Zoomorphology (1982) 100:143–156

Zoomorphology

Ultrastructure of the Tentacles of *Themiste lageniformis* (Sipuncula)

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Summary. An ultrastructural study of the tentacles of *Themiste lageniformis* (Sipuncula) was conducted as part of a larger study of head metamorphosis in the species.

The oral surface of the tentacles is constructed of a multiciliated, pseudostratified, columnar epithelium while the aboral surface is an unciliated, cuboidal epithelium. Intraepidermal mucous cells lie near the junction of the oral and aboral regions. The basal portion of the epidermal cells is embedded in a thick, collagenous extracellular matrix which contains outer circular muscles, inner longitudinal muscles, the main tentacular nerve and its branches. Three tentacular canals are present and are lined by peritoneum. Hemerythrocytes and coelomocytes flow through the lumen of the canals in a regular pattern.

Justification for the designation of the tentacular canals as coelomic rather than vascular is discussed.

A. Introduction

One of the principle functions of the tentacles in adult sipunculans is food capture. Walter (1973) showed that in three mud and coral sand dwelling species the tentacles are used in nonselective deposit feeding to bring sediment and associated food to the mouth. Other forms, such as the sand burrowing species *Sipunculus*, are thought to engulf sediment as they travel through the substratum. Species living in crevices of rocks or similar habitats may employ the tentacles in a form of mucociliary suspension feeding.

The tentacles also function as a surface for gas exchange. Hemerythrin containing cells (Marrian 1927; Florkin 1933) flow through a system of tentacular canals, circum-esophageal sinuses, and one or two blind, compensation sacs in the trunk coelom. Hemocytes also are present in the trunk coelom itself and, in some species it has been shown that the properties of their hemerythrin is different from the hemerythrin in the cells of the tentacular system (Manwell 1963).

0340-6725/82/0100/0143/$02.80

The structure of adult sipunculan tentacles has been studied in a few species but only at the light microscope level (*Golfingia vulgaris*, see Cuénot 1900; *G. elongata*, see Stehle 1953; *Themiste lageniformis*, see Awati and Pradhan 1936; *Sipunculus nudus*, see Metalnikoff 1900). From studies such as these a basic structural plan for the tentacles has emerged. The epidermis is ciliated on the oral surface and unciliated on the aboral surface. Three canals and a tentacular nerve are present. The canals have ciliated linings and contain hemocytes in their lumena. The tentacular canals have been considered to be "blood vessels" by some (Awati and Pradhan 1936; Metalnikoff 1900) and "coelomic cavities" by others (Hyman 1959).

This study describes the structure of the tentacles in an adult sipunculan and has grown out of a larger study of head metamorphosis in the Sipuncula wherein it represents the endpoint of tentacle development. Further, it addresses the tentacular blood vessel-tentacular coelom controversy and provides a structural basis from which a functional analysis of feeding behavior in *Themiste lageniformis* may emerge.

B. Materials and Methods

Specimens of *Themiste lageniformis* Baird 1868, were collected from Boot Toe Point (27° 28.4'N, 80° 18.3'W) near the Fort Pierce Inlet to the Indian River Lagoon on the central east coast of Florida. The worms live between oysters which are present in clusters on intertidal sand bars.

Relaxation of specimens was accomplished by slowly adding 7.5% $MgCl_2$ to the sea water or by adding crystals of $MgCl_2$ to sea water containing animals. When relaxed to the point that they would not retract the head when agitated, the animals were quickly decapitated. The freed heads were immediately fixed for one hour at 4° C in 0.25 M glutaraldehyde with 0.14 M NaCl and buffered to pH 7.4 with 0.2 M Millonig's phosphate. The tissue was rinsed with 0.2 M Millonig's phosphate buffer at 4° C and postfixed at 4° C for one hour with 1% osmium tetroxide in 0.375 M NaCl and 0.1 M Millonig's phosphate buffer. After excess fixative was rinsed off with distilled water the tissue was dehydrated in a graduated ethanol solution series, transferred through three changes of propylene oxide, infiltrated with and embedded in Epon according to the method of Luft (1961).

Thin sections (approximately 60 nm) were cut with a diamond knife on a Porter-Blum MT-2B ultramicrotome, mounted on copper grids, and stained with saturated aqueous uranyl acetate and lead citrate (Reynolds 1963). The tissue sections were observed and photographed with a Zeiss 9S-2 transmission electron microscope.

For scanning electron microscopy the tissue was given a primary fix and buffer wash identical to that described above for TEM preparations. No postfixation was used. The tissue was dehydrated in a graded ethanol solution series, gradually transferred to amyl acetate and then critical point dried. Specimens were glued to glass cover slips and then to aluminum stubs, sputter coated with gold-palladium and observed with a Novascan 30 SEM.

To demonstrate connective tissue components the tissue was fixed in Bouins, dehydrated, embedded in paraffin, sectioned at 5–7 μm thickness, and mounted on slides. Elastin, reticulum, and collagen were differentiated selectively using the method of Humason and Lushbaugh (1969) with the Sirius supra blue FGL-CF (Direct Blue 106, C.I. 51,300) substitution for analine blue. Sirius supra blue FGL-CF (Direct Blue 106. C.I. 51,300) is available from CIBA-GEIGY Corp., Dyestuffs and Chemicals Div., Greensboro, N.C., 27409 under the product name Solophenyl brilliant blue BL.

C. Observations

1. General Description of the Tentacles

The tentacular crown of *Themiste lageniformis* consists of thirty or more unbranched tentacles which are arranged in a cluster around the mouth (Fig. 1).

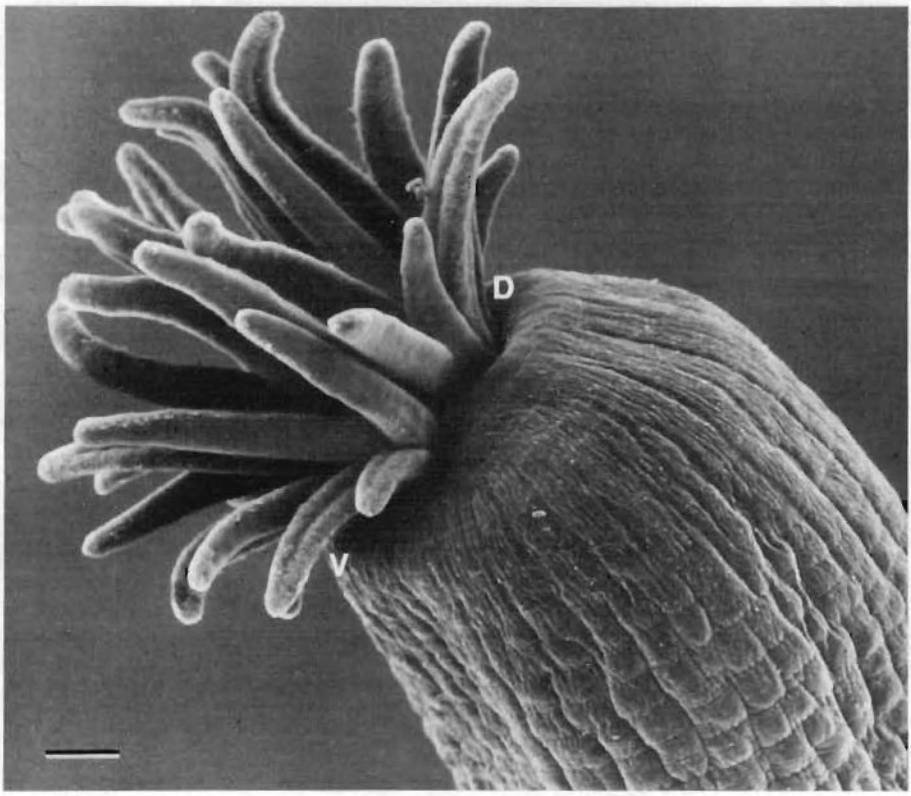

Fig. 1. Scanning electron micrograph of tentacles and anterior introvert of adult *Themiste lageniformis*. *D*, dorsal; *V*, ventral. (Scale 100 μm)

They arise from four main stems; two dorsal to the mouth and two ventral to the mouth. The stems quickly branch and give rise to the blunt, digitate tentacles. In large specimens the length of a tentacle may be 500–800 μm and have a diameter of 80–90 μm.

Awati and Pradhan (1936) designated the side of the tentacle facing the mouth, or the central axis of the crown, as the oral surface and the opposite side as the aboral surface. The oral surface is heavily ciliated and is made slightly concave by a longitudinal furrow (="ciliated groove", Awati and Pradhan 1936) in the midline (Fig. 2A). The aboral surface is convex and bears small papillae and scattered tufts of cilia (Fig. 2B, C). Each tuft consists of five to eight cilia which emerge from a hole in the cuticle approximately 1.5 μm in diameter. Their position on the aboral surface and their distinctive morphology suggest that they are not involved in the production of feeding currents but, instead, they may be sensory.

In transverse section, a tentacle appears as a trinagle with rounded corners (Fig. 3). The oral surface with the median furrow represents one side of the triangle while the other two sides correspond to the convex aboral surface.

Each tentacle is constructed of an outer epithelium which surrounds a thick

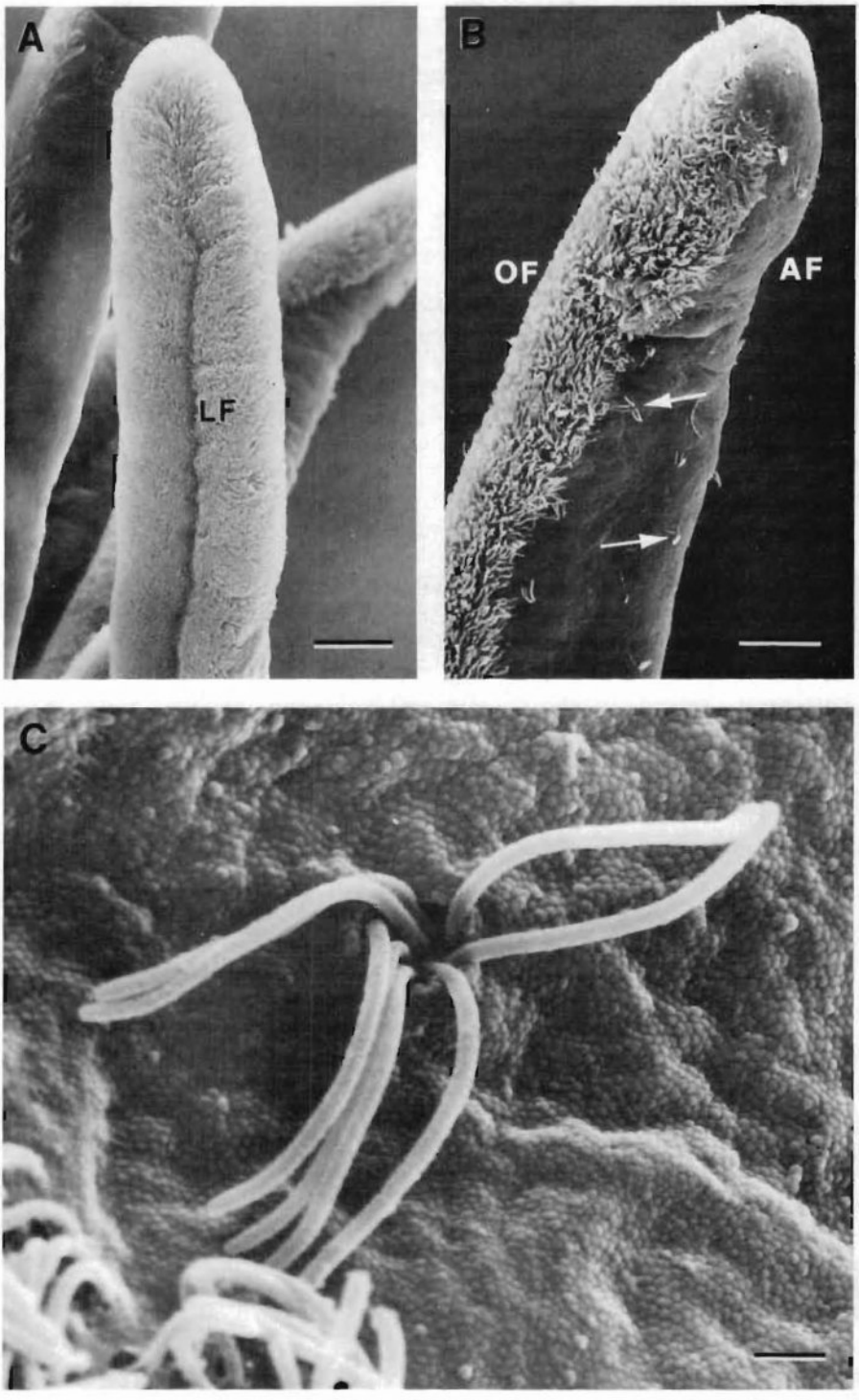

Fig. 2. **A** View of ciliated oral surface of single tentacle showing median longitudinal furrow (*LF*). (Scale 100 μm); **B** Lateral view of tentacle near tip showing ciliated oral face (*OF*) and unciliated aboral face (*AF*) with ciliary tufts (*arrows*). (Scale 20 μm); **C** Enlargement of ciliary tuft on aboral face of tentacle. The cilia emerge from hole in papillated cuticular surface. (Scale 1 μm)

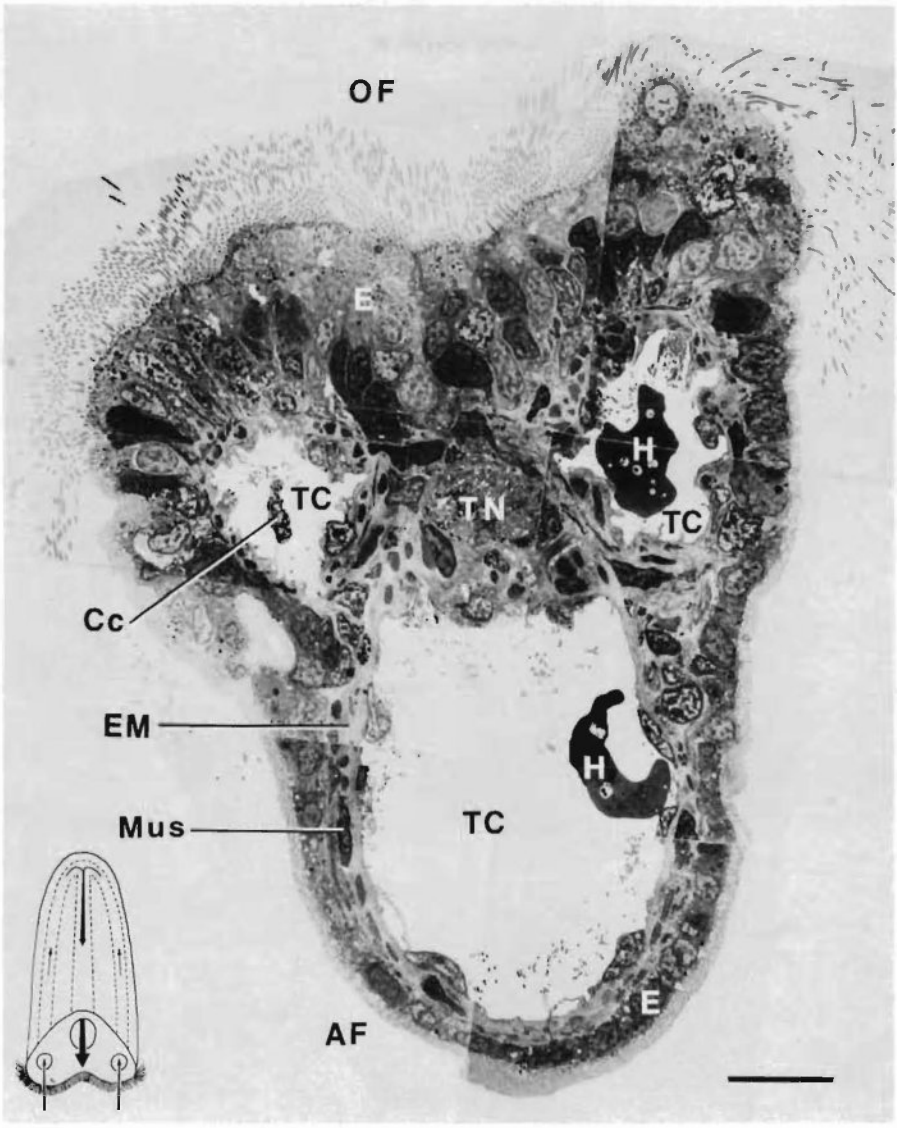

Fig. 3. Transmission electron microscope mosaic of tentacle cross section. *AF*, aboral face; *Cc*, coelomocyte; *E*, epithelium; *EM*, extracellular matrix; *H*, hemocyte; *Mus*, muscle cell; *OF*, oral face; *TC*, tentacular coelom; *TN*, tentacular nerve. (Scale 10 μm). Insert. Representation of distal portion of tentacle in aboral view. Cut face shows tentacular coelomic canals in section. Dotted lines show canals within tissue. Flow of coelomic fluid, hemocytes, and coelomocytes is efferent in two oral canals and afferent in single aboral canal

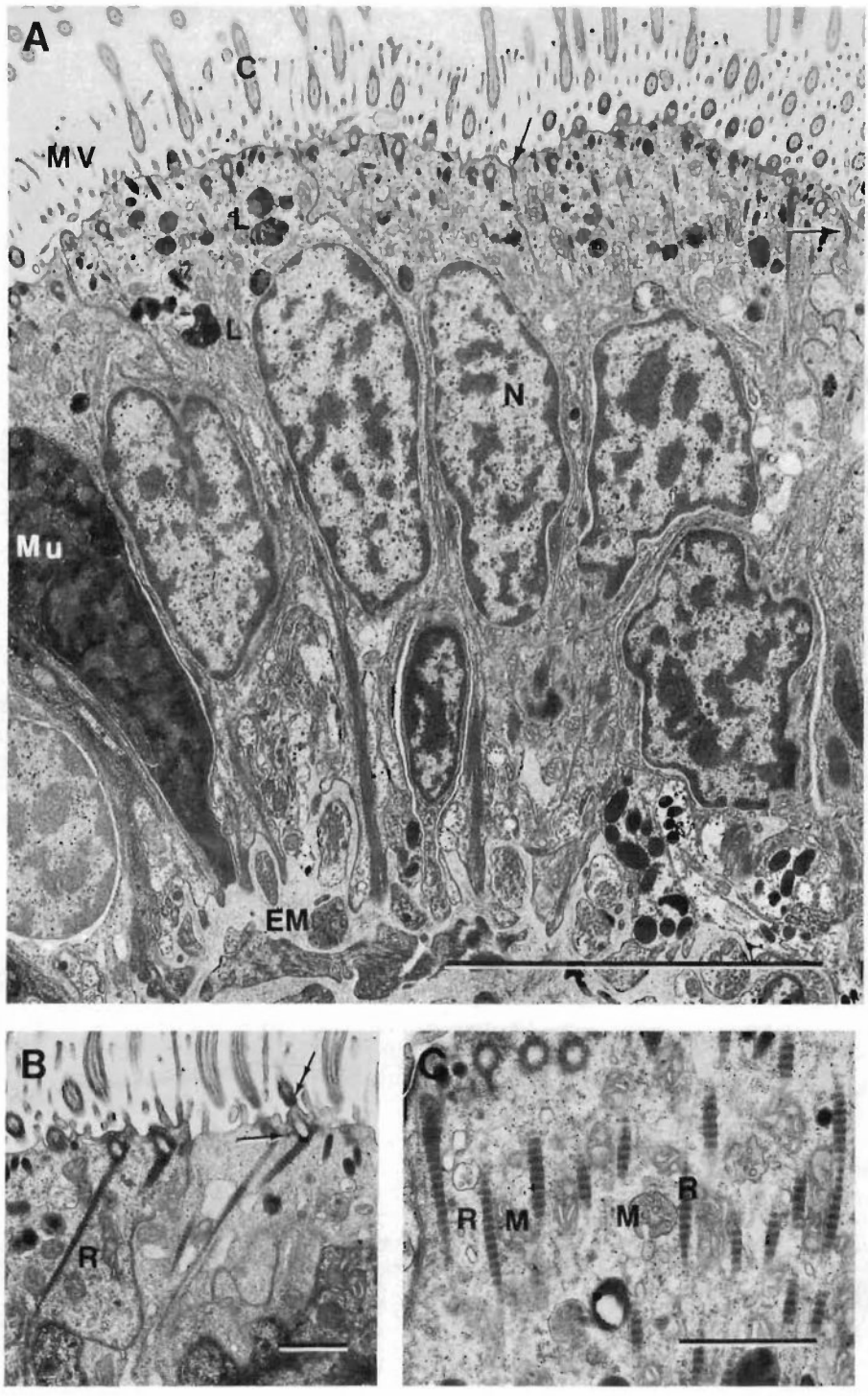

Fig. 4. **A** Pseudostratified, columnar epithelium of oral epidermis overlying extracellular matrix (*EM*) and bearing cilia (*C*) and microvilli (*MV*) at apical surface. *L*, primary lysosome; *Mu*, mucous cell; *N*, nucleus; *arrows*, zonulae adherentes. (Scale 10 μm). **B** Basal body and ciliary rootlet (*R*) of the oral surface cilia. *Single arrow*, basal foot; *double arrow*, basal plate and collar. Scale 1 μm). **C** Ciliary rootlets (*R*) showing 70 nm periodicity and close association of mitochondria (*M*). (Scale 1 μm)

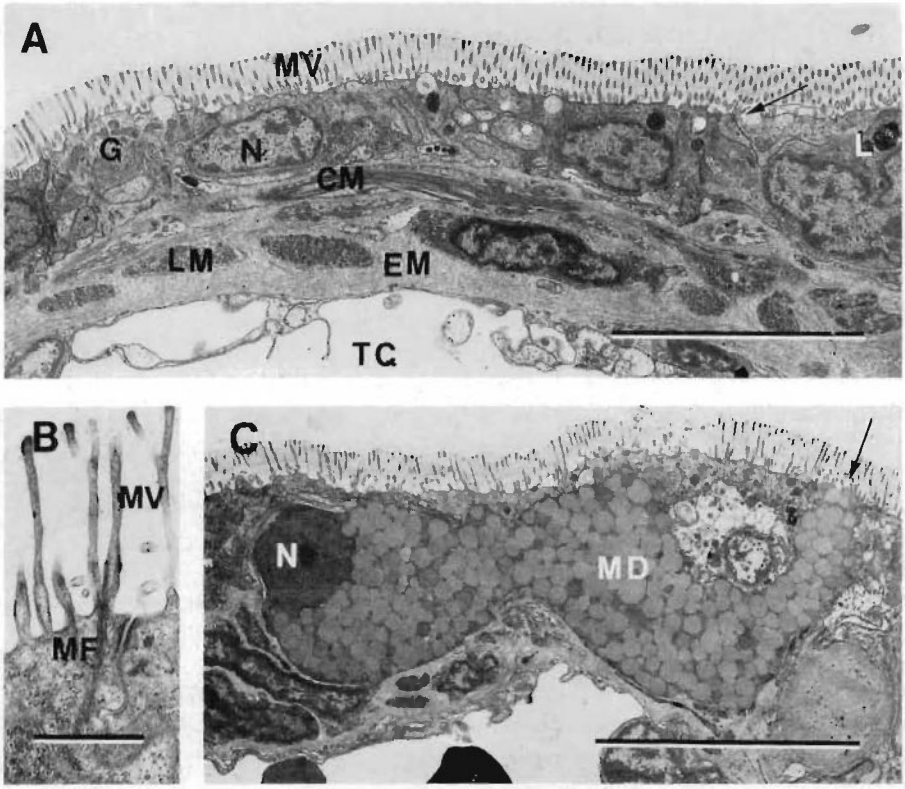

Fig. 5. **A** Unciliated aboral epithelium overlying the extracellular matrix (*EM*) and tentacular canal (*TC*). Outer circular (*CM*) and inner longitudinal muscle bundles (*LM*) are present in the extracellular matrix. *G*, Golgi complex; *L*, primary lysosome; *MV*, microvilli; *N*, nucleus; *arrow*, zonulae adherentes. (Scale 5 μm). **B** Aboral microvilli (*MV*) showing branching character and microfilament bundle (*MF*) extending into the apical cytoplasm. (Scale 1 μm). **C** Intraepidermal mucous cell in the aboral epithelium. Mucous droplets (*MD*) and the nucleus (*N*) are the only conspicuous cytoplasmic structures. Mucous droplets are released at the surface (*arrow*). (Scale 10 μm)

extracellular matrix[1] having muscles and nerves embedded in it. In each corner of the triangle is a tentacular coelomic canal lined by peritoneum. Hemocytes and coelomocytes commonly are found in these canals.

2. *Ultrastructure of the Tentacles*

a) Epidermis. The oral surface is a ciliated, pseudostratified, columnar epithelium (Fig. 4A). The cells are joined to neighboring cells by zonulae adherentes whereas their basal membranes are in contact with the underlying extracellular matrix. The apical surface of each cell bears many microvilli. These are approximately

1 "Extracellular matrix" refers to the same material designated as "connective tissue" in the earlier, light histological literature (Awati and Pradhan 1936; Hyman 1959). "Extracellular matrix" is the perferred term because the region lacks fibroblasts which are characteristic of true connective tissue

1.4 μm long, have bifuracted tips, and are embedded in a cuticular matrix so that only the tips are exposed at the surface. Several cilia are present on the apical surface of each cell. The basal body (Type 2: Pitelka 1974) has a thick basal plate and an electron dense collar which extends distally just inside the peripheral doublet microtubules (Fig. 4B). A short basal foot extends from the basal body. Each basal body has a single rootlet which projects obliquely toward the midline and base of the tentacle and extends approximately 4 μm into the cytoplasm. The rootlet makes an angle of about 100° with the basal body and tapers along its length. Cross striations within the rootlet have a 70 nm periodicity (Fig. 4C).

Oral epithelial cells have a centrally or basally located nucleus. Mitochondria are most abundant in the apical cytoplasm where they often are in contact with the ciliary rootlets (Fig. 4C). Heterogeneous electron-dense bodies (presumably primary lysosomes) are present in the apical cytoplasm (Fig. 4C).

The aboral epidermis is a non-ciliated cuboidal epithelium (Fig. 5A). Zonulae adherentes are present near the apex of adjacent cells. The dimensions of the cells vary from 3–8 μm wide by 2–6 μm high. The cells directly opposite the oral surface generally are short and wide whereas those near the oral surface grade toward the columnar form and are taller and narrower. The free surface of each cell is covered by a microvillar border which is approximately 1.2 μm tall and is embedded in a cuticular matrix. The distal ends of the microvilli project through the cuticle and may be bifurcated. The small papillae seen on the aboral surface (Fig. 2C) probably are the tips of these microvilli. Parallel microfilaments project from the interior of the microvilli into the apical cytoplasm, but a terminal web was not distinguishable (Fig. 5B). Large nuclei, Golgi complexes, mitochondria, and primary lysosomes also are present.

Large intra-epidermal mucous cells are present in the oral epithelium and in the aboral epithelium near the junction of the two tissues (Fig. 3, 5C). These cells are heavily laden with mucous droplets up to 1 μm in diameter. Except for the nucleus and a few mitochondria, the droplets exclude most other organelles. Golgi complexes, for instance, are conspicuously absent. Mucous droplets are released from the mucous cell onto the surface of the tentacle.

b) Extracellular Matrix. Beneath the epidermis is an extracellular matrix which varies in thickness from 0.5 μm to 4.5 μm (Fig. 3). The matrix is composed of a random network of thin fibers which have electron dense bands perpendicular to their long axis. Histochemical analysis has shown that these fibers are primarily collagen. Fibroblasts were not present in the matrix.

Embedded in the extracellular matrix behind the oral epithelium is the main tentacular nerve. It is a bundle of naked nerve fibers and glial cells containing electron-dense particles (Fig. 3, 6). Branches from the main tentacular nerve course through the extracellular matrix and, presumably, innervate various cells in the tentacle.

Muscle cells also are present in the extracellular matrix. The arrangement of the muscles is the same as one finds in the body wall; the outer layer being circular and the inner longitudinal (Fig. 5A). The cells contain mitochondria, large nuclei, and thick myofilaments surrounded by abundant and ran-

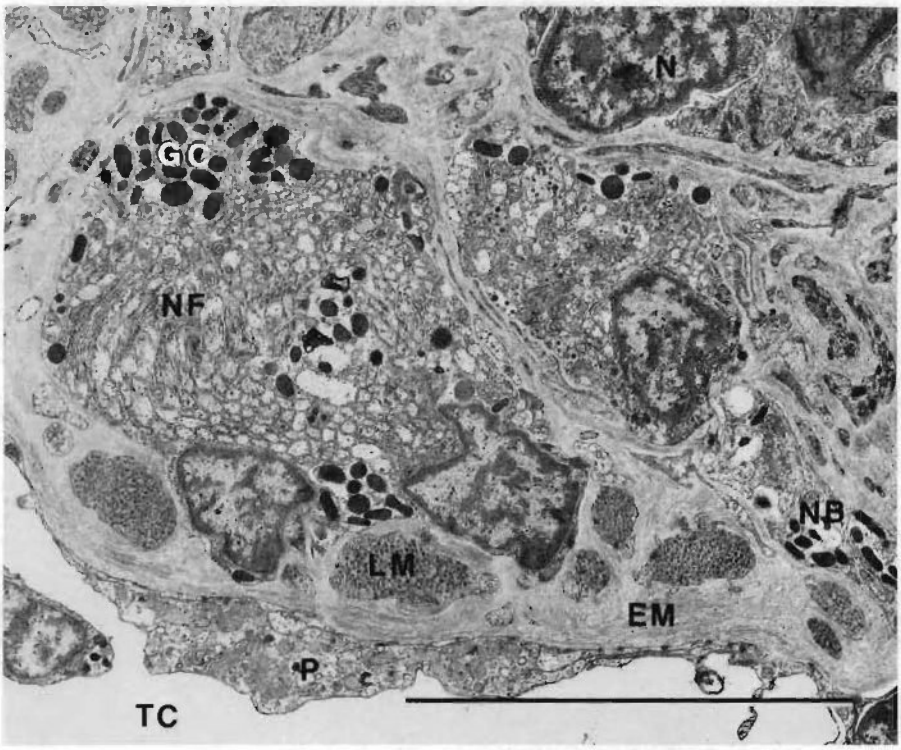

Fig. 6. Cross section through main tentacular nerve showing nerve fibers (*NF*) and glial cells (*GC*). Nerve branches (*NB*) and muscle cells (*LM*) are visible in the extracellular matrix (*EM*). *N*, Nucleus of an oral epithelial cell; *TC*, main tentacular coelom; *P*, peritoneal lining of tentacular coelom. (Scale 10 μm)

domly arranged thin myofilaments. They are considered to be smooth muscles since striations in the myofibril arrangement were not observed.

c) Tentacular Canals. Within the extracellular matrix, near each corner of the triangular tentacle, are large tentacular canals (Fig. 3). Generally the single aboral canal is larger than either of the two oral canals. Each canal is fully lined by a thin layer of squamous peritoneal cells. In many areas the lining is extremely thin, consisting of two closely applied membranes of a single peritoneal cell (Fig. 7A). In other areas local thickening is produced by the presence of the nucleus or other organelles (Fig. 7A). Adjacent peritoneal cells are joined by zonulae adherentes and frequently overlap each other making the lining appear to be two or three cells thick in some sectional views (Fig. 7A).

Certain of the peritoneal cells bear cilia and microvilli, both of which are aggregated into tufts (Fig. 7A, B). Mitochondria are more abundant in these cells. Presumably the cilia are responsible for the flow of coelomic fluid and hemocytes within the canals.

Large nucleated hemocytes are present in the tentacular canals (Fig. 7A). These cells have a dense homogeneous cytoplasm which lacks organelles except for vacuoles. The vacuoles contain electron-dense material which may have

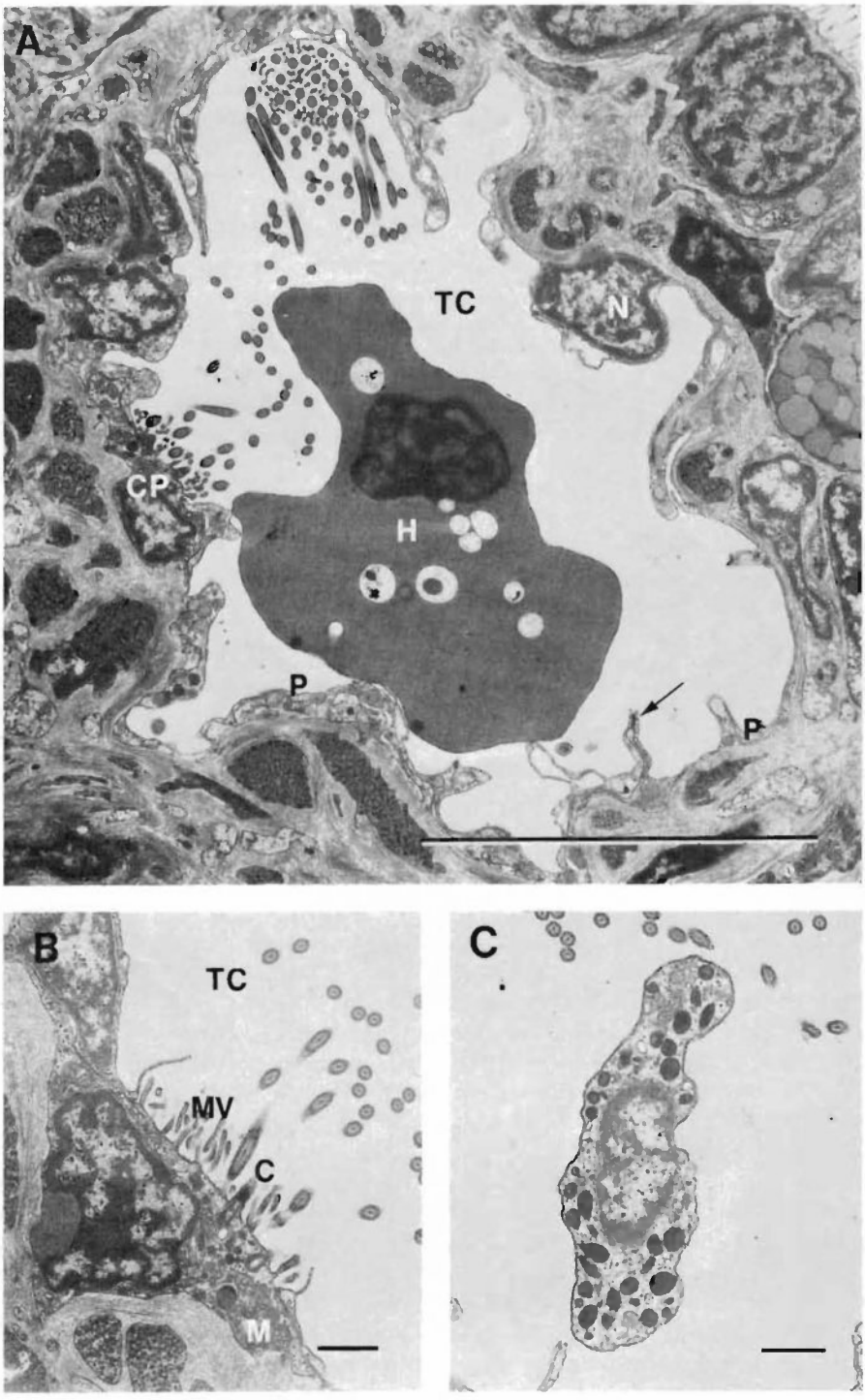

Fig. 7. **A** Cross section through a tentacular coelomic channel (*TC*) with a hemocyte (*H*) in the lumen. *CP*, ciliated peritoneal cell from the lining of the tentacular coelom; arrow, zonulae adherentes. (Scale 10 µm). **B** Ciliated peritoneal cell from the lining of the tentacular coelom (*TC*). *C*, cilia; *M*, mitochondria; *MV*, microvilli. (Scale 1 µm). **C** Section through granular coelomocyte from tentacular coelom. This cell is similar to granulocytes which are typical of trunk coelom. (Scale 1 µm)

been incorporated by phagocytosis. Cells which are more typical of the trunk coelom occasionally are seen in the tentacular canals (Fig. 7C).

3. Flow of Hemocytes in the Tentacular Coelom.

When illuminated with reflected light and observed with a stereo microscope, the flow of cells in the tentacles of living specimens may be seen. The cells are carried in the fluid from the base of the tentacle to its tip in each of the two oral canals. These passages are confluent with each other and with the aboral canal at the tip of the tentacle. The combined volume of fluid from the oral canals returns to the base of the tentacle through the single aboral canal (Fig. 3, insert). At the proximal end of each tentacle the three canals open into a system of interconnecting passages which surround the pharynx. This ring sinus communicates dorsally with a large compensation sac which bears numerous small diverticula and which projects posteriorally on the dorsal side of the esophagus in the coelom. When the head is retracted the hemocytes and fluid are squeezed from the tentacular canals and accumulated in the compensation sac. When the head is extended the retractor muscles relax and the body wall muscles contract. The increased coelomic pressure extends the head and causes hemocytes and fluid in the compensation sac to be forced into the tentacular system where they are circulated by the action of the cilia lining the coelomic channels.

Discussion

The polyciliated character of the oral epithelium in *Themiste lageniformis* is consistent with the hypothesis of Rieger (1976) which states that a monociliated epidermis is characteristic of the lower Metazoa, archicoelomates, and some Achelminth phyla. The only Spiralian which has been found to possess a monociliated epidermis is the polychaete *Owenia fusiformis* (Gardiner 1978), and this is considered to reflect the primitive nature of the species among the Spiralia.

The mechanism of mucous droplet production in the mucous cells of *Themiste* tentacles is unclear. Considering the quantity of mucous in some of these cells one would expect to see conspicuous processing/packaging centers but none were found. That these cells might be holocrine and that the packaging centers have degenerated is possible but unlikely because mucous cell nuclei were not pycnotic and mitochondria were not degenerative. Further, if the mucous is indeed used in feeding (Pilger 1979), holocrine extrusion mechanisms would seem energetically "expensive." Sequential extrusion of individual mucous droplets, as the evidence for *Themiste* suggests, is similar to that reported for the polychaete *Dendronereides heteropoda* (Storch and Welsch 1972).

Baskin (1971) described the fine structure of glial cells in the central nervous system of nereid polychaetes and speculated that they provide support and protection from deformative forces. Similar cells have been recognized in the central nervous system of other polychaetes (Gardiner 1978). Cells associated with the tentacular nerve of *Themiste lageniformis* also fit the description of glial cells. They do not form the thick sheath around the nerve that Baskin (1971) described but neither are they exposed to as much stress as the polychaete ventral nerve.

Hemerythrocytes of *Themiste lageniformis* resemble those of *Sipunculus nudus* (Valembois and Boiledieu 1980) in most respects except that mitoehondria were not seen in *Themiste*. Phagocytosis has been observed in sipuneulan blood cells (Cuénot 1913; Chapheau 1928) and probably accounts for the vacuoles of electron dense partieles in *Themiste* hemocytes.

Tentacular canals have been recognized in all sipunculans where the tentacles have been investigated. The canals are part of a larger system which includes eircumesophageal sinuses and one or two blind sacs projeeting into the trunk coelom. The lumen of this system contains fluid, hemerythrin containing cells (Marrian 1928; Florkin 1933; Manwell 1960) and some coelomocytes. The flow pattern of these cells in the tentacles of *Themiste* is the same as that described elsewhere for this and other sipunculan species (Peebles and Fox 1933; Awati and Pradhan 1936; Stehle 1953). Features generally considered to be important for aquatic respiratory systems (e.g. large evaginated surface area, water flow over the respiratory surface) are present in the sipunculan tentacles. It is not surprising, therefore, that these systems have been referred to as blood vessells (Metalnikoff 1900; Awati and Pradhan 1936). Although this is a correct interpretation from a functional point of view, it is not correct when morphological criteria are applied.

True invertebrate blood vessels usually are invaginations of the coelomic wall into the coelomic space so that the vessel is lined by the basal lamina of the peritoneum. In other cases they are not invaginations but simple spaces in the extracellular matrix underlying the coelomic peritoneum (Nakao 1974; Welsch and Storch 1976). Therefore, invertebrate blood vessels lack the endothelial lining that is characteristic of the vertebrates. The blood vessells of hemichordates (Dilly 1972), Brachiopods (Welsch and Storch 1976; Reed and Cloney 1977), annelids (Nakao 1974; Eckelbarger 1980) and other invertebrate groups clearly illustrate this relationship.

This study regards the lining of the tentacular canals in *Themiste lageniformis* as being a peritoneum and, therefore, the lumen constitutes a true coelomic spaee. Several lines of direct and indirect evidence led to this conclusion. First, the tentacular canals do not fit the morphological criteria of true invertebrate blood vessels in that the lining consists of the apical surfaces of cells which lack a basal lamina and may be elaborated with cilia. Second, the sequence of tissue layers encountered in a cross section view when one reads from the outside of the tentacle to the lumen (cuticle, epithelium, extracellular matrix[2], circular muscles, longitudinal muscles, peritoneum, tentacular lumen) is the same as the sequence encountered when reading from the outside of the body wall to the trunk coelom. Third, the presence of nucleated hemerythrocytes in the tentacular coelom of sipunculans follows the general tendency for these types of cells to be situated in coelomic spaces (Priapula: Mattisson and Fange 1973; Brachiopoda: Welsch and Storch 1976; Polychaeta; Magelonidae[3]: Boilly 1974). That the lining of the sipunculan tentacular canals bears a relationship

2 The muscles in the tentacles are broken up into individual fibers suspended in the extracellular matrix. Body wall muscles form continuous layers which are bounded by the extracellular matrix on the outside and the basal lamina of the peritoneum on the coelomic side

3 Hemerythrocytes are anucleate in *Magelona* (Boilly 1974)

to the coelomic peritoneum also is suggested by information concerning other sipunculans. For *Sipunculus nudus,* Selensky (1908) reported that ciliated cells and fixed urns which were located in the coelomic peritoneum were similar to those in the lining of the tentacular canals and the compensation sac. Ohuye (1942) further reported that both epithelia (coelomic and tentacular canal system) were capable of producing coelomocytes. Similarly, in *Phascolopsis gouldii* these epithelia each produce hemerythrin-containing cells (Manwell 1963). Although circumstantial, these data suggest a functional similarity between coelomic peritoneum and tentacular peritoneum which can be explained best by ontogenetic equivalence.

The development of the tentacular coelom in the Sipuncula and its relationship to the trunk coelom remains undetermined. It is expected, however, that studies of head metamorphosis will provide the answers to these questions.

Acknowledgements. The author gratefully acknowledges the support of a Smithsonian Institution Postdoctoral Fellowship and an Agnes Scott College Faculty Summer Research Fellowship during the course of this study. Sincere gratitude is expressed to Drs. M.E. Rice and E.E. Ruppert for many stimulating discussions and for critical review of this manuscript.

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Received January 26, 1982