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Structure, Ultrastructure, and Function of the Terminal Organ of a Pelagosphera Larva (Sipuncula)

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Summary. The terminal organ, a structure enabling pelagosphera larvae of Sipuncula to form temporary attachments to substrata, was examined behaviorally and with light and electron microscopy for larvae of *Golfingia misakiana*, collected from the Florida Current. The terminal organ appears as a retractile rounded knob with a short neck joining the posterior extremity of the trunk. It can attach larvae directly to substratum or can secrete a tether-like mucus strand about which the organism moves. In unattached larvae, the terminal organ is often placed in the mouth. The terminal organ of a 5.5 day old larva consists of 29 cells: 8 epidermal, 3 mucus, 2 tension-bearing, 5 sensory, 10 retractor muscles and 1 unknown cell. The mucus cells are presumed to release the adhesive material while the microvilli on the tension-bearing cells, with their dense cores of microfilaments, bear the strain. The latter are joined directly to the retractor muscles which originate on the dorsal body wall near the anus. Two of the sensory cells terminate within the cuticle flanking the adhesive pore and are assumed to be cuticle strain receptors. Three sensory cells terminate in cilia that extend posteriorly from the pore. These may function in substratum evaluation prior to temporary attachment, or settlement preceding metamorphosis. The terminal organ is compared to adhesive organs in other soft-bodied metazoans and although it approximates the structure found in some rotifers, it is considered to be independently evolved within the Sipuncula. The terminal organ can be understood as an adaptation in young larvae for protective attachment and facilitation of feeding whereas, in older larvae, it may only function in substrate evaluation prior to settlement.

A. Introduction

The planktonic larval stage in the phylum Sipuncula is known as the pelagosphera. The term was first used by Mingazinni (1905) who, in describing

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an oceanic larva from the South Pacific Ocean, believed it to be a planktonic adult sipunculan. His mistake was soon realized and, more recently, the term pelagosphera has been used to designate the planktonic larval stage of sipunculans that succeeds the trochophore and is characterized by a prominent band of metatrochal cilia utilized by the larva as a locomotory organ (Rice 1967, 1973, 1975, 1978, 1981; Hall and Scheltema 1975; Scheltema 1975; Scheltema and Hall 1975). Most pelagosphera larvae are planktotrophic, although a few are known to be lecithotrophic, having a pelagic or benthi-pelagic stage of short duration (Gerould 1906; Åkesson 1958, 1961a; Rice 1967, 1975). Whether planktotrophic or lecithotrophic, a characteristic feature of nearly all pelagosphera larvae is a terminal organ, commonly used for temporary attachment to a substratum. In larvae of different species and in larvae of the same species, but differing ages, the terminal organ may show varying degrees of prominence and use. When highly developed the terminal organ functions for temporary attachment of the larva to a substratum. When relatively small, the terminal organ is rarely, if ever, used for attachment and has been presumed to be primarily sensory in function (Hatschek 1883).

Only a few previous reports on sipunculan larvae have referred to the histology or structure of the terminal organ and these studies have been on larvae with relatively minute organs. In his studies of the development of *Sipunculus nudus*, Hatschek (1883) described the terminal organ of this larva as an epithelial thickening, usually invaginated, which was provided with tufts of sensory cilia. Åkesson (1961b) described the histology of 4 oceanic pelagospheras from preserved plankton collections of the Galathea expedition off Natal, noting the presence of an invaginated terminal organ, comprised of stratified glandular epithelium, which he compared to a similar but more complex terminal organ in adults of the genera *Xenosiphon* and *Sipunculus*. Secretory cells have also been reported in the terminal organs of the young lecithotrophic larva of *Golfingia elongata* (Åkesson 1961a) and the young planktotrophic larva of *Phascolosoma agassizi* (Rice 1973).

In planktotrophic larvae of the open ocean the degree of development of the terminal organ ranges from a prominent organ by which the larva may attach when a substratum is contacted to a small, relatively insignificant structure usually retracted and not observed to serve as an attachment organ. These larvae are found in the surface waters of all of the major east-west currents in the North and South Atlantic oceans. They may live in the larval stage as long as 8 months (Scheltema and Hall 1975), attaining sizes from one-half to several mm in length. Little is known of the adult affinities of most of these larvae, and in only one instance is there information on both the young and older pelagosphera larval stage of a single species. This species is tentatively identified as *Golfingia misakiana* (Ikeda 1904) and has been reared in the laboratory from oceanic larvae to sexually mature adults (Rice 1978, 1981). From spawnings of these adults, early larval stages have been obtained and studied (see Materials and Methods). The terminal organ of the young larva of *Golfingia misakiana* has been

found to be highly developed as a structure for attachment whereas in the older oceanic larva it is proportionately small and rarely extended.

In this paper we have chosen to examine in detail the behavioral, morphological, and ultrastructural features of the terminal organ of a young pelagosphera of *Golfingia misakiana* of 4 to 5 days after fertilization. In addition, comparative observations will be noted on the older oceanic larva of the same species, but of unknown age. By this multi-faceted investigation we hope to learn more about the function of the organ, particularly at the earlier stage in development, and to clarify the role of the organ in the biology of the pelagosphera larva. The ultrastructural observations also will be compared with those on adhesive organs of other marine invertebrates and a mechanism suggested for larval attachment and release. This is the first intensive study of the terminal organ of sipunculans, and we anticipate that it will serve as a basis for subsequent comparative studies of the structure and significance of this organ in the phylum Sipuncula.

B. Materials and Methods

For this study young pelagosphera larvae were obtained from spawnings of adults reared in the laboratory from oceanic larvae collected in the Florida Current. The oceanic larvae have been described previously and tentatively identified as *Golfingia misakiana* by Rice (1978, 1981). Earlier reports on this larval type are those of Hall and Scheltema (1975) who designated it as Type C and Häcker (1898) who named it *Baccaria oliva*. The adult reared from larvae in the laboratory differs in some morphological and developmental characters from field-collected animals (Rice 1981), although the significance of these differences remains to be analyzed. The species for purposes of this paper is still designated as *Golfingia misakiana* (Ikeda 1904).

Oceanic larvae were collected by plankton tows off the central east coast of Florida 20 to 25 miles offshore from Fort Pierce in the Florida Current, a component of the Gulf Stream system. Tows of 10 to 15 min duration were made with a $^1/_2$ m net of 125 µm mesh. Plankton was transported alive to the laboratory where larvae were sorted and induced to metamorphose by the method of Rice (1978, 1981). After metamorphosis animals were maintained in fine sediment less than 102 µm in diameter in a recirculating system or in dishes in which the water was partially changed 2 to 3 times a week. Sexual maturity was attained in 9 to 10 months.

For spawning, adults were removed from the sediment and placed in fingerbowls. Spawnings of eggs and sperm, obtained from October through March, were transferred to tall covered petri dishes of 500 ml capacity and maintained at room temperature (about 25° C). Three to four days after fertilization the young pelagosphera larvae, resulting from metamorphosis of trochophores, were distributed among clean dishes in concentrations ranging from about 100 to 500 per dish. Usually sea water was allowed to stand in these dishes 2 to 3 days prior to transfer so that a thin biological film covered the bottom. Young larvae were fed an algal mixture, including *Phaeodactylum* sp., *Chlorella autotrophica*, *Dunaliella salina*, *Isochrysis galbana*, and *Asterionella glacialis*.

Specimens were prepared for transmission electron microscopy by relaxation in 10% ethanol in sea water, fixation in 2.5% glutaraldehyde buffered with Millonig's phosphate buffer at room temperature, postfixation in phosphate-buffered 2% osmium tetroxide, dehydration in an alcohol and propylene oxide or acetone series, and embedment in Epon 812. Serial thin sections were obtained for 5 larvae (4.5 and 5.5 days old) and were collected on Formvar filmed slot grids. For scanning electron microscopy specimens were fixed in 2.5% phosphate-buffered glutaraldehyde, dehydrated in alcohol and acetone, dried in a Denton critical point drier (CO_2) and coated with gold paladium in a Technics Hummer I sputter-coater.

C. Results

1. Morphological and Behavioral Observations of Live Larvae

In developmental stages reared in the laboratory, we observe the first appearance of the terminal organ at the premetamorphosis stage of the trochophore three days following fertilization. Arising as an evagination of the posterior body wall, it appears as a bulge or protuberance of the posterior extremity of the trunk. By $4^1/_2$ days, at the time of metamorphosis of the trochophore to the pelagosphera larva, the terminal organ is well-formed, resembling a posterior rounded knob connected to the body by a more narrow, elongate neck (Figs. 1–4). At this time larvae begin to attach by the terminal organ to the bottom of the culture dishes, although the majority continues to swim for one or more days, some moving throughout the culture dish and others swimming near the bottom. Within a few days, usually by 6 days of age, most of the larvae are firmly attached.

The shape of the terminal organ is changeable in living larvae, depending on the degree of extension and retraction of the body. Although usually knob-shaped with an elongate neck, it may be stretched into a cylindrical or club-shaped organ. The neck of the organ has considerable flexibility and extensibility, allowing the larva to move about its point of attachment in all directions. Whether attached or free, the terminal organ can be retracted into the trunk of the larva either entirely or in part. On maximal retraction, the posterior body wall continuous with the neck of the organ is also withdrawn into the trunk, leaving a prominent depression in the posterior body in the region of the retracted organ. In a larva with retracted

Fig. 1. Photomicrograph of living pelagosphera larvae 11 days after fertilization. Spawnings giving rise to these larvae and to those in Figs. 2, 3, and 4 were from adults reared in the laboratory from oceanic larvae collected in the Florida Current and tentatively identified as *Golfingia misakiana*. The larvae are attached by their terminal organs (*to*) to debris. Note that the terminal organ of the middle larva is retracted

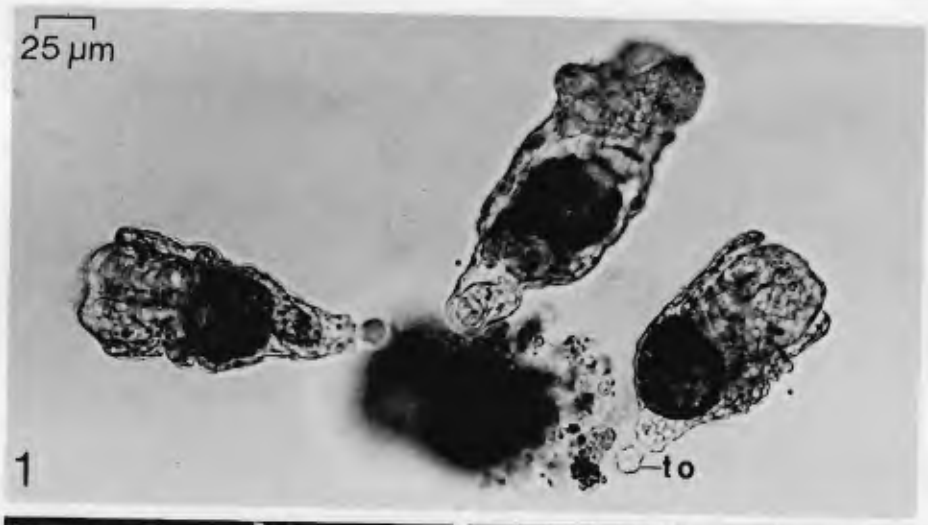
Fig. 2. Scanning electron micrograph of a pelagosphera larva 11 days after fertilization. Ventrolateral view. *mt*, metatrochal band of cilia; *to*, terminal organ

Fig. 3. Scanning electron micrograph of the terminal organ of a pelagosphera larva 11 days after fertilization

Fig. 4. Photomicrograph of terminal organ of a living pelagosphera larva $6^1/_2$ days after fertilization. Note attachment strand (*as*) that serves to attach the young larva to a substratum

Fig. 5. Scanning electron micrograph of larva of unknown age collected from oceanic plankton of the Florida Current. This larval type when reared in the laboratory gives rise to an adult tentatively identified as *Golfingia misakiana*. The arrow points to the terminal organ which is relatively small in comparison with that of the younger larva of the same species shown in Fig. 2. In contrast to the smooth cuticle of the young larva, that of the older oceanic larva is covered with prominent papillae. *mt*, metatrochal band of cilia

Fig. 6. A higher magnification of the posterior region of the larva in Fig. 5 shows the terminal organ (*arrow*) and its relation to the surrounding cuticular papillae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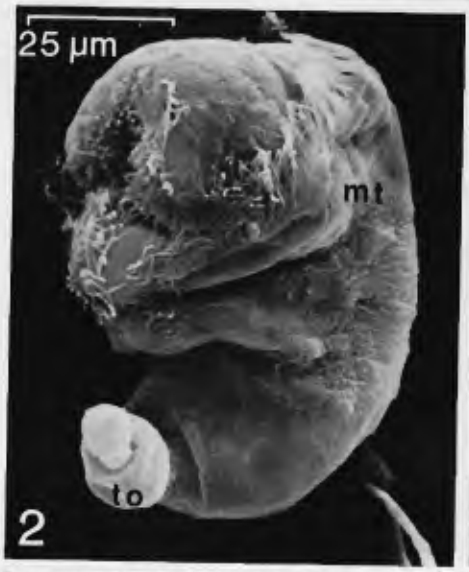
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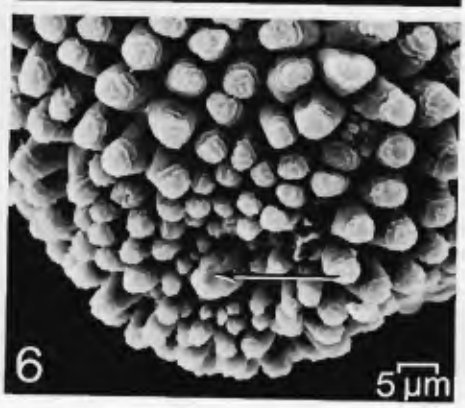
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terminal organ, the posterior body is shortened and rounded, whereas in a larva with extruded terminal organ, the body is attenuated, the region immediately anterior to the terminal organ being frequently tightly constricted. Retraction of the terminal organ is dependent on the contraction of a pair of posterior retractor muscles (described below); extension, on the other hand, is apparently brought about by the contraction of the musculature of the posterior body wall, forcing the extrusion of the organ.

Larvae have been observed in the laboratory to exhibit a variety of behavioral activities. An attached larva can stretch out at any angle from its point of attachment. It can extend straight upward, perpendicular to the bottom; in this position we believe the larva can feed on suspended matter in the water column by directing particles into the mouth through ciliary activity of the ventral head and metatrochal regions. The larva can also bend its body in a C-shape so that the ventral surface of the head is applied to the bottom of the dish. In this latter position larvae have been observed to feed by grazing on diatoms and bottom detritus in the vicinity of their attachments. Larvae are capable of turning in complete circles about their points of attachment. Although usually attached, larvae can release themselves and swim freely through the water. Most commonly free larvae remain near the bottom of the culture dish. Here they are observed to move along either parallel to the bottom or with ventral head applied to the bottom and posterior end upward, apparently feeding on bottom detritus. Unattached larvae may remain relatively quiescent on the bottom. Frequently they are observed to curl the body so that the terminal organ is placed in or near the mouth; the function of this behavior, characteristic of all sipunculan larvae, is not understood.

In attempting to determine the manner of larval attachment, we have removed larvae from culture dishes along with debris to which they are attached for examination under the compound microscope. In some instances we have observed a thin strand of material, designated as the attachment strand, which joins the tip of the terminal organ to the available substratum (Fig. 4). The strand is flexible, bending or folding upon itself as the larva moves in different directions, or extending out straight as the larva moves away from its attachment. It functions as a tether, limiting the distance the larva is able to move. There is no evidence that the strand is elastic. The attachment strand is difficult to detect because of its minute dimensions and because it is often obscured by surrounding debris. We have not been able to determine, therefore, whether this is the usual or only an occasional mode of attachment of the terminal organ. That the terminal organ produces an adhesive secretion is evident from the adherent debris commonly surrounding the organ. Algal cells, fecal matter, and other extraneous material accumulate around the terminal organ and frequently remain attached to it even after its release from the substratum. The firmness of attachment varies considerably among larvae in a culture dish. Some are readily dislodged by suction of a pipette fitted with a rubber bulb, whereas others can be dislodged only by scraping off the film or debris to which they are attached. When suction is applied to those most tightly

attached, they retract the anterior body into the trunk and spin like a top on their strongly cemented points of attachment.

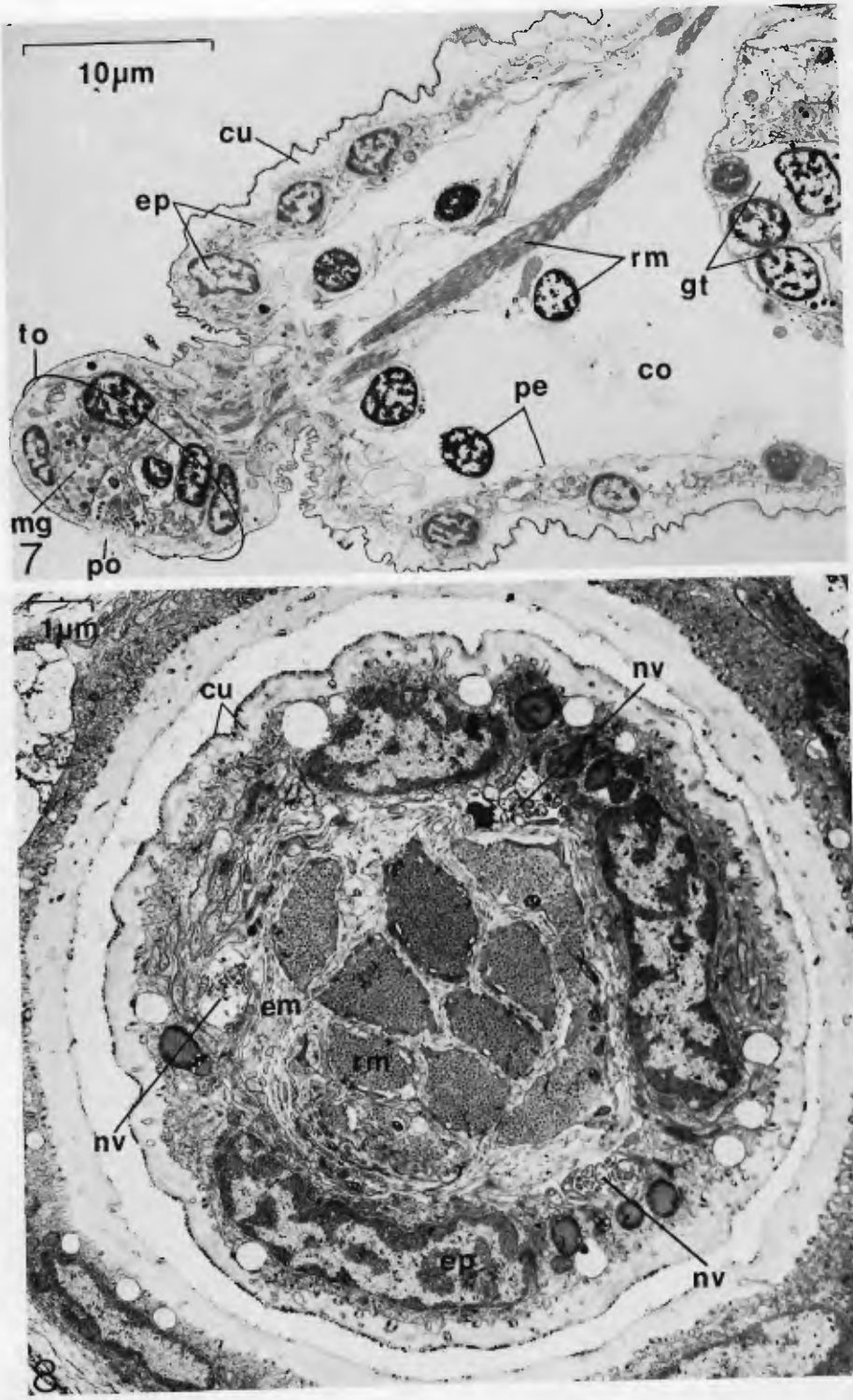
Once attached, larvae remain in one position for long periods. Patient observation of cultures under the dissecting microscope has revealed an occasional release and reattachment of a few individual larvae. Larvae observed to release have been in a feeding position bent over with ventral head applied to substratum. At release the attachment is abruptly disrupted and the terminal organ directed upward away from the substratum. The larva then swims away, usually remaining close to the substratum, and frequently moves along with ventral head on the substratum, apparently feeding as it progresses. Prior to reattachment the larva ceases swimming, remaining quiescent on the bottom. On one occasion attachment occurred after the larva had moved around in a circle with terminal organ extended toward the bottom and head directed upward. If the larva is not moving actively around its point of attachment, it is not easy to determine whether attachment has taken place. It has been assumed that attachment has occurred if the larva is not dislodged by jarring of the dish or by currents of water directed toward it by means of a pipette.

Incidental observations in the laboratory indicate that successful attachment is dependent on availability of an appropriate substratum. Larvae will not attach to sand grains or fine sediments, and when introduced to dishes containing such substrata, they continue to swim for a few days, then die. The larvae settle readily, however, in glass culture dishes in which sea water has been allowed to stand for a day or two and a thin film of undetermined origin has developed on the bottom of the dish.

II. Electron Microscopic Observations

1. General Organization of the Terminal Organ and Trunk (Figs. 7, 8). When viewed with the transmission electron microscope, the terminal organ of the 5.5 day larva appears as a prominent, bulb-shaped outgrowth of the posterior body wall (Fig. 7). It lacks, however, both the peritoneal epithelium and a continuation of the coelom, i.e. it is a compact organ. The distal pore of the terminal organ is a circular interruption of the outer two layers of the overlying cuticle (4–5 μm diam) that receives the necks of the various gland and sensory cells associated with the organ. The bulbous part of the terminal organ joins the trunk by a narrow stem through which pass neurites and 10 well-developed retractor muscle cells (Fig. 8).

The body wall of the trunk of the 5.5 day larva resembles that of the terminal organ except that the cuticle is thicker (0.5–1.8 μm, Fig. 21) and the musculature is not as strongly developed. Apart from a well-developed sphincter muscle associated with the hindgut, the body wall muscles consist only of outer circular muscle cells (40–50 nm thick in transverse section situated just below the epidermis) and the coelomic epithelium, or peritoneum, which forms the longitudinal body wall musculature (0.6–4.0 μm thick in non-nuclear regions, Fig. 21). Myofilaments were not observed in perito-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neal cells of older larvae where a layer of longitudinal body wall muscles is present (unpublished observations).

2. Cellular Organization of the Terminal Organ (Figs. 7–30). The bulbous portion of the terminal organ is composed of 29 cells which include: 8 epidermal cells, 3 mucus cells, 2 tension-bearing cells, 5 sensory cells, 1 internal cell of unknown function and 10 retractor muscle cells. We will offer a short description of each of the cell types below.

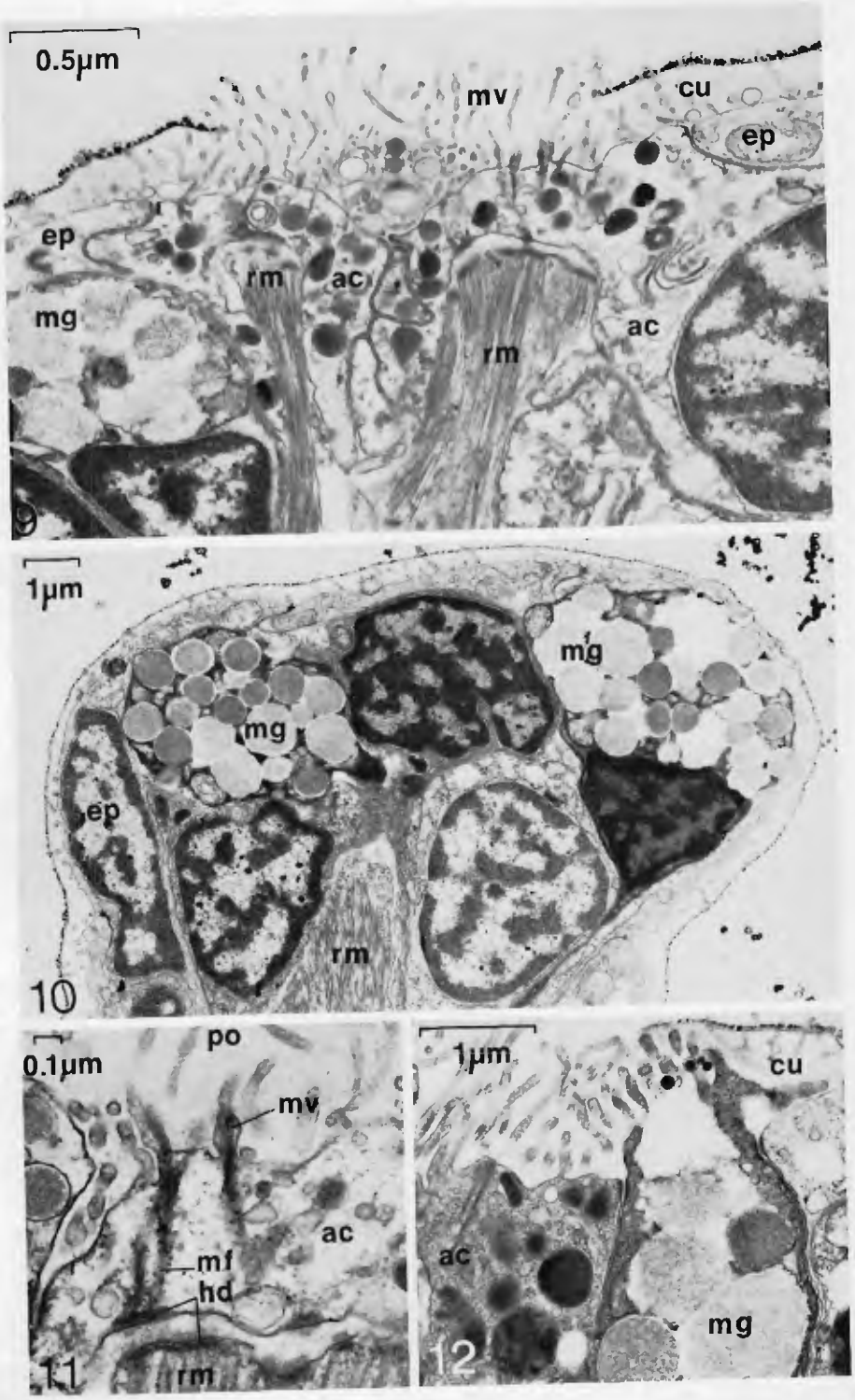
The pore of the terminal organ is a distally situated dimple that is formed by the microvillar borders of the necks of 2 mucus gland cells (in the 5.5 day larva), 3 sensory cells and the apical borders of the 2 tension-bearing cells (Figs. 7, 27–29). Seven cells, therefore, are involved in the formation and functioning of the adhesive pore of the terminal organ in the 5.5 day larva.

3. Epidermal Cells (Figs. 8–10, 25, 26). The epidermis of the terminal organ forms a thin (0.9–1.4 μm) covering over the organ; it secretes the cuticle and elaborates microvilli that extend through the cuticle (Fig. 21). The microvilli are occasionally branched. The epidermal cells are interjoined by apical zonulae adhaerentes followed basally by septate desmosomes.

The cuticle of the terminal organ of the 5.5 day larva is thin (0.3–0.7 μm) and consists of 3 layers. The innermost part of the cuticle is formed by a basal zone of fibrous material that resembles surface coat material (Fig. 21) and is quite unlike the basal cuticular zone of older larvae and adults that possess a meshwork of collagen fibers (unpublished observations). Apically, there is a dense layer resembling a membrane (10 nm thick; Figs. 9, 12) and an outermost epicuticular layer of electron dense material (40–70 nm thick; oceanic larva, see Fig. 19). Microvilli from the epidermal cells pass through the cuticle at intervals, forming slightly bulbous tips with radiating fibers of surface coat material extending into the external environment.

The Golgi apparatus of each cell (as many as 3 Golgi bodies/cell) is situated in the perinuclear region near the apical plasmalemma. Small (170 nm) dense vesicles are produced by the Golgi apparatus that may fuse immediately below the plasmalemma to form larger (400–600 nm) vesicles containing loosely consolidated material of varying densities. These vesicles are in close association with the apical plasmalemmata of all the epidermal cells but we have not observed any vesicles releasing their contents into the developing cuticle. Similar vesicles are present throughout the trunk epidermis (Fig. 21). It is also possible that these 400–600 nm vesicles may be involved in uptake rather than release of material but we have no experi-

Figs. 7–8. TEMs of pelagosphera larvae. **Fig. 7.** Sagittal TEM section of a 4.5 day larva. Note accelerated differentiation of the terminal organ compared with the trunk body wall. **Fig. 8.** Transverse TEM section of a planktonic pelagosphera of undetermined age. It is partly retracted into the trunk. *co*, coelom; *cu*, cuticle; *em*, extracellular matrix; *ep*, epidermis; *gt*, gut; *mg*, mucus gland; *nv*, neurite bundles; *pe*, peritoneum; *po*, pore of terminal organ; *rm*, retractor muscles; *to*, terminal organ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mental data to resolve the issue. The epidermal cells were also noted to contain RER, mitochondria and an occasional lysosome (1.4 μm).

4. Mucus Gland Cells (Figs. 10, 12, 16, 27). The terminal organ contains 3 secretory cells that produce granules we identify structurally as mucus (maximum diameter 1.2 μm, Figs. 10, 12). The cells are large (10.5–11.7 μm long) and irregularly shaped with a nucleus situated nearest the proximal end of each cell and a short neck extending distally to the pore of the terminal organ (2 cells only). The cytoplasm of the cells is packed with mucus vesicles that are released at the pore through an apical collar (0.7 μm diameter) of 12 microvilli (0.8–1.0 μm long each, Fig. 12). The microvilli do not appear to have cores of microfilaments. One mucus cell is situated ventrally in the left half of the terminal organ and opens ventrolaterally into the pore. The second, and slightly larger, mucus cell is dorsally situated from the midline to right side of the terminal organ and opens dorsally into the pore. The third is situated medially in the right half of the organ. A gland neck extending to the pore of the terminal organ was not found in any of our sections associated with this third mucus cell. We assume that the neck appears later in development. Each mucus cell contains perinuclear RER and at least one Golgi body between the nucleus and the mucus granules. Additionally, the nuclei of both mucus cells contain distinctively electron-dense chromatin, a fact that distinguishes them immediately from nuclei of other cells in the terminal organ in our electron micrographs (Fig. 10).

Two of the mucus cells were found to be innervated basally (Fig. 27).

5. Tension-Bearing Cells (Figs. 9, 11, 16, 28). Two cells form the primary tension-bearing elements of the terminal organ pore (Figs. 9, 11, 16). The perikarya of both cells are situated immediately internal to the pore, one to the left of the pore and slightly ventral in position, and one to the right of the pore and slightly dorsal in position. The apical surfaces of these cells form most of the surface area of the pore. Other cells involved with the pore contribute only narrow necks. Many microvilli (170 nm long) with dense cores of microfilaments issue from apical membranes of these cells (Figs. 11, 16).

The basal surfaces of both tension-bearing cells are deeply invaginated just below the pore region to permit the insertion of retractor muscles on a point immediately internal to the pore itself (Fig. 11). As a result of this invagination, the distance between the apical and basal membranes of the

Figs. 9–12. Sagittal TEM sections of terminal organs. **Fig. 9.** Pore, tension-bearing cells and retractor muscle cell integration of 4.5 day larva. **Fig. 10.** Mucus cells of 5.5 day larva. **Fig. 11.** Detail of tension-bearing cell of 4.5 day larva. **Fig. 12.** Mucus gland neck of 5.5 day larva. Compare the microvilli with cores of microfilaments on the tension-bearing cell with the microvilli lacking such cores surrounding the gland neck. *ac*, tension-bearing cells; *cu*, cuticle; *ep*, epidermis; *hd*, hemidesmosomes; *mf*, microfilaments; *mg*, mucus granules; *mv*, microvilli; *po*, pore of terminal organ; *rm*, retractor muscles

tension-bearing cells below the pore is as little as 1.0 μm. Two muscle cells extend into the basal invagination of each cell and form a series of junctional complexes with the basal plasmalemmata of the tension-bearing cells across the extracellular matrix. These junctions resemble hemidesmosomes (Fig. 11). Bundles of microfilaments extend apically from the basal junctional complexes on the tension-bearing cells to the cores of the microvilli protruding into the pore of the organ (Figs. 11, 16).

The apical cytoplasm of these tension-bearing cells is moderately dense with 350–880 nm vesicles containing uniformly electron-opaque material (Figs. 9, 11, 16, 18). These vesicles are again RER/Golgi-derived. The dorsalmost cell contained two Golgi bodies and the more ventral cell, one. The innervation of these cells has not been determined.

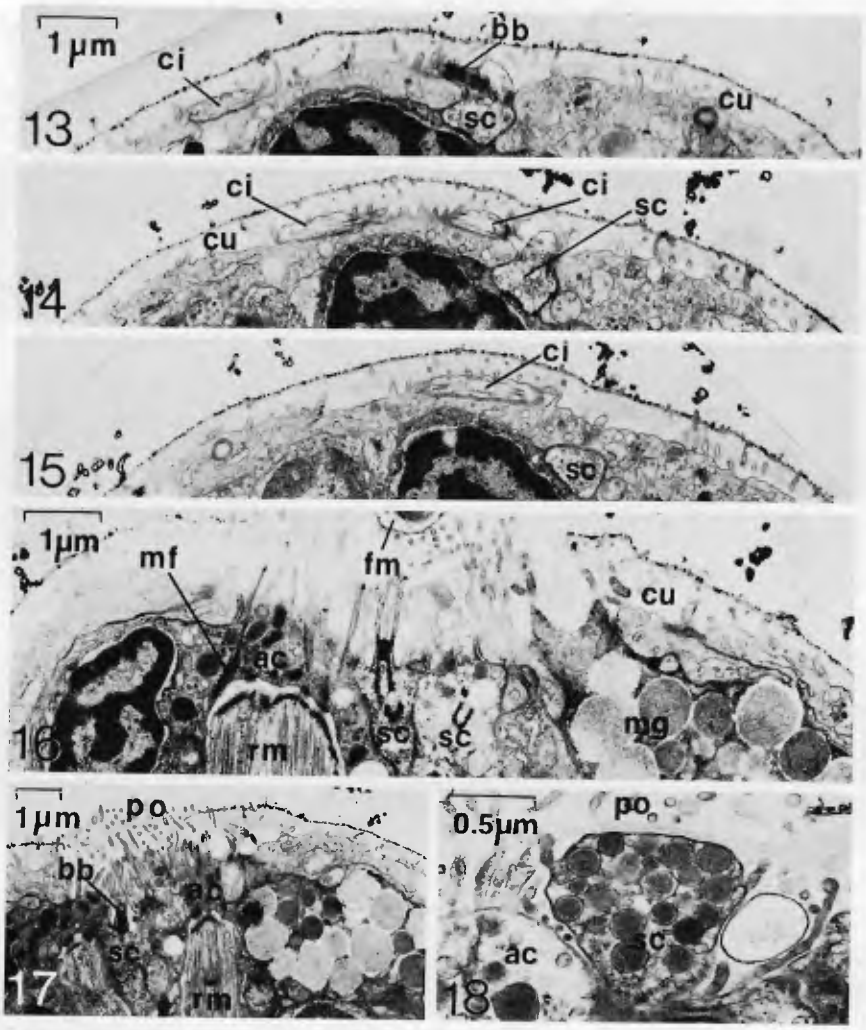
6. Sensory Cells (Figs. 12–18, 29, 30). Five sensory cells occur within the terminal organ of the 5.5 day larva. Three open into the pore of the organ and two are associated with the cuticle flanking the pore on the left and right sides. Four of the 5 cells are secondary sensory cells and 1 is a primary sensory cell.

Three flask-shaped cells are situated in the bulb of the terminal organ, one dorsal and on the left side of the organ, one medial in position, and one ventral on the right side of the organ (Figs. 16–18, 29). All 3 possess basal nuclei and are innervated by basal synaptic boutons. Each cell possesses a long narrow neck that terminates in a collar of microvilli at the terminal organ pore. A single cilium extends into the pore from the microvillar collars of both the ventral and medial cells (Figs. 16, 17). A cilium is absent from the dorsal cell but a diplosome is present immediately internal to the microvillar collar of this cell and its presence may indicate that ciliogenesis has not yet occurred (Fig. 16). All 3 cells produce RER/Golgi-derived vesicles with electron-dense material mostly 200–300 nm in diameter but some as large as 600 nm have been noted. These vesicles closely resemble those produced by the tension-bearing cells discussed above.

Two additional sensory cells occur in the terminal organ and each possesses a dendritic ending associated with the basal layer of the cuticle. These endings do not extend into the pore of the terminal organ but are immediately lateral to it. Each cell possesses a single Golgi body situated adjacent to the dendritic side of the nucleus that packages uniform electron-dense material into small vesicles ranging in size from 350 nm to one large vesicle 1 μm in diameter. Many of the vesicles are electron-lucent, a fact that may indicate inadequate fixation of the material in some of the vesicles.

One of these cells, a secondary sensory cell, is situated on the right side of the terminal organ pore (Figs. 12–14, 30). It terminates apically in a cilium that passes dorsally through the basal cuticular layer from a position to just below the pore to a position just above the pore. This intracuticular cilium vertically flanks the right side of the terminal organ pore.

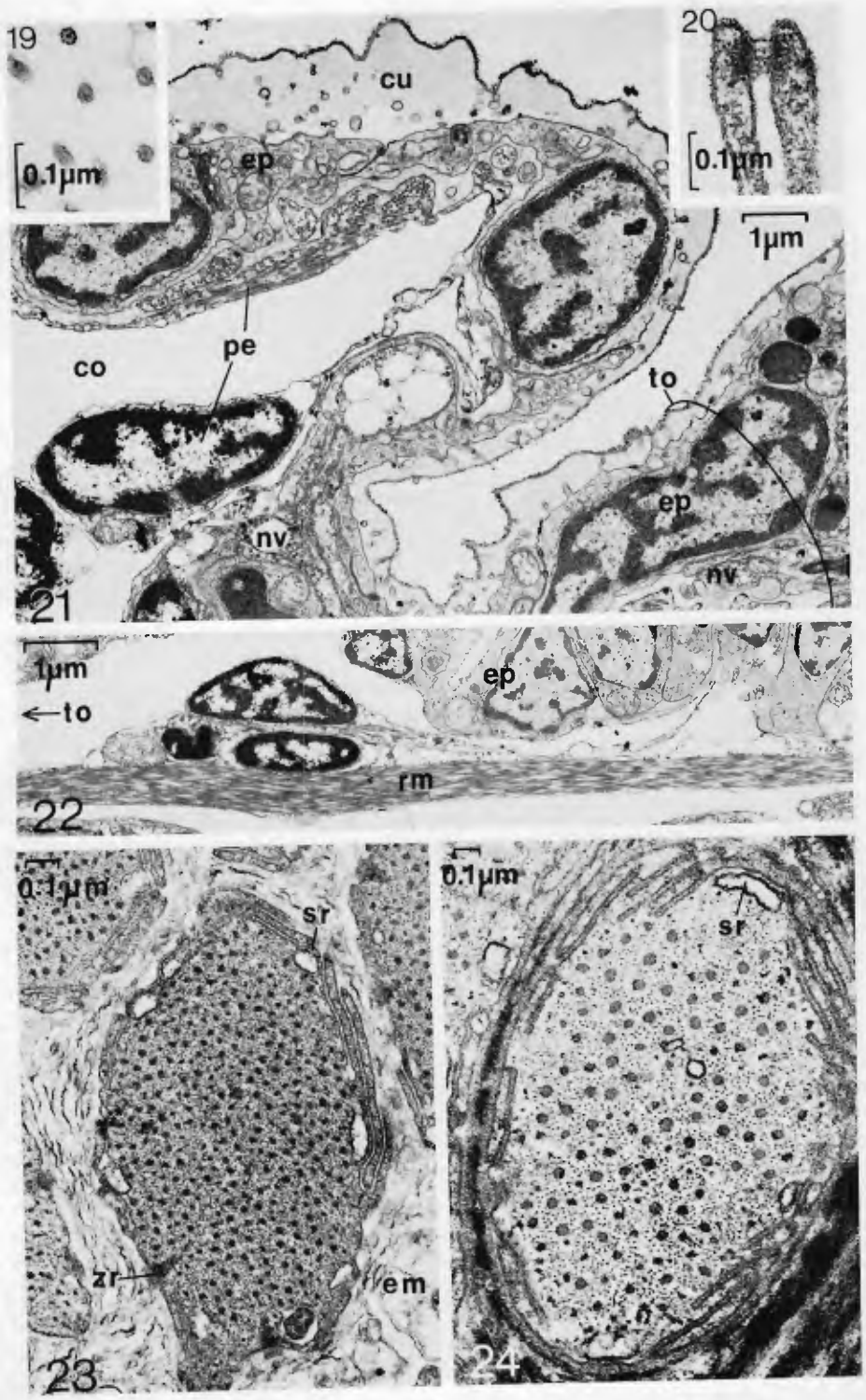
The second presumed proprioceptive cell, a primary sensory cell, is situated dorsally and medially within the terminal organ (Fig. 30). A long dendrite extends from the perikaryon to the right side of the pore where it



Figs. 13–18. Sensory cells of terminal organ of 5.5 day larva. Sagittal TEM sections of the organ. **Figs. 13–15.** Serial thin sections showing disposition of intracuticular sensory cilium. **Fig. 16.** Two pore-associated sensory cells. Note foreign material adhering to tension-bearing cell microvilli. **Fig. 17.** Detail of third pore-associated sensory cell. **Fig. 18.** Secretion granules in pore-associated sensory cell. *ac*, tension-bearing cell; *bb*, basal body; *ci*, cilium; *cu* cuticle; *fm*, foreign material; *mf*, microfilaments; *mg*, mucus granules; *po*, pore of terminal organ; *rm*, retractor muscles; *sc*, sensory cell

terminates in a narrow neck (600 nm) containing a basal body that protrudes into the basal cuticular layer. We assume that this basal body will induce ciliogenesis at a later stage of development.

7. Internal Cell (Fig. 26). One internal cell, of unclear function, is found ventrally in the terminal organ bulb immediately below the epidermis in the 5.5 day larva.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8. Retractor Muscle Cells (Figs. 7–10, 22, 23, 28). Ten spindle-shaped retractor muscle cells enter the terminal organ and form junctions with 16 of the 18 non-muscle cells. The retractor muscle cells extend from the terminal organ through the dorsal part of the coelom to insert on the body wall on each side of the hindgut (length 70 µm in a relaxed, fixed specimen; maximum diameter 1.5–1.75 µm). All the perikarya of the retractor muscle cells are situated in the coelom (Fig. 7).

The retractor muscles appear smooth (non-striated) when examined by light-microscopy. Electron microscopy also indicates that the muscles are smooth. Unlike typical invertebrate smooth muscle however, where thin filaments anchor to sarcomeral dense bodies and to sites on the sarcolemma (Reuter 1977), Z-rods are present in these muscles, as is more typical of the various forms of obliquely-striated muscle (Knapp 1978). The Z-rods radiate centripetally from their origins on the sarcolemma of the cells which are oval to circular in transverse section (Figs. 23, 24). The Z-rods do not, however, show the regular ordering that occurs commonly in obliquely-striated muscle of many annelids (Wissocq and Boilly 1977). These muscles, therefore, seem to be somewhat intermediate between typical invertebrate obliquely-striated and smooth types.

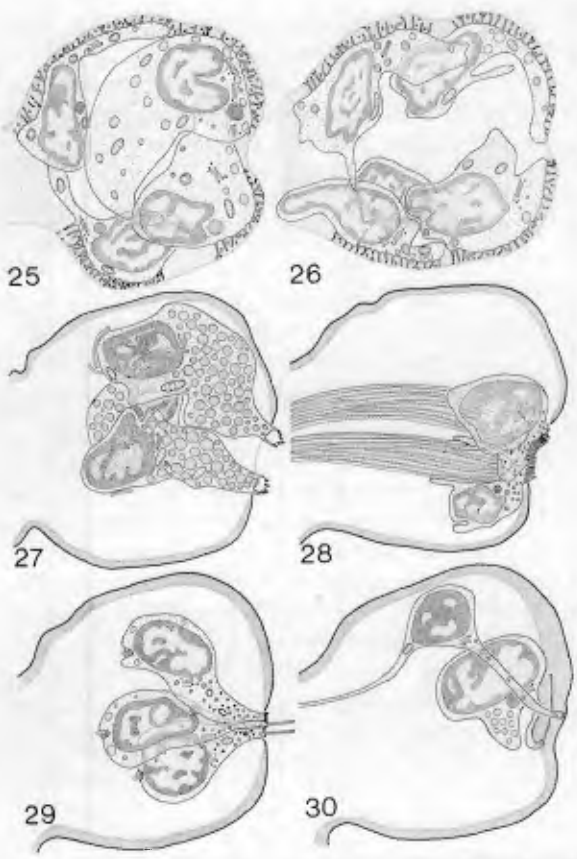
Thick (30–50 nm) and thin (5–6 nm) myofilaments are distributed throughout the sarcoplasm in the non-nuclear parts of the cells (Figs. 23, 24). The ratio of thin to thick filaments is approximately 12–14:1. The lengths of the tapered thick filaments were difficult to determine because they appear to wind in and out of the plane of our longitudinal sections. The length of one filament measured at least 3 µm, but we are uncertain whether this represents its total length.

The retractor muscle cells of the 5.5 day larva possess an excitation-contraction coupling system that consists only of subsarcolemmal cisternae of sarcoplasmic reticulum coupled with the sarcolemma to form so-called peripheral couplings (Oliphant and Clancy 1972). The retractor muscles of an older, oceanic larva of *G. misakiana* showed T-shaped invaginations of the sarcolemma (Fig. 23) that make diadic contacts with the SR. These retractor muscle cells were 6–7 µm in diameter.

The observed rate of retraction of the terminal organ in the 5.5 day larva was 220 µm/s (one observation).

9. Innervation of the Terminal Organ (Figs. 2, 15). Three subepidermal bundles of 8–10 neurites each enter the neck and bulb of the terminal organ

Figs. 19–24. TEMs of pelagosphera larvae. **Fig. 19.** Epicuticular filaments of planktonic pelagosphera of undetermined age. **Fig. 20.** Junctional complex between peritoneal cells of the larva in Fig. 19. The peritoneal cells are non-myoepithelial at this stage. **Fig. 21.** Sagittal section through the posterior trunk and terminal organ of 5.5 day larva. Note that what is designated as the peritoneal lining is the longitudinal trunk musculature at this stage of development. **Fig. 22.** Sagittal section of coelomic part of retractor muscle of terminal organ in 5.5 day larva. **Fig. 23.** Transverse section of a terminal organ retractor muscle in planktonic larva of undetermined age. **Fig. 24.** Transverse section of a longitudinal trunk muscle of larva in Fig. 23. Note the ingrowth of the sarcolemma in these older larvae. *co*, coelom; *cu*, cuticle; *ep*, epidermis; *ne*, neurites; *pe*, peritoneum; *rm*, retractor muscles; *sr*, sarcoplasmic reticulum; *to*, terminal organ; *zr*, *z*-rods



Figs. 25–30. Sagittal reconstruction of a cellular anatomy of the terminal organ of 5.5 day larva from serial TEM sections. Most nerve and muscle cells omitted. **Figs. 25, 26.** Epidermal cells and internal cell (Fig. 26). Gaps indicate missing data. **Fig. 27.** Three mucus gland cells. **Fig. 28.** Two tension-bearing cells. **Fig. 29.** Three pore-associated sensory cells. **Fig. 30.** Two cuticle-associated sensory cells

from the trunk (Fig. 8). One bundle is ventrally situated and continuous with the ventral nerve cord. The other two bundles are lateral to dorsolateral in position, one on the right side of the organ, the other on the left. The neurites from the left and right bundles extend into the trunk and then curve ventrally where they probably join the ventral nerve cord from the right and left sides of the body.

D. Discussion

1. Functional Integration of Cells in the Terminal Organ as an Organ of Larval Adhesion

Although all cells in the terminal organ, except the muscle cells, are ectodermal derivatives, it is apparent that only the 8 epidermal cells function to maintain the integrity of the organ and to secrete the cuticle (Figs. 25, 26).

The mucus cells are implicated as the adhesive producing cells because of their considerable volume of vesicles, the relation of the gland necks to the pore of the terminal organ, and the observed breakdown of vesicles into the pore (Fig. 12). Mucus is known to possess adhesive properties (fluidity, surface activity, cohesion: Dahlquist 1977; Tyler 1976; Rieger and Tyler 1979). We presume that the observed attachment strand (pp. 6) secreted by these larvae is a mucus thread. That attached larvae can spin while anchored on their attachment strands suggests that either intermolecular shearing occurs within the strand or, more likely, that the strand simply twists as the animal rotates.

The tension-bearing structures appear to be the tension-bearing cells and perhaps part of the surrounding cuticle. This interpretation seems reasonable because the apical surfaces of the anchor-cells constitute most of the pore surface and their microvilli contain bundles of microfilaments that extend basally to join directly to the retractor muscles via junctions across the extracellular matrix.

The two sensory cells with intracuticular cilia would seem to function as cuticular strain receptors, a possibility supported not only by the general position of the cilium of one cell and the basal body of the other but by the orthogonal orientation of the cilium of one cell and the basal body of the other within the cuticle (Fig. 30). This orientation of receptoral cilia flanking the pore could allow the animals to monitor cuticular strain in any direction.

As discussed earlier in the paper, the retractor muscles are responsible for retraction of the terminal organ into the trunk of the animal, flexion of the terminal organ and for bearing tension when the larva is attached to a substratum.

The specific function, or functions, of the 3 remaining sensory cells associated with the pore of the terminal organ are uncertain. Because larvae are selective in the substrata to which they will attach, it is possible that these receptors evaluate the suitability of substrata for adhesion. Alternatively, the spatial relationship of their cilia to the pore of the terminal organ suggests that they may be incorporated into the attachment strand as it is secreted and could serve therefore to monitor strain in the tether.

The most difficult aspect of function to determine is the mode of release of the terminal organ from the substratum. On this point, our data do not allow us to decide whether this event occurs mechanically or chemically, or both. Mechanical release might be accomplished by muscular contraction, as in a sudden flexion of the terminal organ, or swimming movements that could tear the larva from its point of attachment. Evidence for the possibility of chemical release is the observation of numerous secretory vesicles in the anchor cells and in the 3 pore-associated sensory cells. These could possibly function as chemical releasers.

II. Comparison of the Terminal Organ with Adhesive Organs in Lower Metazoa

Discrete adhesive organs relying on mucus secreting cells are documented in Acoela and Nemertodermatida among the Turbellaria, in Nemertea,

Gnathostomulida, and perhaps some Nematoda and Rotifera (see Rieger and Tyler 1979 for review). Of these, the terminal organ of the pelagosphera is generally similar to the rotifer adhesive organ (*Philodina*, Dickson and Mercer 1966). Both share the following characteristics: (1) possession of retractile, posteriorly-situated organs. (2) Mucoid material is released from multiple, symmetrically oriented glands at distinct pores. (3) Retractor muscle bands extend from the trunk into the adhesive organ to form junctions with the tension-bearing cells. (4) Variants of obliquely-striated muscle are present. The adhesive organ of *Philodina*, however, differs in its organization from the pelagosphera organ in the following ways: (1) the organ terminates in two toes. (2) There are 12 gland cells with a longitudinal array of microtubules immediately below the plasmalemma of each cell neck and the necks do not terminate in a collar of microvilli. (3) The epidermis lacks an extracellular cuticle and microvilli. The cells instead produce a dense apical layer of filaments resembling a terminal web. (4) The anchor cells show microvillar-like processes but the cores contain a fiber resembling a ciliary rootlet. The processes may therefore be modified cilia rather than true microvilli. (5) A layer of circular muscle is present proximally in the organ. This comparison indicates that although the rotiferan and sipunculan attachment organs share similarities at a general level of comparison, they are decidedly dissimilar on closer examination. In the absence of any known designs to link the disparate organizations of these two organs, we conclude that there is a low probability of homology between the two, i.e. it appears likely that sipunculans and rotifers independently evolved their adhesive organs.

The other major design of adhesive organs in lower Metazoa is the duo-gland adhesive organ described originally by Tyler (1976). These organs consist minimally of two gland cell types, a viscid gland secreting the adhesive substance and a releasing gland secreting the chemical releaser. Although we cannot rule out the simultaneous occurrence of a duo-gland mechanism with a mucus adhering mechanism in the terminal organ system, we consider this unlikely for the following reasons: (1) there are no established examples of the simultaneous occurrence of these two systems in a single adhesive organ in any lower metazoan. (2) We have not seen evidence of release of any vesicles except those of the mucus glands of the pelagosphera. (3) On the basis of size, the vesicles observed in the anchoring cells and in the three pore-associated sensory cells qualify as viscid granules but we have not found any cells that contain vesicles that compare in size (0.1–0.2 μm) to previously described releasing granules (Tyler 1976; Martin 1978). We conclude, on the basis of current comparative data, that the terminal organ adhesive system of the pelagosphera larva is a design unique to the Sipuncula that shares similarities to adhesive organs of other metazoans only at the most general levels of comparison.

III. Significance of the Terminal Organ in Larval Biology and Life Histories of Sipunculans

The terminal organ plays an important role in larval biology by enchancing the efficiency of larval feeding and enabling the exploitation of different

sources of food. By attaching to the substratum, the larva is able to feed both on the bottom surrounding its point of attachment as well as from the overlying water column. While feeding on the bottom, the larval body is bent so that the ventral head and mouth region are applied to the substratum, thus enabling the animal to graze on detrital material, algal coverings and bacterial films. The area of grazing in this attached position is limited by the extensibility of the larval body; however, the temporary nature of the attachment allows the larva to release itself and move to new and different feeding areas. When feeding from the water column, the larva maintains an upright position from its point of attachment with the ciliated mouth region directed upward to feed by a ciliary mucus mechanism on suspended particulate matter.

A more direct contribution to the feeding process may be the provision of a source of food through the secretory activities of the terminal organ. Surrounding the attachment of the organ there frequently accumulates a considerable amount of adherent debris held together by mucus secretions and consisting of particulate matter, feces, and bacteria. The mucus secretions, while entrapping particulate matter, also provide a medium for bacterial growth. A characteristic behavioral pattern of all sipunculan larvae is the placement of the terminal organ in the mouth, and, although the significance of this behavior has not been documented, one result could be the ingestion of some of the adherent matter. Other possible functions of this behavior include a transfer of secretions (Jägersten 1963) or communication between head and terminal regions as a means of testing the substratum prior to either attachment or settlement.

Sensory structures alluded to earlier in this discussion may function in the selectivity of a substratum for attachment in younger larvae. That some selectivity does occur is evidenced by the failure of young larvae to attach to sand grains or other granular material. Also young larvae are less likely to attach to the bottom of a clean glass dish than to one which has accumulated a thin film presumably of microorganismal origin. For older larvae the sensory complex of the terminal organ could conceivably be utilized in the selection of a site for settlement, burrowing and subsequent metamorphosis.

The changes in the terminal organ of *Golfingia misakiana* in prominence and function during the course of larval developmental history reflect ecological adaptations and behavioral modifications that are important in a consideration of the overall life histories pattern of species. The young larval stage of *Golfingia misakiana* is well adapted for a benthic existence. The strong attachment to the substratum, afforded by the terminal organ, prevents it from being swept away by currents and allows it to feed from the bottom and the overlying water. As the larva grows the terminal organ is proportionately reduced relative to body size and its function in attachment is lost. At this stage the larva could then be transported by currents into the surface waters where it is commonly found in the major currents of the open ocean. Whereas the younger stage is adapted for feeding and growth, the older larva, with its well developed metatrochal band of swimming cilia, is well adapted for dispersal (Rice 1981). Although the attach-

ment of the terminal organ is lost in the older larva, it is speculated that the sensory function is retained possibly for substratum testing in preparation for settlement. An evaluation must await further ultrastructural studies of the organ in older larvae.

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