APICAL GLAND CELLS — No. 3	-	-	-	-	-	-	-	+++ NYS	-
	DIGESTIVE CELL — Cytoplasm	-	-	-	-	-	-	-	++ NYS	-
	DIGESTIVE CELL — Secretory Granules	-	-	-	-	-	-	-	+++ NYS	-
	EPITHELIO-MUSCULAR CELL — Hyonome	-	-	-	-	-	-	-	+++ NYS	-
	EPITHELIO-MUSCULAR CELL — Vacuolar Contents in Starvation	Some ++	Some ++	Some +	-	-	?	-	Some ++ PAS Some + AB	Some + PAS Some + AB
	EPITHELIO-MUSCULAR CELL — Vacuolar Contents after Feeding	Some ++	Some +	Some ++	Some ++	Some ++	Some +++	Some ++	Most ++ PAS +++ NYS	Most + PAS + NYS

- NO REACTION: + WEAK REACTION: ++ MODERATE REACTION: +++ STRONG REACTION: ? DOUBTFUL REACTION

Table 1: Reactions of the body wall constituents of **S. tenella** to tests for carbohydrates.

TABLE 2 REACTIONS FOR RNA, PROTEIN & LIPID

TISSUE CONSTITUENT / HISTOCHEMICAL TEST		PYRONIN STAINING		MERCURY BROMPHENOL BLUE	NAPHTHOL YELLOW S	MILLON	SAKAGUCHI	DMAB-NITRITE	ELASTIN	RETICULIN	OIL RED O
		No RNase	RNase								
ECTODERM											
CUTICLE	Apical & Middle Hydranth Regions	-	-	-	++	-	-	-	-	-	-
	Basal Hydranth Region	-	-	-	-	-	-	-	-	-	-
PERISARC	Inner Layer	-	-	+	+++	-	-	-	-	-	-
	Outer Layer	-	-	-	-	-	-	-	-	-	-
EPITHELIO-MUSCULAR CELL	Myoneme	-	-	+	+++	++	-	-	-	-	-
	Cell Body	++	-	-	+	-	-	-	-	-	▪
MESOGLOEA											
	No. 1	-	-	+	++	-	-	-	-	-	-
	No. 2	-	-	-	-	-	-	-	-	-	-
	No. 3	++	-	+	+++	+ to ++	-	-	-	-	-
ENDODERM											
DIGESTIVE CELL	Cytoplasm	+++	-	-	++	-	-	-	-	-	-
	Secretory Granules	-	-	++	+++	+++	-	++	-	-	-
EPITHELIO-MUSCULAR CELL	Myoneme	-	-	+	+++	++	-	-	-	-	-
	Vacuolar Contents in Starvation	-	-	-	-	-	-	-	-	-	-
	Vacuolar Contents after Feeding	-	-	Some ++	Some +++	Some ++	-	-	-	-	-

- NO REACTION: + WEAK REACTION: ++ MODERATE REACTION: +++ STRONG REACTION

Table 2: Reactions of the body wall constituents of **S. tenella** to tests for RNA, protein, and lipid.

The tentacles are solid, with a core of endodermal cells. These are all epitheliomuscular cells which do not bear a flagellum. A longitudinal section of a tentacle shows two layers of endodermal cells in close contact. The cytoplasm and nucleus of the cells of each layer are distally placed and thus are in close proximity (Pl. 4, Fig 1, EN). In transverse section the endodermal cells are seen to be radially arranged, with the cytoplasm and nucleus of each cell close to the centre of the tentacle (Pl. 4, Fig. 2).

B. HISTOCHEMICAL OBSERVATIONS: Histochemical results are summarised in tables 1 and 2. Features not able to be expressed in the tables or those not readily seen from the tables are described below.

ECTODERM. 12-18 hrs after feeding many of the epitheliomuscular cells of the ectoderm show an intensely PAS positive substance in the form of irregularly shaped bodies. These are removed by digestion with diastase for 30 mins. (Pl. 2, Figs 4A, 4B), and in table 1 are denoted as "PAS +++ INC.". Others of the same type of cell contain very fine granules which are also intensely PAS positive (Pl. 1, Fig. 6). These granules are denoted as "PAS +++ FG" in table 1. They are resistant to diastase digestion, but are removed by pepsin digestion. Their presence seems unaffected by the feeding state of the animal.

At the base of the hydranth, the ectodermal cells contain coarse granules which stain intensely with Mowry's colloidal iron reagent. This is not shown in table 1 (see Pl. 5, Figs. 1A and 1B).

The nerve cells stain strongly with pyronin in the methyl green/pyronin technique. This is prevented by digestion with RNase.

The small round cells found at the base of the ectoderm display moderate pyroninophilia which is removed by RNase digestion.

MESOGLOEA. In the PAS/AB/NYS test before pepsin digestion the mesogloea colours an intense red with here and there patches of deep blue (Pl. 4, Figs. 4A, 4B); however this appearance is reversed after pepsin digestion, that is, the mesogloea appears blue with patches of red.

ENDODERM. Intensely PAS positive structures which are diastase labile are found in the endodermal epitheliomuscular cells a short time after feeding. This is not shown in table 1. Animals sectioned 12-18 hrs. after feeding with *Artemia* show fragments of *Artemia* exoskeleton, of various sizes, within endodermal cells of the basal and middle hydranth regions. These fragments stain with the PAS and AB techniques.

Three types of gland cell can be distinguished histochemically in the apical hydranth region. One type (type 1) is only found immediately surrounding the mouth. It is very finely granular and intensely PAS positive. Another type (type 2) extends from a region just below the mouth throughout the apical region of the hydranth. It mostly appears as a "network" structure and stains moderately strongly with the PAS test but intensely with the AB and Mowry colloidal iron tests (Pl. 3, Figs. 3A, 3B). The third gland cell type stains strongly with NYS. It is also finely granular, and is found around the mouth region. Types 1 and 2 appear to correspond to the gland cells which stain with aniline blue in Mallory/Azan preparations, while type 3 corresponds to eosinophil glands described earlier.

DISCUSSION

ECTODERM. The coelenterates of the Order Hydroida have long been divided into two groups depending on whether or not the hydranths are enclosed within a chitinous periderm (Hyman, 1940). In the calyptoblastic (thecate) hydroids the perisarc of the stems expands at polyp bases to form cup-like structures (hydrothecae) which loosely enclose the hydranths. In the gymnoblastic (athecate) hydroids, the perisarc of stems (if present) ends abruptly at the base of hydranths which are thus naked. The results of the present study indicate, however, that in *S. tenella* a periderm which is continuous with the perisarc at the hydranth base is present on the hydranth. The periderm is visible only in histological preparations but it is unlikely to be artifact since different fixatives preserve it in the same form and confer upon it similar staining properties, both histological and histochemical. It is much thinner than the perisarc, and is very closely applied to the surface of the ectodermal cells (Pl. 2, Fig. 1, C) except in some places where it projects from the hydranth as a tangled mass (Pl. 1, Fig. 4, C). It is therefore quite different in morphology to the periderm forming the hydrothecae of calyptoblastic hydroids, and in order to avoid a confusion of terms it will for the time being be called a cuticle.

Rudall (1955) states that *Protohydra* (a naked hydroid) produces a delicate periderm, but he does not elaborate on its nature. Also, the electron microscope reveals a thin cuticle covering the surface of *Hydra* (Hess, 1961). Manton (1940) shows a cuticle which is very similar to that of *S. tenella* to be present on the athecate hydroid *Myriothela penola*. Cowden (1965) studied the cytology of the gymnoblastic hydroid *Pennaria tiarella*, but makes no mention of the presence of a periderm on the hydranth. However an examination of some of his figures, especially photographs of PAS/AB, and acrolein/Schiff (for protein) stained sections shows a thin, dense line at the free surface of the ectoderm which appears very similar to the cuticle of *S. tenella*. It would be interesting to know if a similar covering is present on the hydranths of gymnoblastic hydroids in general or if it is confined to those animals cited above. As Rudall has stated, " . . . . it is a question as to whether the whole mechanism of periderm formation is completely eliminated in naked forms." (Rudall, 1955, p.52).

The cuticle appears to be very flexible, for on contraction of the hydranth it is thrown into folds (Pl. 4, Fig. 4A, C). Such folding must deform the ectodermal cells, especially their distal ends, but to what extent this occurs has not as yet been determined.

Histochemical tests indicate that the cuticle varies in composition in different regions. On the apical and middle regions of the hydranth it appears to be composed of a mucoprotein, or neutral mucopolysaccharide (according to the carbohydrate classification of Pearse (1960)), since it gives a positive reaction in the PAS test and with NYS. However since staining by these methods is abolished by digestion with pepsin, it is likely that the polysaccharide component is firmly bound to protein, and it may be deduced that a mucoprotein is the substance present. At the basal region of the hydranth the cuticle seems to be composed of an inner layer of mucoprotein (which stains less intensely with PAS and NYS than that of the apical and middle regions), together with an outer layer of an acidic mucopolysaccharide. These two layers are well differentiated in the perisarc in the region of annulations at the polyp base where they are both considerably thicker. Here, the inner layer stains only weakly with the PAS test, and more strongly with NYS. Staining is still abolished by pepsin digestion. The outer layer stains strongly in tests for acidic mucopolysaccharides (Pl. 5, Fig. 2). A double layered perisarc has been described by Berrill (1949) in the stolons of *Obelia*. As in the present study, the layering is evident in the region of annulations at the hydranth base, and Berrill considers that the inner perisarc layer of closely packed lamellae is laid down subsequent to the outer layer of loose annular lamellae. No histochemical staining was attempted however, so that detailed comparison between the perisarc of *Obelia* and that of *S. tenella* is not possible.

The periderm of hydroids has long been described as being chitinous. Rudall (1955), by means of X-ray diffraction, observed the presence of chitin in *Tubularia* colonies and in other hydrozoans. The diffraction patterns obtained were different from the patterns shown by purified arthropod chitin, but that of *Tubularia* approached the standard arthropod chitin pattern more closely than the other hydrozoans studied. *Syncoryne tenella* is commonly called a "tubularian hydroid" (Hyman, 1940) and it is reasonable to suppose that it's perisarc is chitinous. This supposition does not go against the histochemical findings of the present study, at least as far as the inner perisarc layer is concerned: Pearse (1960) considers chitin to be usually PAS negative but positive in tests for protein if it is present as protein complexes, and it has been seen that the inner perisarc layer is only weakly PAS positive, and stains with NYS. He further states that chitin does not occur in the pure form in nature but is always mixed with calcium carbonate, or protein, or both. Chitin does not, according to Pearse, stain in tests for acidic mucopolysaccharide, so that the outer perisarc layer in *S. tenella* may not be chitinous, or may contain only very small amounts of chitin. Similarly, the cuticle of the apical and middle regions of *S. tenella*, being intensely PAS positive, may not be chitinous or may contain small amounts only. A third possibility is that the outer perisarc layer and the cuticle may contain "non-typical" chitin. It is noteworthy that Rudall (1955) found that the chitin of millipore colonies showed non-typical X-ray diffraction patterns which he considered likely to indicate a second principal constituent.

Berrill (1949) considers the perisarc of *Obelia* to be secreted by a specialized ectodermal cell and not by the general epidermis since various regions, for example, the tentacles, are free of perisarc. He described ovoid cells containing iron haematoxylin staining granules in the epidermis of the growing stolon tip, and the stem epidermis. In the present study, however, the perisarc (at least the inner perisarc layer) and the hydranth cuticle appear to originate by secretion of substances from the general ectodermal cells. These cells were found to contain fine PAS positive granules (Pl. 1, Fig. 6) in their cytoplasm. As with the cuticle, the PAS positivity was removed by pepsin digestion which suggests that the cuticle and these ectodermal granules have a similar chemical composition consisting of a polysaccharide/protein complex.

The acidic mucopolysaccharide granules seen in the ectoderm at the hydranth base in the present study (Pl. 5, Fig. 1B) quite possibly correspond to the iron haematoxylin staining granules described by Berrill

(1949), since basic dyes such as haematein (the true dye substance of haematoxylin) have a great affinity for acidic tissue substances. However, the use of iron mordants can modify the haematoxylin staining reaction so that virtually all tissue components may be stained, and because of this no great significance can be placed on the above mentioned possibility.

In the present study there remains the question as to whether the general ectodermal cell in the region of perisarc annulations is capable of secreting both the acidic mucopolysaccharide substance(s) of the outer perisarc layer and the mucoprotein substance(s) of the inner layer, or whether specialized cells perform the former function. A further question is whether the 2 layered structure of the perisarc described here is confined to regions of annulations, or whether it is the general pattern of perisarc structure. Until this is known, it would be premature to speculate on the properties which the 2 layered structure might confer on the perisarc, and thus on the hydrocaulus.

The small masses of cuticle which have been seen to project from the hydroid (Pl. 1, Fig. 4, C) are peculiar structures. It is possible that they are artifacts due to separation of the cuticle from the underlying ectoderm during processing. If they are not artifacts, and in the living animal serve some particular function, this function is not at present apparent. The cuticle as a whole can only be supposed, at this time, to perform a protective role.

The other PAS positive, but distase labile ectodermal bodies (Pl. 2, Figs. 4A and 4B) are concluded to be glycogen. They were abundant in both ectoderm and endoderm a short time after feeding, but not often seen in specimens fixed and sectioned after a period of starvation. Hyman (1940) states that excess food coelenterates is stored in the gastrodermis (endoderm) chiefly as fat but that glycogen may be stored without change. In the present study no lipid was detected in either epithelial layer, but the glycogen deposits already mentioned may serve as a food reserve. Both epithelial layers, since they possess muscle fibres, would presumably require reserves of food for muscular contraction as well as other metabolic activities. It is possible that lipid may be stored in other parts of the colony such as hydrocaulus.

The ectodermal cells also contain moderate amounts of RNA (Pl. 2, Figs. 3A, 3B), indicating that protein synthesis occurs within them. This is probably of two main types: Synthesis of proteins to maintain the contractile appartus of the cell, and synthesis of the protein moiety of the secretion granules which form the cuticle. Staining for protein by NYS in ectodermal cells is weak, except for the muscle fibres, but it is possible that proteins within ectodermal cells lack sufficient basic groups to bind NYS; Deitch (1955) considers that NYS is bound by E-basic groups of lysine, arginine, and histidine in tissue sections. There is, however, a distinct increase in the amount of protein in the ectodermal cells immediately surrounding the mouth of the hydranth, an observation also made by Cowden (1965) for *Pennaria* hydranths.

It is notable that at the base of the polyp, where the inner proteinaceous perisarc layer is much thicker than is the cuticle, the underlying ectodermal cells contain more RNA (as judged by RNase reversible pyroninophilia), and they stain more densely with NYS.

Results indicate that the cnidoblasts present in the tentacles of *S. tenella* arrive there by migration through the ectoderm of the hydrocaulus and hydranth. Cnidoblasts bearing mature nematocysts are found in the head and shaft of tentacles, in the hydranth and in the hydrocaulus. Cnidoblasts with immature nemotocysts are present only in the hydranth and hydrocaulus, not the tentacles. Further, the nematocysts in tentacle shaft and hydrocaulus, and often those in the hydranth, are orientated with their long axis parallel to the surface of the ectoderm. This suggests that they are in transit rather than in the sites in which they will be used. There is no indication of a "nematocyst replacement zone" in the tentacle ectoderm immediately beneath the terminal cap as was found by Cowden (1965) for *Pennaria* capitate tentacles.

Cnidoblast migration has been described in *Hydra* (Lenhoff, 1959; cited in Picken & Skaer, 1966). Also the early observations of Hadzi (1907, in Picken and Skaer, 1966) on the migration of ectodermal cnidoblasts in *Tubularia* from perisarc covered regions of the hydrocaulus have been confirmed by more recent studies (Tardent, 1962, and Tardent & Eymann, 1959; both cited in Picken & Skaer, 1966). In *Tubularia*, however, the supply of cnidoblasts to the hydranth is discontinuous and the renewal of the nematocyst stock coincides with regeneration of a new hydranth (Picken & Skaer, 1966). During this process large numbers of cnidoblasts accumulate at the primordial area, so that the replacement of cnidoblasts is here linked with normal, regular autotomy of the hydranth. However, Mackie (1966) showed that in fully grown *Tubularia crocea* many weeks old, there exists a capacity for large scale cellular proliferation (as evidenced by, for example, regrowth of amputated tentacles), and for production of nematocysts and digestive enzymes. Mackie points out that such hydranths had been using up nematocysts and digestive products in the daily process of feeding throughout their lives. The "normal, regular autotomy" of hydranths did not occur in Mackie's cultures of *Tubularia*, and he states that in gymnoblastic hydroids such autotomy has only been observed in the laboratory under wholly unnatural conditions. He concludes that it is very probable that the hydranths of *Tubularia*, and perhaps of gymnoblastic hydroids in general, can live indefinitely.

In the present study sections of *S. tenella* buds (hydranth primordia) show clusters of cnidoblasts with developing nematocysts in the ectoderm, and it is noteworthy that in the mature hydranth no clusters of cnidoblasts are visible except in the capitate tentacles. It seems probable, therefore, that nematocysts are initially provided in *S. tenella* hydranths by a similar process to that described above for *Tubularia*, and that in the mature hydranth other migratory mechanisms are involved. Further work on this problem is desirable, especially the examination of sections of hydranths at different stages of development. One of the nematocysts seen in this study (Pl. 4, Fig. 6B) presents a curious internal structure. No explanation of the function of such a structure can be given at present. It seems unlikely to be artifact, because of its clarity, and contrasts markedly with the nematocyst shown in Pl. 4, Fig. 6A. This latter nematocyst capsule appears to have been flattened, or compressed during preparation and does not show the internal structure of the first. It resembles a stenotele which has lost its barbs.

The hydroids characteristically possess undifferentiated cells (interstitial cells) which are able to differentiate into any of the cell types when required (Hyman, 1940). The clusters of interstitial cells seen in

Hydra have long served as a "model" for the way in which these cells are organized with respect to the epithelial layers. However, nothing resembling the clusters of interstitial cells which occur in *Hydra* are found in *S. tenella,* and if any kind of cell in *S. tenella* is to be called an interstitial cell there seem to be only two contenders. These are the small cells which occur singly at the base of the ectoderm, and the cells also occurring singly which have already been termed nerve cells. Several factors indicate that the former may be interstitial cells. Firstly, the cells are small, and round in sections, as are the interstitial cells of *Hydra;* the cells have no cytoplasmic extensions as are seen in nerve cells. Secondly, the moderate RNase labile pyroninophilia of the cytoplasm of the cells indicates the presence of RNA which Singer (1952) and Slautterback (1961) show is characteristic of interstitial cells. Thirdly, the morphology of the nucleus in the cells is very similar to that of cnidoblasts with mature or developing nematocysts. The cells found singly at the base of the ectoderm in *Pennaria* (Cowden, 1965) are similar in morphology to bipolar nerve cells when seen in section. Cowden found these cells to have a high level of nucleolar and cytoplasmic RNA, and on this basis identified them as interstitial cells. However it is characteristic of nerve cells to have high levels of RNA also. He states that the "interstitial" cells were not numerous, and that no epidermal concentrations of these cells was ever observed. He found difficulty in identifying nerve cells, and did not describe them.

The fact that interstitial cells in *S. tenella* occur singly and not in clusters indicates that they may migrate from a region of proliferation, and in the present study the only mitotic figures observed occurred in the ectoderm at the base of the hydranth. The increased RNA content of cells in this region may indicate increased metabolic activity and perhaps support the view that it is a region of cell proliferation. However in this region the small interstitial cells do not appear more numerous than in other regions. Haynes & Burnett (1963) have demonstrated that in *Hydra* the mucous cells of the gastroderm have the capacity to dedifferentiate into interstitial cells and redifferentiate into other cell types including ectodermal cells. Also Slautterback (in Lenhoff & Loomis, 1961, p.314) states that when the pedal disc of *Hydra* is amputated, the secretory cells are soon replaced by partial dedifferentiation of cnidoblasts followed by differentiation into secretory cells. It seems that "totipotency" may be a property of more cells than just interstitial cells. A similar mechanism of dedifferentiation, if it occurred in either ectoderm or endoderm of *S. tenella,* could also explain the sparse distribution of interstitial cells. Cowden (1965) thinks it possible that in the nematocyst replacement zone of *Pennaria* capitate tentacles, ectodermal cells may differentiate into interstitial cells which subsequently redifferentiate into cnidoblasts.

MESOGLOEA. The mesogloea of hydroids, especially that of the polyps (as opposed to hydromedusae) continues to be a very difficult subject for study. In polyps the mesogloea is thin and difficult, if not impossible, to isolate, so that its chemical composition must be inferred from its staining reactions. Even in *Hydra,* a hydroid which has been very extensively studied (Lenhoff and Loomis, 1961), the structure of the mesogloea is still not well known. Very little work has been done on the mesogloea of other hydroid polyps.

The mesogloea of *S. tenella* stains blue in the Mallory and Mallory/Azan methods. Hess, Cohen, and Robson (1957) noted a similar result for *Hydra* mesogloea, and believed it to indicate an affinity with the mesogloea of other coelenterates and with collagens. Rudall (1955) however examined whole freeze dried *Hydra* specimens by X-rays but could not detect any collagen. He concluded that collagen is either absent or at most minutely present in hydrozoan polyps.

The fibrous nature of the mesogloea evident from this study is very similar to that found by Cowden (1965) for *Pennaria.* Chapman (1953, 1966) showed the mesogloea of hydromedusae to be fibrous, and Hess, Cohen and Robson (1957) found that the mesogloea of freeze dried formalin fixed *Hydra* sometimes exhibited fibres visible in the light microscope. In the electron microscope, however, they found the mesogloea to be finely granular, with some evidence of very fine fibrils which certainly could not be seen with the light microscope. An electron microscope study of *S. tenella* is planned by the present author, and it will be interesting to discover if the mesogloeal fibres seen with the light microscope are visible in electron micrographs.

The staining reaction of the mesogloea in the PAS/AB/NYS test suggests that it is composed predominantly of PAS positive but pepsin labile mucoprotein, together with acidic mucopolysaccharide. Further, the sites of PAS positivity following pepsin digestion indicate a non-protein bound constituent, perhaps neutral mucopolysaccharide. The failure to exhibit metachromasia after toluidine blue staining (when examined in DPX) could indicate a low degree of sulphation of the acidic mucopolysaccharides present (Pearse, 1960; Bergeron & Singer 1958) or that the positions of the binding sites (carboxyl, sulphate) do not favour the expression of metachromasy (Bergeron & Singer, 1958).

Although it seems that the mesogloea must originate from the cell layers, Chapman (1966) believes that in hydrozoans there is no evidence of any special intracellular precursors in ectoderm or endoderm. However the present study reveals mucoprotein granules in many ectodermal epitheliomuscular cells in *S. tenella.* It is possible that these mucoprotein granules are mesogloeal precursors, as well as cuticle precursors, but the difficulty of chemical analysis of the delicate cuticle and mesogloea probably will not allow the testing of such a hypothesis. In any case, the present results certainly lend weight to Chapman's (1966, p.165) statement that "The secretion, on the outside of the ectoderm of various Hydrozoa, of the aminopolysaccharide chitin and on the inside of the mucoprotein and collagenous mesogloea are perhaps not as far removed from one another chemically as was first considered."

As Chapman (1966) says, there has never been much doubt that the function of the mesogloea is to stick the cells and cell layers together and to provide a flexible bed to which muscle fibres can be attached. The folding of the mesogloeal layer in contact with the endodermal cells on contraction of the ectodermal muscles (Pl. 4, Fig. 5, M) is very similar to the process of buckling which occurs in sea anemones (Batham & Pantin, 1951), and may indicate similar mechanical properties of the mesogloea in the two groups.

ENDODERM: There is a marked histological and histochemical specialization of the endoderm in the basal, middle, and apical hydranth

regions. This has also been observed in *Pennaria* (Cowden, 1965). Such specialization is in full accord with the concept of digestion in hydroids. In these animals, and in many other hydrozoans, after food has been ingested, digestion occurs in two phases (Hyman, 1940). The first phase is one of extracellular digestion, in which ingested food is reduced to a kind of "broth" containing fragments and liquid in the coelenteric cavity. The second phase is one of intracellular digestion following the active ingestion by certain cells lining the coelenteron of the broth produced by the first digestive phase.

In the apical region of *S. tenella* histochemical tests indicate the presence of three types of gland cells. Type 1 appears to have a mucoprotein secretion, type 2 a predominantly acidic mucopolysaccharide secretion, and type 3 a proteinaceous secretion. In addition, the type 2 gland cell may contain a neutral mucopolysaccharide, or mucoprotein, as it displays moderate PAS positivity, a feature not characteristic of acidic mucopolysaccharides (Pearse, 1960). Cowden (1965) recognises two types of gland in the pharynx region of *Pennaria*, one which is both PAS and AB positive, and another which stains for protein.

The function of each of these different secretions would be difficult to determine. They possibly act independently, or perhaps together, probably to lubricate the pharynx region during the swallowing of stunned prey. The villated appearance of the pharynx (that is, the endoderm of the apical region) in transverse section has been reported also by Cowden (1965) and Wineera (1968) in other athecate hydroids.

The middle endoderm region is one of both extracellular and intracellular digestion. The gland cells here are probably cells which secrete the enzymes necessary for extracellular digestion. The cytoplasm of these cells is rich in RNA (Pl. 2, Figs. 3A, 3B) and they contain large protein granules which stain in the Millon test for tyrosine and in the DMAB-nitrite test for tryptophan. In fact their morphology and staining reactions are strikingly similar to those of vertebrate pancreas acinar cells, an observation made by Cowden (1965) for similar secretory cells in the middle hydranth region of *Pennaria*. While Cowden observed the "digestive cell" granules to be both PAS and acrolein/Schiff (protein) positive, in the present study the granules were PAS negative. The timing of the secretory cycle, or rather, the cycle of secretory granule synthesis, also suggests that these cells secrete the enzymes for extracellular digestion. In starved animals the cells are full of protein staining granules while epitheliomuscular cells are very vacuolated. Shortly after feeding (12-18 hrs.) the secretory cells are empty of granules, or nearly so, while adjacent epitheliomuscular cells contain many vacuoles with protein and polysaccharide staining contents.

These results also show that the endodermal epitheliomuscular cells are the site of intracellular digestion. The "food vacuoles" resemble those of protozoans, and it has been stated (Hyman, 1940) that intracellular digestion proceeds in regular protozoan fashion. Hyman also remarks that extracellular digestion is purely proteolytic, while in the food vacuoles the digestion of protein, fats, and in some cases carbohydrates occurs. The endoderm of the basal hydranth region, since it consists wholly of epitheliomuscular cells, is capable only of intracellular digestion. The products of extracellular digestion appear to be transported to other parts of the colony also, since 12-18 hrs. after feeding, the hydrocaulus contains

food vacuoles in the endodermal cells. During periods of starvation the vacuolar contents of endodermal cells probably represent residual food wastes to be excreted. Burnett (1961) describes similar bodies in *Hydra* which persist during starvation. She concludes that they probably represent some type of excretory crystal.

Hyman (1940) describes two types of solid tentacle in hydroid polyps. One type (present in the Tubulariidae) has a core formed by several rows of endodermal cells. This type is quite different from the tentacles of *S. tenella,* and will not be discussed further. The other type has a core formed by a single row of highly vacuolated, stiff cylindrical cells. Cowden (1965) also describes the solid core of *Pennaria* capitate and filiform tentacles as being composed of a single row of endodermal epitheliomuscular cells. To support these statements Hyman has published illustrations, and Cowden photographs, depicting longitudinal sections of solid tentacles. When these are compared with longitudinal sections of the tentacles present in *S. tenella* (Pl. 4, Fig. 1) the resemblance to the endodermal cores described by Hyman is striking. However the present author disagrees with the concept of the tentacle core cells as advocated by Hyman and Cowden, for three reasons. Firstly, regarding the formation of "cylindrical cells' it is possible that such cells could be formed at the tentacle base by coalescence of cells around the perimeter. But this process would call for massive cellular and subcellular reorganization. Secondly, assuming cylindrical cells could be formed, these cells would possess a base, (that is, the surface in contact with the mesogloea) but no apex; the "base" of the cell would completely envelop it. Now all of the other endodermal epitheliomuscular cells in the hydranth possess basal muscle fibres, and there seems no reason why the muscle fibres of the tentacle endodermal cells also should not be basal in position. Accepting this, the muscle fibre could then possibly be circular (because the cell base is circular), or perhaps there could be a series of fibres (one for each cell used in the formation of the cylindrical cell) each running for a small distance around the cell base. Both alternatives seem far to complex as solutions. The third objection to the "cylindrical cell" concept is that in the polyp the epitheliomuscular cells of the endoderm are also digestive cells and actively engulf particles. It is difficult to understand how the tentacle endodermal cells would accomplish this. If they, however, have lost their digestive function, then it is reasonable to suppose that they are specifically a distinct type of endodermal cell. In this case nutrition supposedly is sustained by diffusion from other cells. However the tentacles are often very long, and it is difficult to see the process of diffusion as being efficient enough to serve the cells in the distal tentacle regions, especially since the tentacles are such active structures in the important process of feeding.

Results from the present study suggest a much simpler arrangement of endodermal cells in solid tentacles which is obtained without the need for gross mophological changes in the cells, and which is still consistent with the appearance of these tentacles in longitudinal sections as described by Hyman (1940) and Cowden (1965) : It is evident from Pl. 4, Fig. 1, which shows a longitudinal section of a tentacle base, that the polyp endoderm immediately above and below the tentacle base curves into the tentacle, so that in longitudinal section the tentacle endoderm is seen to be 2 layers thick. It has already been noted above that the endodermal

epitheliomuscular cells have cytoplasm and nucleus distally placed and they possess basal vacuoles (see RESULTS: ENDODERM). In longitudinal sections of tentacles the contact of the two endodermal layers results in a mass of cytoplasm and nuclei in the centre of the tentacle with vacuolated areas between this cytoplasm and the mesogloea (Pl. 4, Fig. 1). It can be seen that this arrangement gives the appearance figured by Hyman (1940) and Cowden (1965). If this explanation for the structure of the tentacle core is correct, it could be expected that the process which causes it, occurs around the whole perimeter of a tentacle base, and not just in the plane of the longitudinal section. It follows from this that a transverse section of a tentacle, particularly near the base, should show a radial arrangement of endodermal cells. This has been observed in the present study (Pl. 4, Fig. 2). The only modification necessary to the epitheliomuscular cells would be the loss of flagella. In the present study also, food vacuoles have been observed in the distal endodermal cells of a tentacle (Pl. 4, Fig. 3). It seems probable that food particles could reach such distally placed cells more easily with the arrangement of tentacle endoderm proposed here, perhaps by flattening of the endodermal cells to form a temporary narrow passage down the centre of the tentacle. If this did occur, the tentacles could be described as not being strictly solid all of the time. Further work is necessary on this problem of food transport in tentacles, but the present evidence strongly suggests that the tentacle core of solid tentacles is formed by ordinary columnar endodermal cells and not by cylindrical cells as described by Hyman and Cowden.

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Dr. Patricia M. Ralph of the Zoology Department, Victoria University of Wellington, has shown an interest in this work which greatly assisted its completion.

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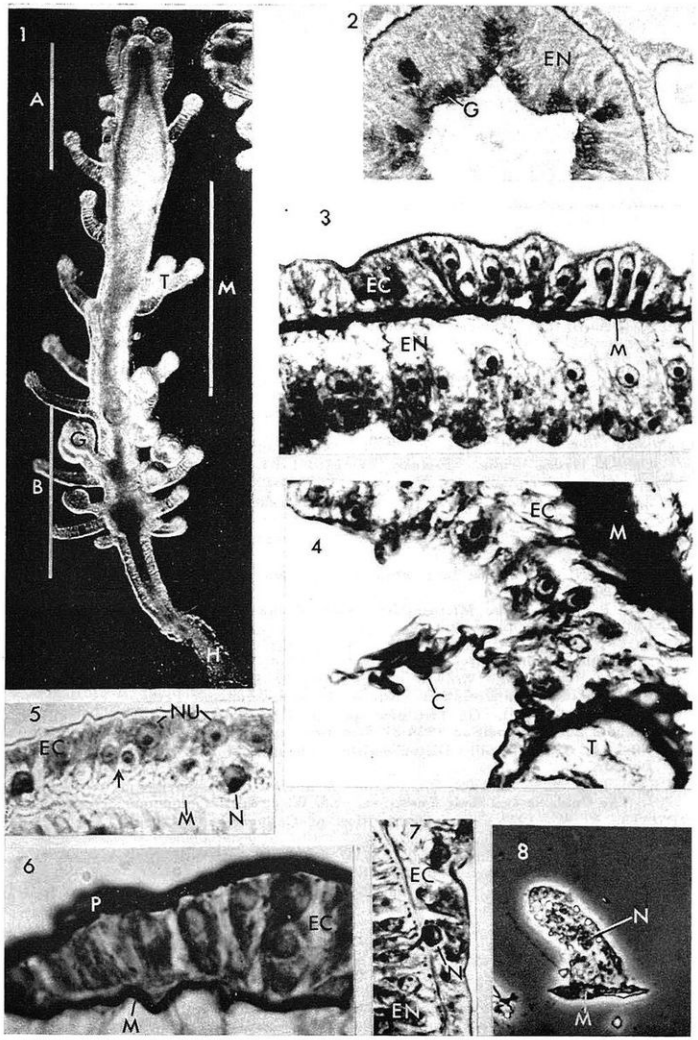


Plate 1—Fig. 1: A hydranth of **S. tenella,** from life, as seen under dark field illumination. x 100.
A, M, B, regions corresponding to the apical, middle and basal regions, respectively, seen in longitudinal sections of hydranths; G, developing gonophore; H, hydrocaulus; T, tentacle.

Fig. 2: Transverse section through apical hydranth region. Note villated endoderm with gland cells situated at periphery of villi. PAS/AB/NYS stain, x 410.
EN, endoderm; G, gland cells.

Fig. 3: Longitudinal section through basal hydranth region. Note columnar ectodermal cells, and vacuolated endodermal cells. Many endodermal cells contain food vacuoles. Mallory/Azan stain, x 1,000.
EC, ectoderm; EN, endoderm; M, mesogloea.

Fig. 4: Transverse section of hydranth in apical region to show cuticle hanging from ectoderm. Mallory/Azan stain, x 1,000.
C, cuticle; EC, ectoderm; M, mesogloea; T, tentacle base.

Fig. 5: Transverse section of ectoderm and mesogloea in apical region, showing small cells (arrows) considered to be interstitial cells. Delafield's haematoxylin and eosin preparation, x 1,000.
EC, ectoderm; M, mesogloea; N, nerve cell; NU, nuclei of epitheliomuscular cells.

Fig. 6: Longitudinal section of hydrocaulus at base of hydranth, stained with PAS/haematoxylin. The perisarc, mesogloea, and ectodermal cell granules stain intensely with Schiff's reagent. x 2,000.
EC, ectoderm; M, mesogloea; P, perisarc.

Fig. 7: Longitudinal section of hydranth in apical region showing nerve cell at base of ectoderm with nerve process crossing mesogloea to endoderm. Heidenhain's iron haematoxylin stain, x 1,000.
EC, ectoderm; EN, endoderm; N, nerve cell.

Fig. 8: An endodermal epitheliomuscular cell from a dissociation preparation. The flagellum is not visible. Phase contrast photomicrograph, x 700.
M, myoneme; N, nucleus.

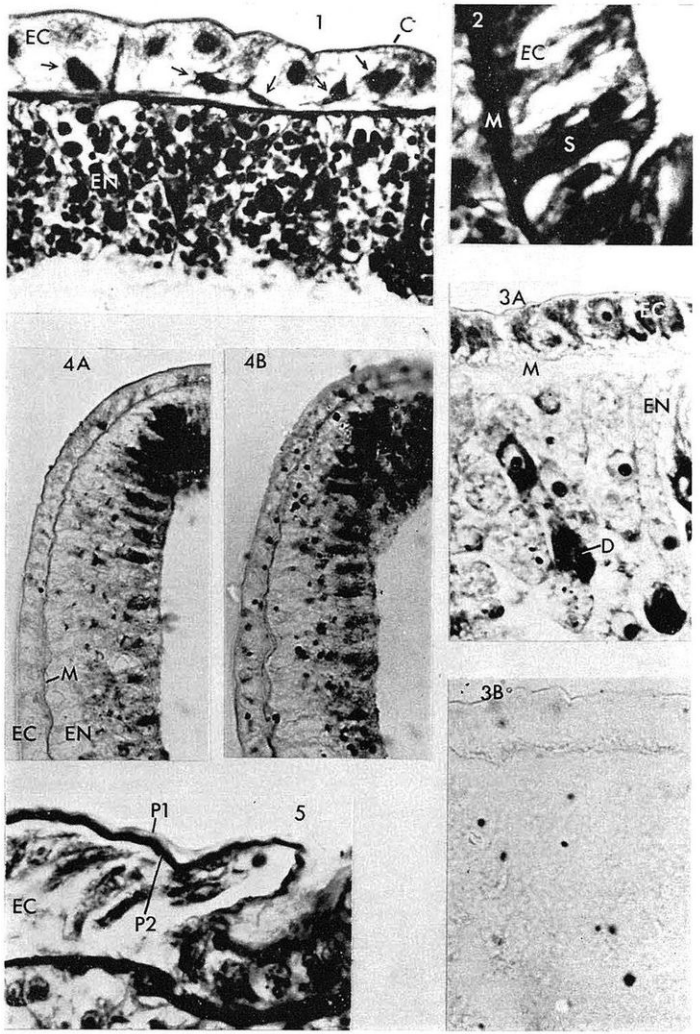


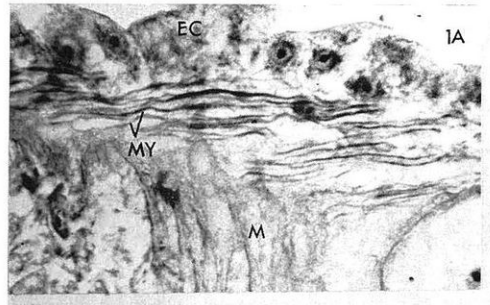
Plate 2—Fig. 1: Longitudinal section of hydranth body wall. Specimen fixed in Formol/Calcium, stained with Mallory/Azan. Nerve cells (arrowed) show clearly. Note cuticle, and densely staining food vacuoles in endoderm. x 1,100.
C, cuticle; EC, ectoderm; EN, endoderm.

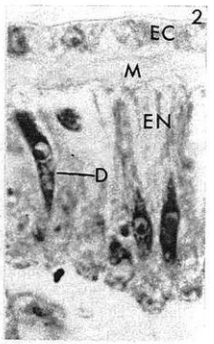
Fig. 2: Region of ectoderm adjacent to that shown in Pl. 2, Fig. 1, from the same preparation. Note hair like processes extending from sensory cell. x 1,650.
EC, ectoderm; M, mesogloea; S, sensory cell.

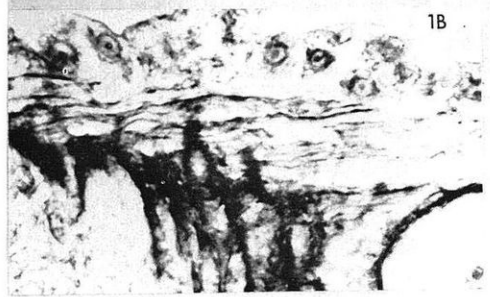
Figs. 3A, 3B: Transverse serial sections through body wall of middle hydranth region. Methyl green/pyronin stain, without (A) and with (B) prior RNase digestion. x 1,130.
D, digestive cell; EC, ectoderm; EN, endoderm; M, mesogloea.

Figs. 4A, 4B: Longitudinal serial sections through hypostome. PAS test, with (A) and without (B) prior diastase digestion. Many darkly staining bodies have been removed from the ectoderm and endoderm by diastase digestion. Those remaining represent hypostomal gland cells. x 830.
EC, ectoderm; EN, endoderm; M, mesogloea.

Fig. 5: Longitudinal section of hydrocaulus at hydranth base to show 2-layered structure of perisarc. Mallory/Azan stain, x 1,130.
EC, ectoderm; P1, outer perisarc layer; P2, inner perisarc layer.







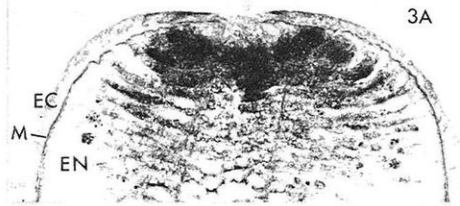


Plate 3—Figs. 1A and 1B: Tangential section of hydranth body wall, Mallory/Azan stain. Fig. 1A photographed through a deep blue filter; note densely coloured myonemes of ectodermal cells (MY), and lightly coloured mesogloea (M). Fig. 1B, same section photographed through red filter; mesogloea colours deeply because it is stained with aniline blue; myonemes not clearly seen. x 1,050.
EC, ectoderm; M, mesogloea; MY, myonemes.

Fig. 2: Longitudinal section of middle region of hydranth body wall, showing digestive cells, x 1,000. Delafield's haematoxylin and eosin stain.
D, digestive cell; EC, ectoderm; EN, endoderm; M, mesogloea.

Figs. 3A, 3B: Longitudinal section of mouth region. PAS/AB/NYS stain. Fig. 3A photographed through deep blue filter to emphasize the gland cells which stain with Schiff's reagent. Fig. 3B photographed through red filter to emphasize the gland cells which stain with AB. x 720.
EC, ectoderm; EN, endoderm; M, mesogloea.

Fig. 4: Whole mount of hydrocaulus beneath polyp, photographed by phase contrast microscopy. Soft tissues were removed with a weak "Janola" solution. Note the numerous nematomysts (arrows). x 625.

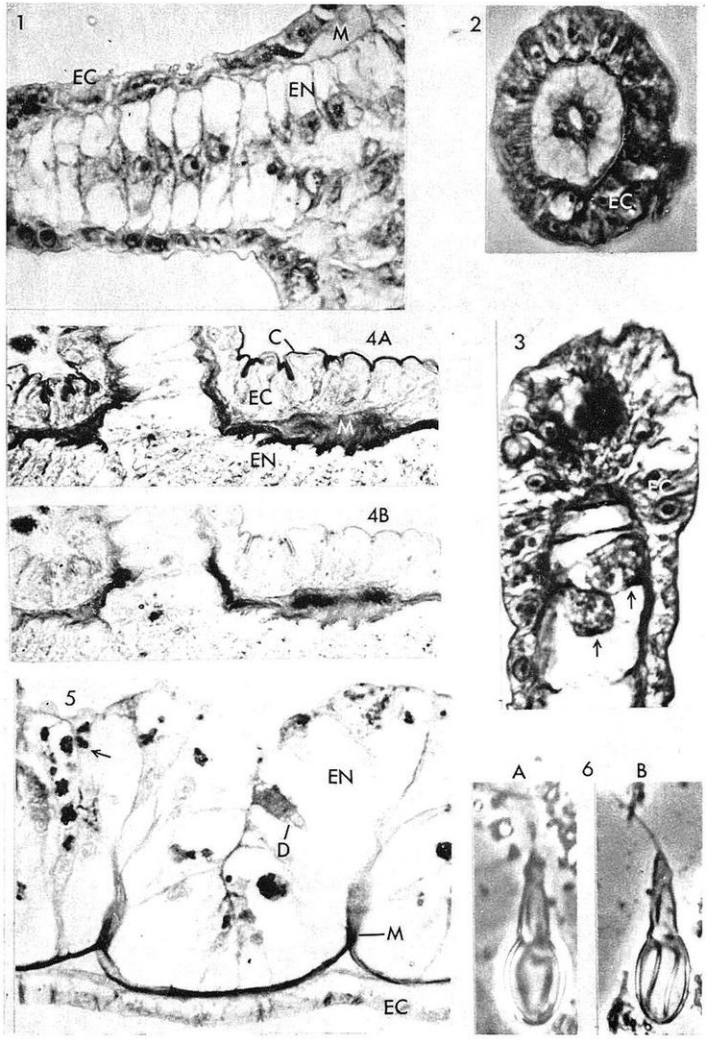


Plate 4—Fig. 1: Longitudinal section of tentacle. Delafield's haematoxylin and eosin preparation, x 1,000.
EC, ectoderm; EN, endoderm; M, mesogloea.

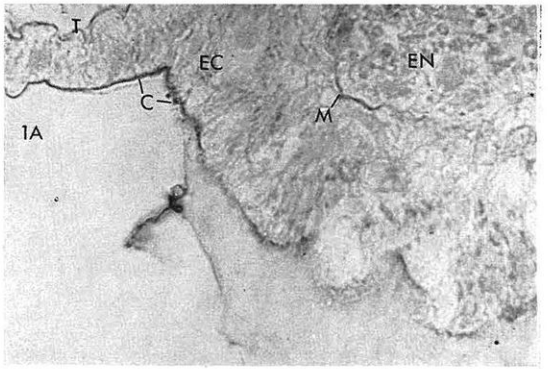
Fig. 2: Transverse section of a tentacle near its base. The endodermal cells appear radially arranged, around a central cavity. PAS/AB/NYS stain, phase contrast photomicrograph, x 900.
EC, ectoderm.

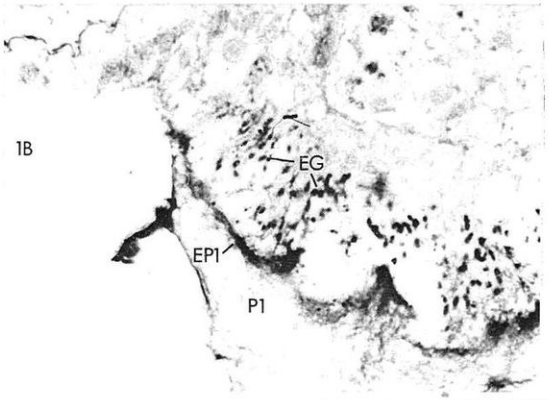
Fig. 3: Longitudinal section of distal region of tentacle, showing endodermal cells containing food vacuoles (arrows). Mallory/Azan stain, x 1,100.
EC, ectoderm.

Figs. 4A, 4B: Transverse section of hydranth body wall and tentacle base. PAS/Mowry colloidal iron stain. Fig. 4A photographed through green filter to emphasize the Schiff staining of the mesogloea. Fig. 4B photographed through red filter to show mesogloeal regions which stain blue (these show darkly in the photograph). Note the folding of the cuticle in Fig. 4A. x 750.
C, cuticle; EC, ectoderm; EN, endoderm; M, mesogloea.

Fig. 5: Longitudinal section of hydranth body wall in middle region. Animal fixed without anaesthetization after a period of starvation. Note buckling of mesogloea (M), and aggregates of small dark bodies (arrow) in vacuolated endodermal cells. PAS/AB/NYS stain, x 1,000.
D, digestive cell; EC, ectoderm; EN, endoderm; M, mesogloea.

Figs. 6A & 6B: Nematocysts from dissociation preparations photographed by phase contrast illumination, x 1,000.





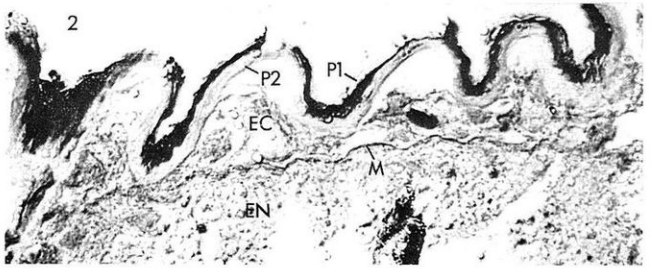


Plate 5—Figs. 1A & 1B: Oblique section through polyp base. PAS/Mowry colloidal iron stain, x 1,100. Fig. 1A photographed through deep blue filter to emphasize the Schiff-staining cuticle. Fig. 1B photographed through red filter to emphasize the Mowry colloidal iron-staining outer perisarc layer and ectodermal granules.
C, cuticle; EC, ectoderm; EG, ectodermal granules; EN, endoderm; EP1, cut edge of outer perisarc layer; M, mesogloea; P1, outer perisarc layer; T, tentacle.

Fig. 2: Longitudinal section of hydrocaulus in region of annulations beneath a polyp. PAS/Mowry colloidal iron stain, photographed by Nomarski interference illumination, x 900.
EC, ectoderm; EN, endoderm; M, mesogloea; P1, outer perisarc layer; P2, inner perisarc layer.