Chapter 7

Sipuncula

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INTRODUCTION

Sipunculans are a phylum of nonsegmented coelomate marine worms. Distributed throughout the oceans of the world, they range from polar seas to the tropics and from intertidal waters to abyssal depths. They occur beneath rocks, in crevices, among the roots of sea grasses or byssal threads of mussels, in oyster beds, or burrowed in sandy and muddy sediments. Many species are found associated with coral reefs, where they inhabit borings of their own formation in coral rock or calcareous rubble. The size of sipunculan species varies from a length of 1 mm to more than 300 mm. The absence of segmentation in both developmental and adult stages distinguishes sipunculans from the closely allied group of annelid worms.

GROSS MORPHOLOGY

The body of a sipunculan is divided into two regions: a thickened posterior trunk and a narrower anterior introvert that can be retracted into the trunk (Fig. 1). At the distal end of the introvert a crown of tentacles usually surrounds the terminal mouth. The introvert commonly bears cuticular elaborations or projections such as hooks, spines, and papillae.

Internally there is a spacious coelomic cavity (Fig. 2) filled with fluid in which coelomocytes and developing gametes are suspended. Retractor muscles extend through the body cavity from an attachment near the base of the tentacles to an attachment on the body wall in the trunk. When contracted these muscles withdraw the introvert into the trunk. The digestive tract consists of an elongate esophagus that extends the length of the introvert into the trunk, an intestinal spiral with a descending loop and ascending loop coiled around one another, and finally, the rectum, which terminates in a dorsal anus, usually at the base of the introvert. A pair of metanephridia, reduced in some species to a single organ, is suspended in the coelomic cavity from ventrolateral attachments in the anterior trunk. A ventral nerve cord, median and unpaired, joins an anterior supraesophageal ganglion or brain by means of circumesophageal connectives. A contractile vessel, a blind sac sometimes with extensions known as villi, lies along the length of the esophagus and unites anteriorly with a ring canal at the base
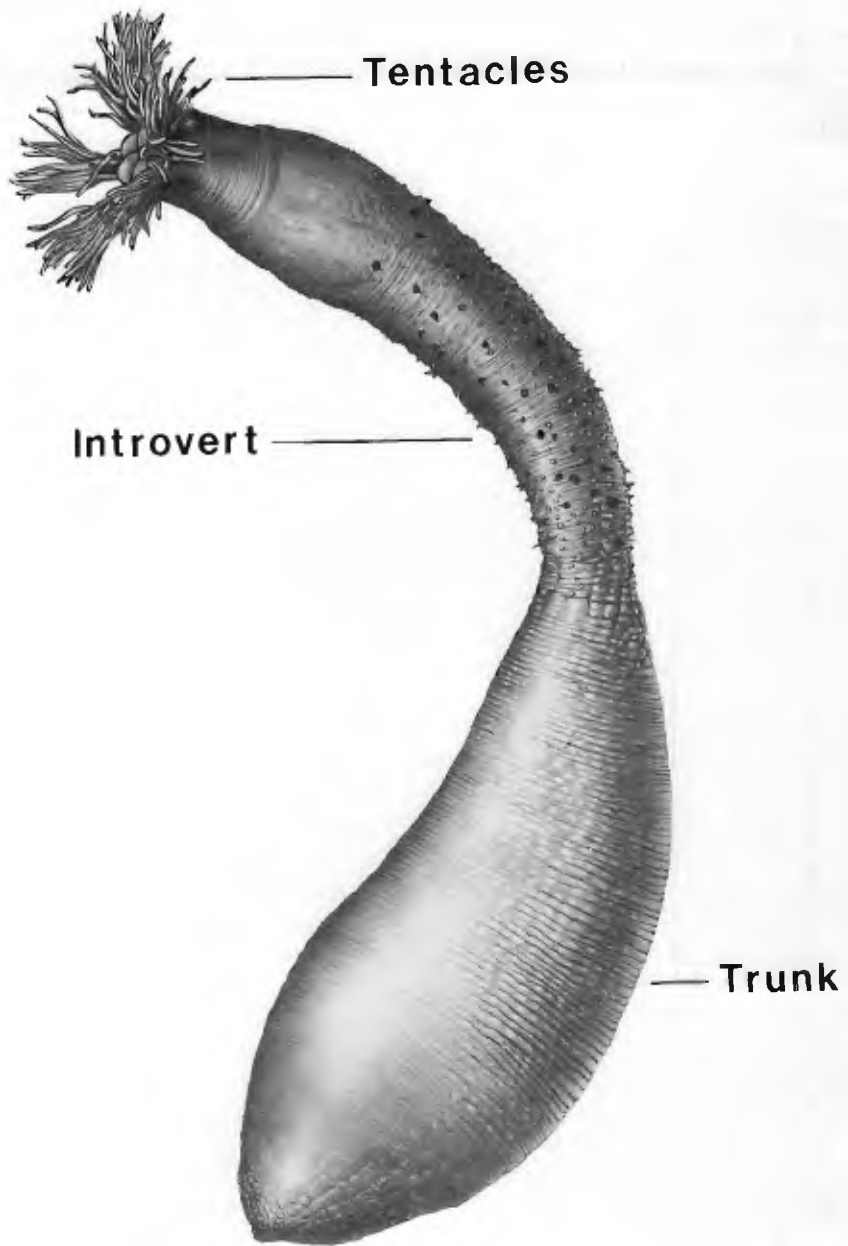
Fig. 1. External anatomy of *Themiste alutacea*. (Illustrated by Carolyn B. Gast.)

of the tentacles. The ring canal, in turn, is continuous with canals in the tentacles. The gonad, either ovary or testis, occurs as a thin strand of tissue at the base of the ventral retractor muscles, extending beneath the ventral nerve cord between the two muscles. Gametes are released from the gonad at an early stage into the coelom, where they undergo most of their development as freely floating cells. At the completion of development they are taken into the nephridium by way of the nephrostome, where they are stored for a short period before being released via the nephridiopore to the surrounding seawater.

SYSTEMATICS

The most recent classifications of the Sipuncula are those of Stephen and Edmonds (1972), who divided the group into four families, and of Gibbs and Cutler (1987), who defined two classes, four orders, and six families. The latter classification was based on a phylogenetic analysis in which contemporary phylogenetic methodology was applied to produce an evolutionary tree (Cutler and Gibbs, 1985) (Fig. 3). Stephen and Edmonds, in their review of the literature, recorded 320 species, whereas Gibbs and Cutler, after a series of taxonomic revisions by Cutler and colleagues (cf. references in Gibbs and Cutler, 1987), reduced that number to 206.

Using 9 distinguishing characters, Gibbs and Cutler described 17 genera (Table 1). They defined genera by the presence or absence of characters and by a unique combination of the designated characters. Examples of the generic characters are given in Figures 4 through 15, which illustrate external and internal anatomies of representative species from 6 of the 17 described genera, including each of the 6 families.

The family Sipunculidae includes the large, sand-burrowing genera *Sipunculus, Xenosiphon, Siphonosoma, Siphonomecus,* and *Phascolopsis.* With the exception of *Phascolopsis,* these genera exhibit banding in both the circular and longitudinal musculatures of the body wall and extensions of coelomic sacs or canals into the body wall. In *Phascolopsis* only longitudinal musculature is banded, and coelomic extensions are absent. The body surface, for the most part, is smooth, but marked with grooves that reflect the internal banding of the musculature.

In Golfingiidae, Phascolionidae, and Themistidae, the body wall musculature is continuous or smooth. Phascolionidae, comprised of the genera *Phascolion* and *Onchnesoma,* is characterized by a fusion, in varying degrees, of the retractor muscles and a reduction in the number of nephridia from two to one. Themistidae, with the single genus *Themiste,* has two retractor muscles and two nephridia and is distinguished by a well-developed tentacular crown of elongate tentacles arising in four to eight stems from the oral disc. The three genera of Golfingiidae (*Golfingia, Nephasoma,* and *Thysanocardia*) lack the distinctive tentacular crown of *Themiste* and the modifications of retractor muscles and nephridial number of species of Phascolionidae, although they share other characters with the two groups (Table 1).

Tentacles of Aspidosiphonidae and Phascolosomatidae, unlike those of other sipunculans, are arranged in an arc dorsal to the mouth, rather than in a circle surrounding the mouth (Figs. 16–21). In these two families, the margin of the oral ridge is expanded as a ridge, the cephalic collar. Aspidosiphonidae is further distinguished by the presence at the anterior end of the trunk of a hardened structure termed a shield. In two genera, *Aspidosiphon* and *Lithacrosiphon,* the shield, composed of a horny protein (not chitin), displaces the introvert to a ventral position (Fig. 8), whereas in the third, *Cloeosiphon,* the shield is composed of calcareous units and the introvert remains in line with the longitudinal axis. In rock-boring species the shield may serve as an operculum, closing the mouth of the burrow when the introvert is withdrawn. Phascolosomatidae lack the shield, but generally have well-developed papillae that are concentrated at the anterior end of the trunk or base of the introvert as well as at the

CV
Es
An
Rec
SpM
N
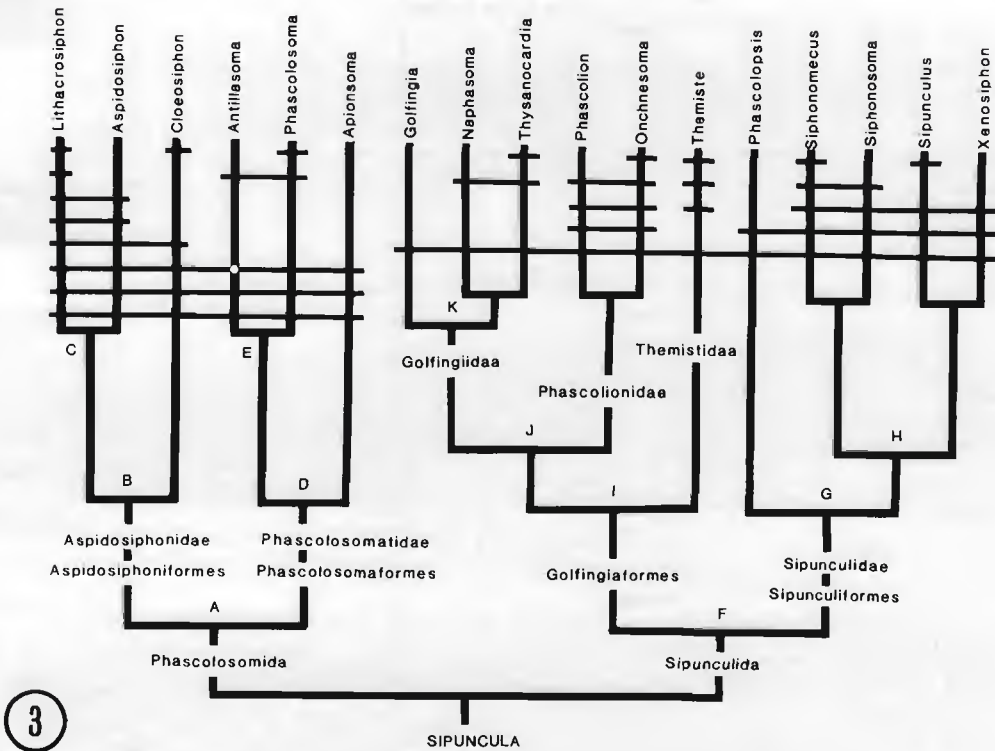FM
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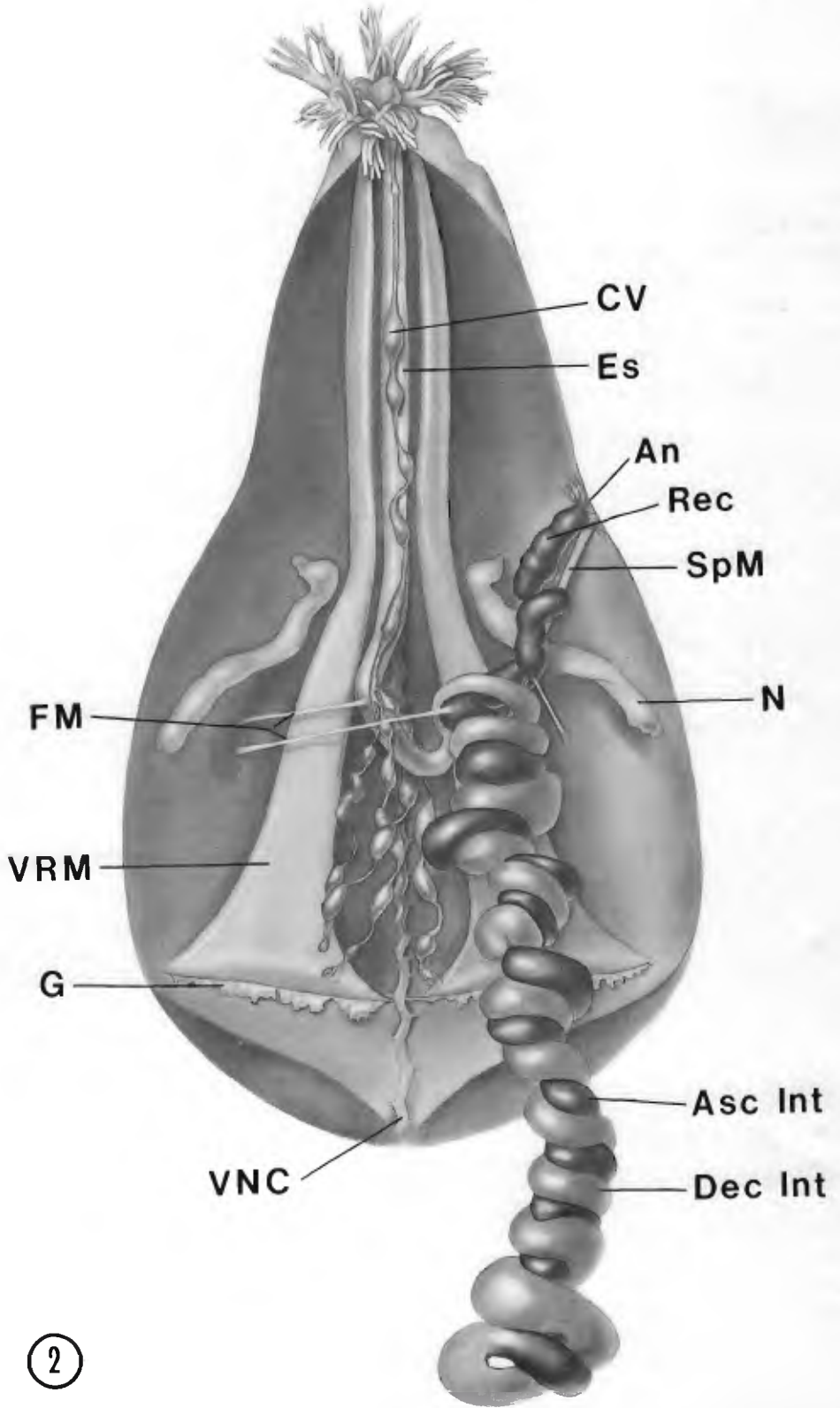
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Fig. 3. Phylogenetic tree of the genera of Sipuncula. Synapomorphies are shown by the horizontal bars. This is derived from cladograms generated by several phylogenetic methods, none of which would resolve it this completely. (From Cutler and Gibbs, 1985.)

posterior extremity of the body (Fig. 9). In both of these families, the introvert is commonly ornamented by hooks that may occur in rings anteriorly and be irregularly scattered more posteriorly. Considerable variation is found at the specific level in shape and dentation of hooks (Fig. 22–27). Although introvert hooks occur in other families, their arrangement is usually scattered rather than in rings and their shape straight and spine-like rather than curved (Fig. 28). The function of introvert hooks is not clear, but they have been implicated in food-gathering and, in rock-boring species, as an aid in burrow formation (Rice, 1969).

Papillary protrusions of the body surface are found in all families, although best developed in the Phascolosomatidae. Shape, size, and distribution of papillae vary among species and genera, as well as along the anteroposterior axis of a single individual (Figs. 29–34). Papillae are associated with glandular and sensory epidermal organs that open through permanent pores in the cuticle.

BODY WALL

The body wall of a sipunculan consists of an outer cuticle, beneath which lie an epidermis, dermis, circular muscle layer, longitudinal muscle layer, and, lining the coelom, a

Fig. 2. Internal anatomy of *Themiste alutacea*. An, anus; Asc Int, ascending intestine (darker shading); CV, contractile vessel with villi or branches in trunk region; Des Int, descending intestine (lighter shading); Es, esophagus; FM, fixing muscle; G, gonad; N, nephridium; Rec, rectum; SpM, spindle muscle; VNC, ventral nerve cord; VRM, ventral retractor muscle. (Illustrated by Carolyn B. Gast.)

TABLE 1. Summary of Characters in Sipunculan Genera

	Presence of anal shield	Type of tentacle arrangement[a]	Type of introvert hook when present[b]	Presence of banding in longitudinal muscle layer	Presence of canals or sacs in body wall	No. of retractor muscles apparent	Spindle muscle attached posteriorly	No. of nephridia	Presence of villi on contractile vessel	No. of species
Sipunculus	–	S	—	+[c]	+	4	–	2	–	10
Xenosiphon	–	S	—	+[c]	+	4	–	2	–	1
Siphonosoma	–	S	S	+[c]	+	4	+	2	+/–[i]	10
Siphonomecus	–	S	S	+[c]	+	2	+	2	–	1
Phascolopsis	–	S	—	+	–	4	–	2	–	1
Golfingia	–	S	S	–	–	4	–	2	–	12
Nephasoma	–	S	S	–	–	2	–	2	–	23
Thysanocardia	–	S	—	–	–	2	–	2	+	3
Phascolion	–	S	S	–	–	4[c]	–[g]	1	–[i]	25
Onchnesoma	–	S	—	–	–	2[f]	–[g]	1	–	4
Themiste	–	(S)	S	–	–	2	–	2	+	25
Apionsoma	–	P	P	–	–	4	+	2	–	6
Phascolosoma	–	P	P	+	–	4	+[h]	2	–	36
Antillesoma	–	P	—	+	–	4	+	2	+	1
Aspidosiphon	+	P	P	–/+[d]	–	2	+	2	–	45
Cloeosiphon	+	P	P	–	–	2	+	2	–	1
Lithacrosiphon	+	P	P	+	–	2	+	2	–	2

[a]S, Sipunculidea form, tentacles surround mouth; P, Phascolosomatidea form, tentacles in arc dorsal to mouth.
[b]S, Sipunculidea form; hooks simple, scattered; P, Phascolosomatidea form; hooks curved, in rings on anterior introvert.
[c]Circular muscle layer also banded.
[d]Present Aspidosiphon (Paraspidosiphon).
[e]Often strongly fused so that number appears to be fewer (3, 2, or 1).
[f]Column is entire but thought to be composed of two fused muscles.
[g]Spindle muscle absent.
[h]Unattached posteriorly in Phascolosoma pectinatum.
[i]Absent in some species.
[j]Present in Phascolion cirratum.
(From Gibbs and Cutler, 1987.)

peritoneal layer (Fig. 35). In families Sipunculidae, Phascolosomatidae, and, in part, Aspidosiphonidae, the longitudinal muscle layer is divided into bundles (Figs. 36, 37). In most members of the family Sipunculidae, the circular musculature is also in bundles and there are extensions from the coelom into the body wall (Fig. 37).

Cuticle

The cuticle is an extracellular, elastic layer that covers the external surfaces of the sipunculan body. It varies in thickness, depending on the species, the region of the body, and the extent of contraction or extension of the animal. Thickness is greatest on the anterior trunk at the base of the introvert and in the posterior body. The thinnest region is that covering the introvert, particularly the most anterior introvert, and the tentacles. Cuticular elaborations include hooks, spines, platelets or scales that are condensations in the cuticle

often associated with papillary protrusions, and the shields of the anterior trunk in the Aspidosiphonidae (Figs. 8, 22–28). On gross examination the cuticle appears externally to be smooth and glistening in some species of *Golfingia*, grooved in transverse and/or longitudinal furrows reflecting arrangement of body wall musculature in *Sipunculus*, rough and uneven due to the numerous papillary protrusions in most species of *Phascolosoma*, or thickened by cuticular platelets in *Antillesoma*.

The cuticle is composed of layers of fiber bundles arranged at right angles to one another and inclined at an angle of approximately 45° to the longitudinal axis of the body (Figs. 38–46). First described from light microscopic studies on *Sipunculus nudus* (Andreae, 1882; Metalnikoff, 1900) and *Siphonosoma cumanense* (Shitamori, 1936), this construction has been further delineated in more recent ultrastructural investigations on
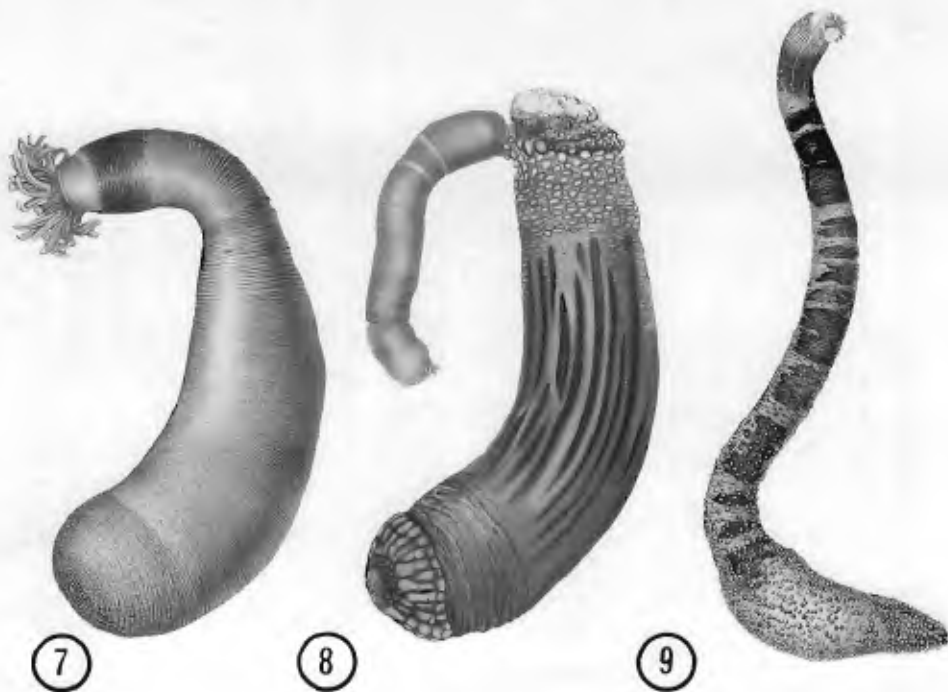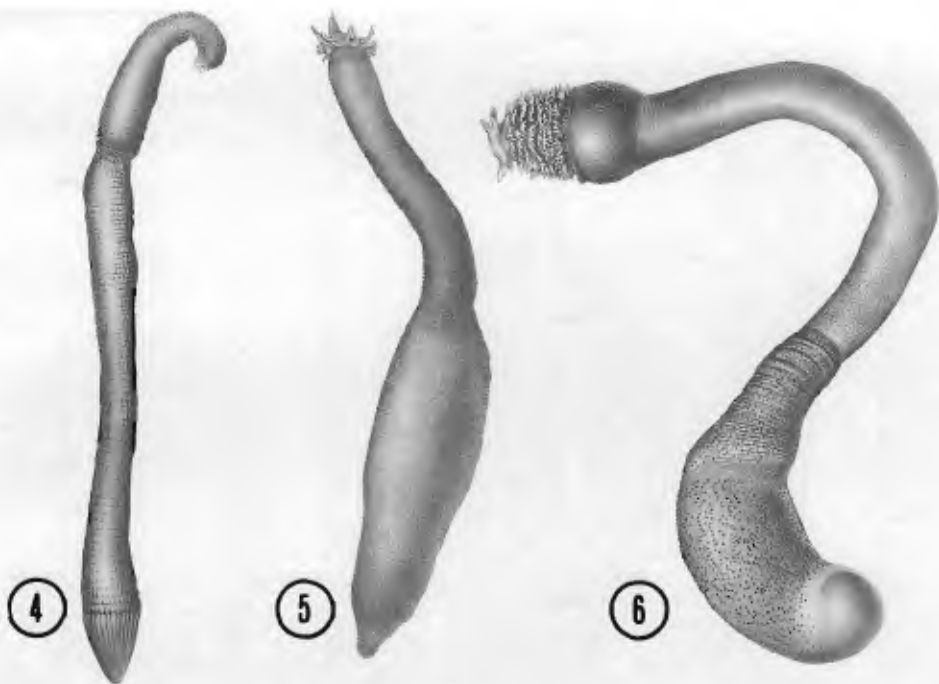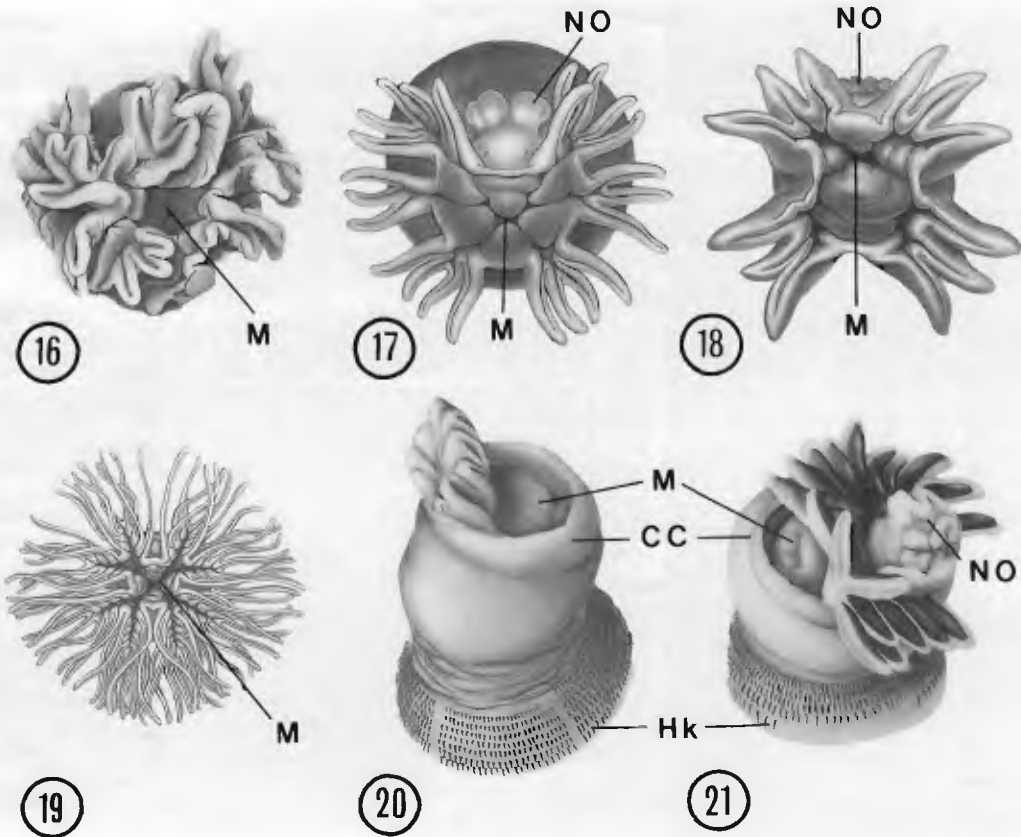
Figs. 4–9. External anatomy of representative species of the six families of Sipuncula. (Illustrated by Charissa Baker, Figs. 4, 8, 9, and Carolyn B. Gast, Figs. 5–7.)
Fig. 4. *Sipunculus nudus* (Sipunculidae).
Fig. 5. *Nephasoma pellucida* (Golfingiidae).
Fig. 6. *Phascolion cryptus (Phascolionidae).*
Fig. 7. *Themiste lageniformis* (Themistidae).
Fig. 8. *Aspidosiphon steenstrupii* (Aspidosiphonidae).
Fig. 9. *Phascolosoma varians* (Phascolosomatidae).

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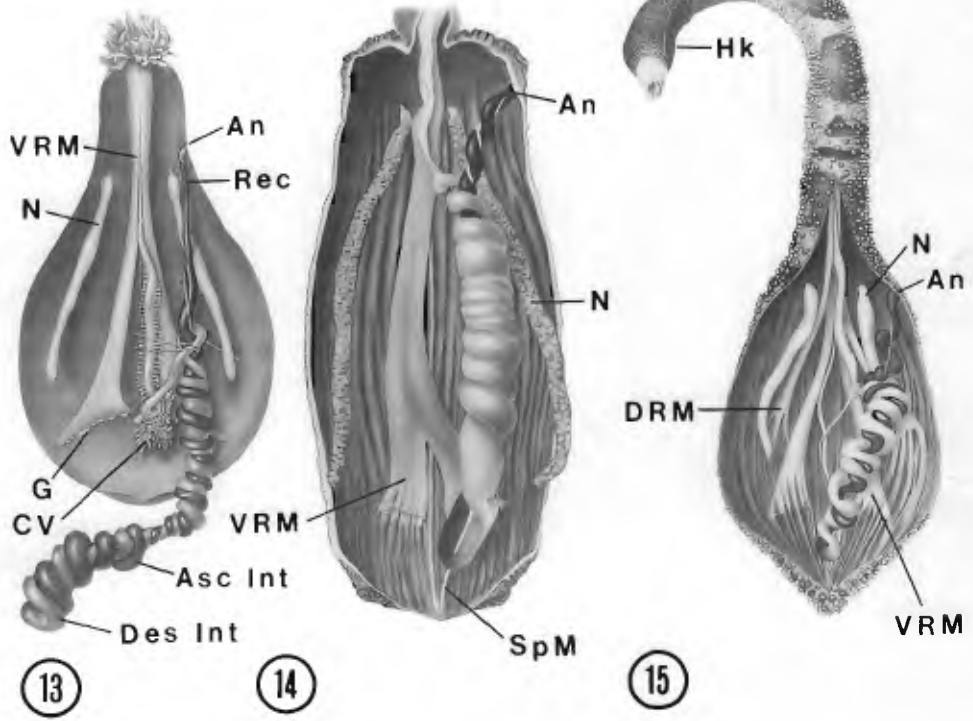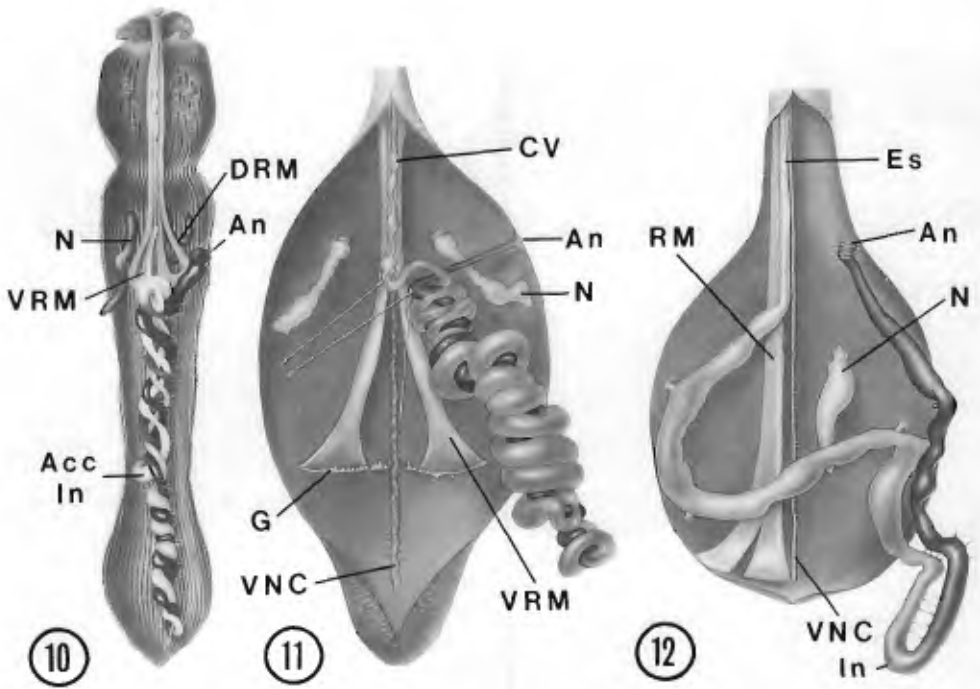
Figs. 16–21. Head regions of representative species of the six families of Sipuncula showing tentacle arrangement, oral disc, and mouth. CC, cephalic collar; Hk, introvert hooks; M, mouth region within the oral disc; NO, nuchal organ. (Illustrated by: Charissa Baker, Figs. 16, 20, 21, and Carolyn B. Gast, Figs. 17–19; From Rice, 1992.)
Fig. 16. *Sipunculus nudus* (Sipunculidae).

Fig. 17. *Nephasoma pellucida* (Golfingiidae). Brain and eye-spots are visible through thin cuticle.
Fig. 18. *Phascolion cryptus* (Phascolionidae).
Fig. 19. *Themiste lageniformis* (Themistidae).
Fig. 20. *Aspidosiphon steenstrupii* (Aspidosiphonidae).
Fig. 21. *Phascolosoma varians* (Phascolosomatidae).

Figs. 10–15. Internal anatomy of representative species of the six families of Sipuncula. Acc In, accessory postesophageal loop of intestine; An, anus; Asc Int, ascending intestine (dark shading); CV, contractile vessel (with villi); Des Int, descending intestine; DRM, dorsal retractor muscle; Es, esophagus; G, gonad; Hk, introvert hooks; In, intestinal loop; N, nephridium; Rec, rectum; RM, retractor muscle; SpM, spindle muscle; VNC, ventral nerve cord; VRM, ventral retractor muscle. (Illustrated by: Charissa Baker, Figs. 10, 14, 15, and Carolyn B. Gast, Figs. 11–13.)
Fig. 10. *Sipunculus nudus* (Sipunculidae).
Fig. 11. *Nephasoma pellucida* (Golfingiidae).
Fig. 12. *Phascolion cryptus* (Phascolionidae).
Fig. 13. *Themiste lageniformis* (Themistidae).
Fig. 14. *Aspidosiphon steenstrupii* (Aspidosiphonidae).
Fig. 15. *Phascolosoma varians* (Phascolosomatidae).

Phascolion strombus (Moritz and Storch, 1970), *Sipunculus nudus*, and *Golfingia vulgaris* (Goffinet et al., 1978). Three regions have been defined, the innermost of which is thickest and most highly stratified. The intermediate region varies in thickness and is composed of a disorganized array of fibers, sometimes assembled in clusters or small disoriented bundles. The third and outermost layer is a thin, irregular, electron-dense border (Figs. 38–45). The basic structure of the
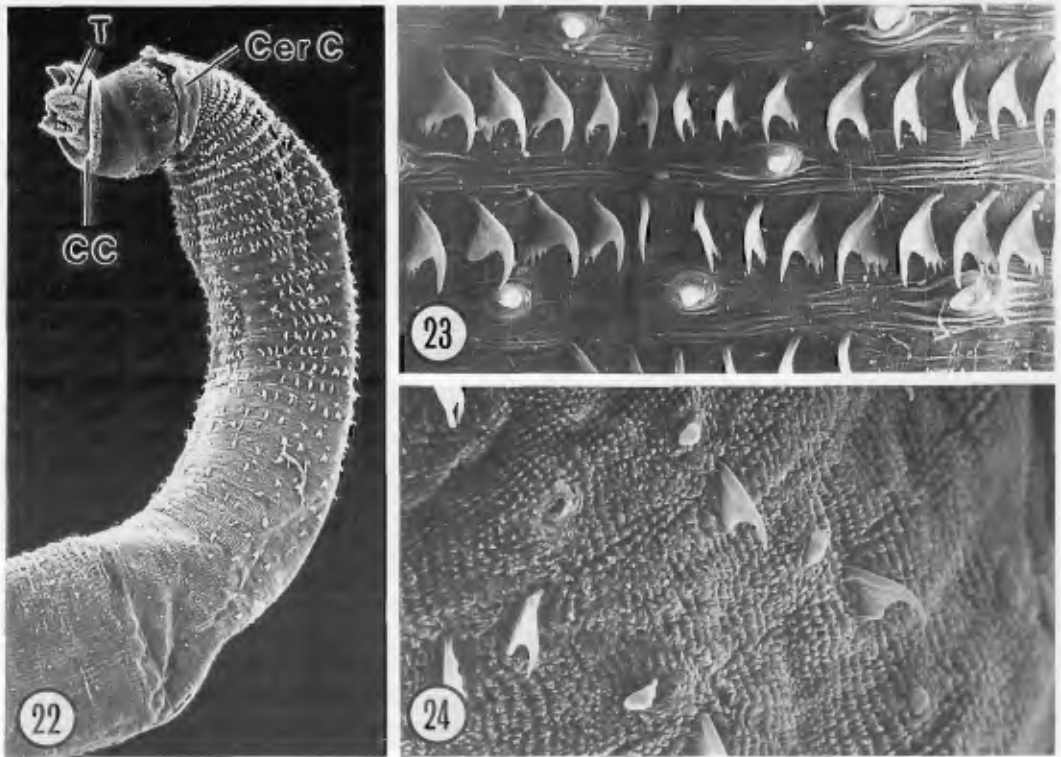
Figs. 22–24.   The introvert of *Apionsoma misakiana* (Phasco-
losomatidae). SEM.
Fig. 22.   Introvert and head, showing tentacles (T), cephalic
collar (CC), cervical collar (CerC), rings of hooks on anterior
introvert, and scattered hooks on more posterior introvert. ×60.

Fig. 23.   Rows of hooks and papillae on the anterior introvert.
Note curvature of hooks and basal spine-like processes. ×600.
Fig. 24.   Scattered hooks on the posterior introvert. Posterior
hooks lack basal processes and extreme curvature. ×600.

cuticle is the same, but specific variations oc-
cur in the microvillar system, the compact-
ness of fibrils within fiber bundles, and the
regularity of the arrangement of the bundles.
In *Sipunculus nudus* the microvillar system is
well developed, consisting of regularly
spaced microvilli extending perpendicularly
from the epidermis to the outer cuticle. Mi-
crovilli are more apparent in the thinner and
less dense cuticle of the introvert (Fig. 41),
where, in depressions within cuticular swell-
ings, they may penetrate the external border
of the cuticle. A second structure that resem-
bles a microvillus, but is of greater diameter,
penetrates the cuticle in a helicoidal rather
than a perpendicular path. In *Phascolion*

strombus (Moritz and Storch, 1970), *Golfin-
gia vulgaris* (Goffinet et al., 1978), and
Nephasoma pellucida (author's observa-
tions), microvilli extend only a short distance
from the epidermis into the fibrous layer, no
farther than one-third of the width of the strat-
ified region. Similarly, tonofilaments may ex-
tend from the epidermal cells farther into the
cuticle in *Sipunculus nudus* than in the other
three species. Unlike *Phascolion strombus*
and *Sipunculus nudus*, in which the orthogo-
nal arrangement of compressed layers of par-
allel fiber bundles is quite consistent, *Golfin-
gia vulgaris* and *Nephasoma pellucida* show a
breakdown in the regularity of successive lay-
ers, particularly in the more distal region of
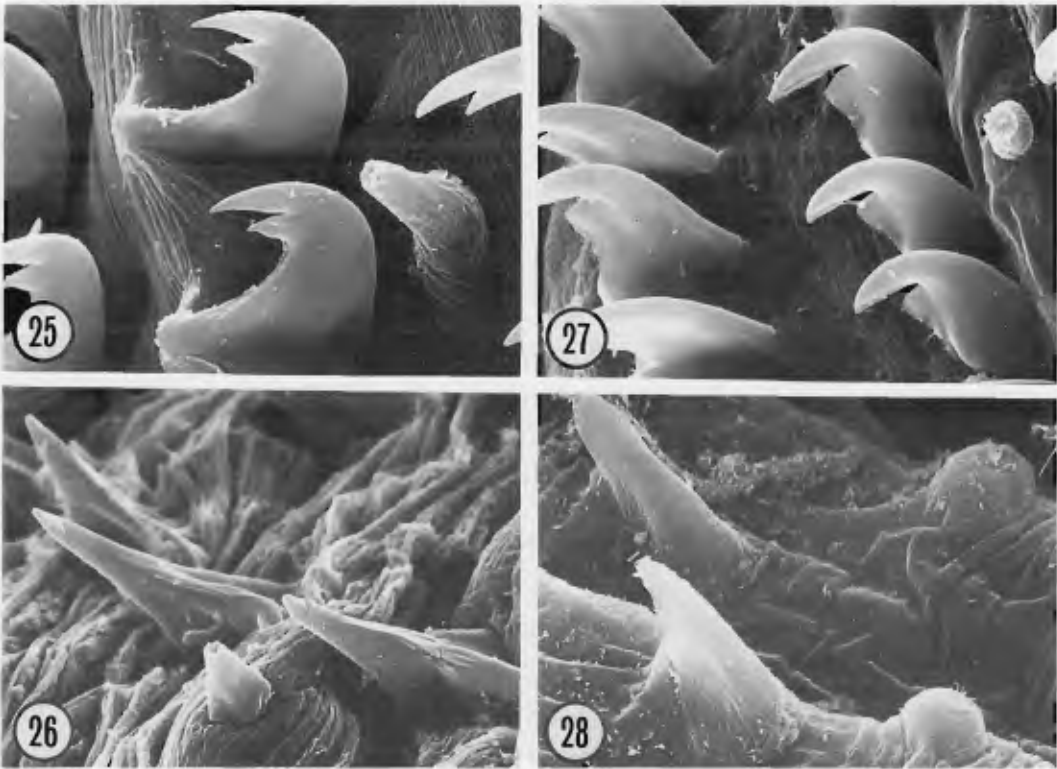
Figs. 25–28. Hooks, spines, and papillae of the introvert of different species, showing variations in structure and form. SEM.
Fig. 25. Bidentate, curved and laterally compressed hooks and elongated papilla from anterior introvert of *Aspidosiphon brocki*. ×2,000.
Fig. 26. Spine-like, conical hooks and conical-shaped papilla

from posterior introvert of *Aspidosiphon brocki*. ×1,550.
Fig. 27. Bidentate, curved, and laterally compressed hooks and rounded papilla from anterior introvert of *Phascolosoma perlucens*. ×1,000.
Fig. 28. Spine-like, simple hooks and rounded papillae from anterior introvert of a juvenile *Nephasoma pellucida*. ×2,750.

the stratified layer. In the latter two species, small electron-dense granules (48 nm in *Golfingia vulgaris*) are scattered throughout the cuticular matrix, and in *Nephasoma pellucida* the bundles are not as tightly compressed and the granulofilamentous matrix is relatively more abundant (Figs. 42–45). The fiber bundles are formed by compactly arranged electron-dense fibrils in all species studied except *Sipunculus nudus* in which the fiber bundles are electron lucent and the fibrils not evident.

Although no periodic substructure indicative of collagen has been identified in the fibrous material of the sipunculan cuticle, the presence of collagen has been demonstrated

in a number of species by histochemical tests and by the chemical isolation of a soluble trichloroacetic fraction identified as collagen. (Voss-Foucart et al., 1977, 1978). In *Sipunculus nudus, Golfingia vulgaris*, and *Aspidosiphon clavatus* (=*Aspidosiphon muelleri*) the cuticular collagen was found to be similar to that of two polychaetes, *Arenicola marina* and *Pectinaria koreni* (Voss-Foucart et al., 1978). The polypeptide fraction remaining after the extraction of collagen differed in the hardened shield of *Aspidosiphon clavatus* by the presence of an additional polypeptide proposed to have a possible role in the hardening process of the shield. A specific enzymatic
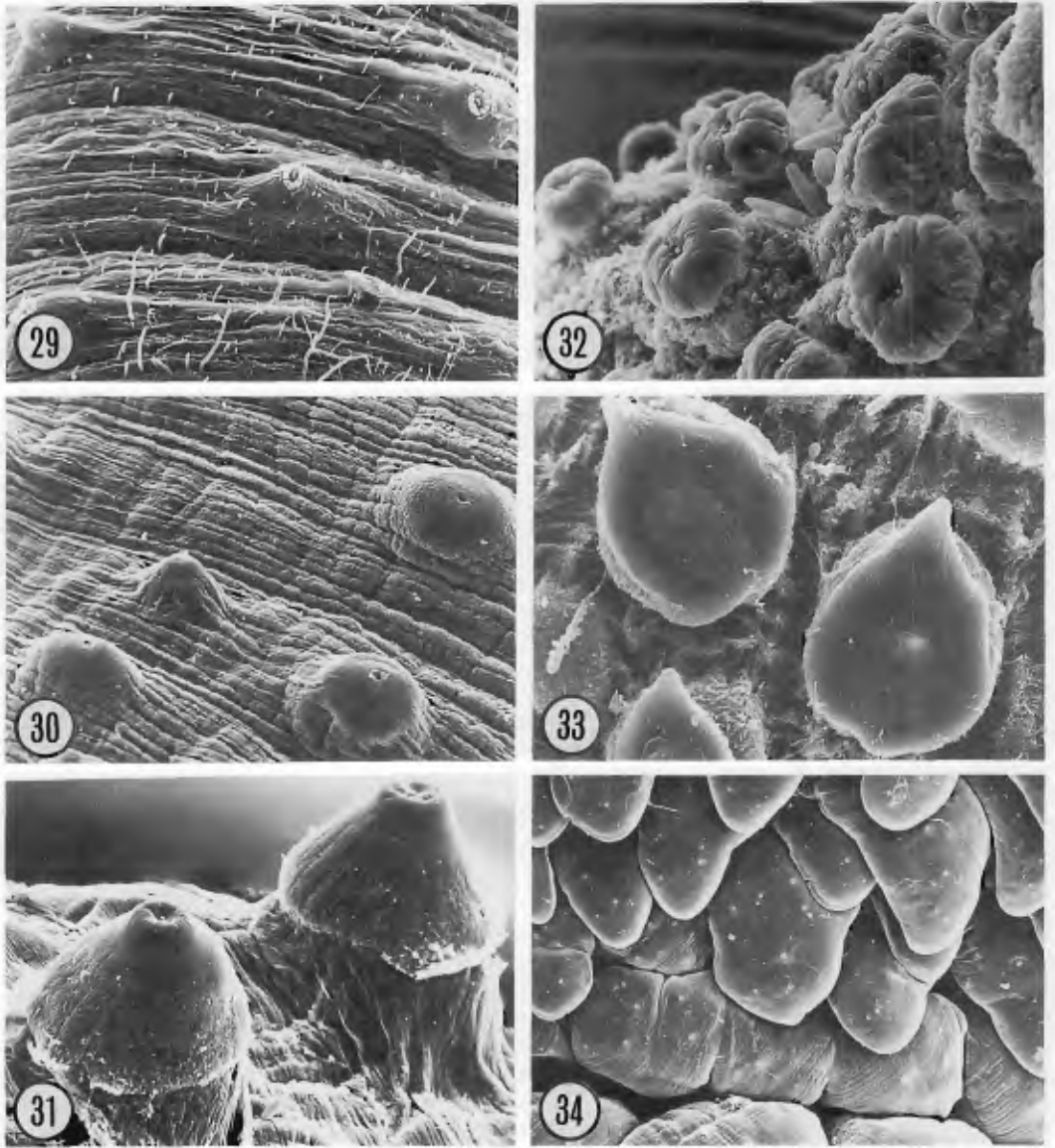
Figs. 29–34. Papillae from introverts and trunks of various species showing intra- and interspecific diversity in form and structure. SEM.
Fig. 29. Papillae from anterior introvert of *Phascolosoma perlucens*, with sensory processes protruding from central pore surrounded by distinctive collar, ×580.
Fig. 30. Dome-shaped papillae from posterior introvert of *Phascolosoma perlucens*. ×368.
Fig. 31. Cone-shaped papillae from posterior trunk of *Phascolosoma perlucens*. ×440.
Fig. 32. Unusual flower-like papillae on posterior trunk of *Apionsoma misakiana*. ×796.
Fig. 33. Holdfast or attachment papillae characteristic of the genus *Phascolion* on anterior trunk of *Phascolion cryptus*. ×320.
Fig. 34. Subtriangular, scale-like papillae on distal introvert of *Sipunculus nudus* and junction with nonpapillar region. Note that each papilla bears several pores. ×120.
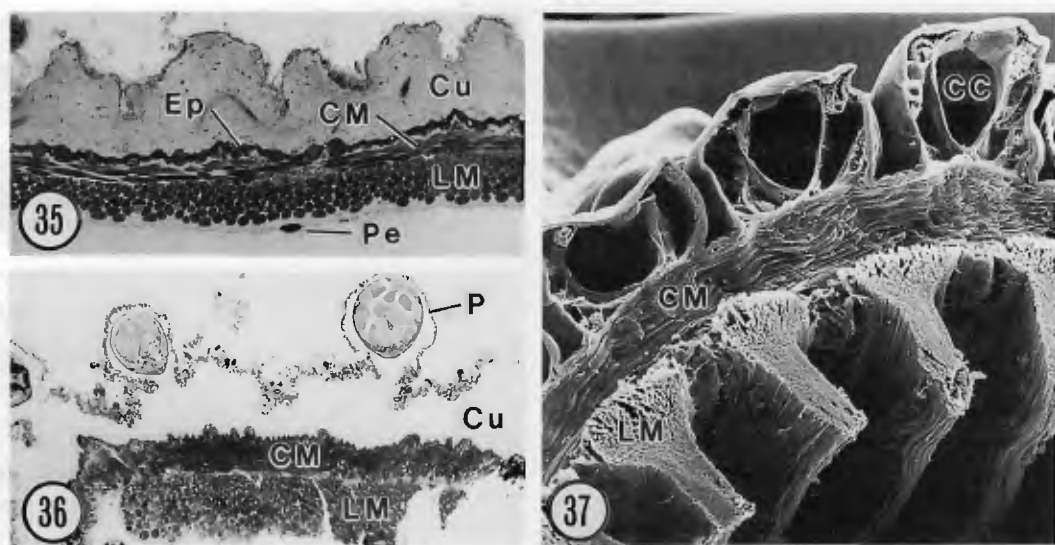
Figs. 35-37. Transverse sections of body walls, showing different muscle and cuticular arrangements. CC, coelomic canals; CM, circular muscle layer; Cu, cuticle; Ep, epidermis; LM, longitudinal muscle layer; P, papilla with epidermal organ; Pe, peritoneum.
Fig. 35. Light micrograph of *Nephasoma pellucida*. Inner longitudinal muscle layer is smooth, i.e., not subdivided into bundles. ×368.
Fig. 36. Light micrograph of *Phascolosoma turnerae* through posterior trunk with epidermal organs protruding into cuticular papillae and longitudinal muscle layer in bundles. ×71.
Fig. 37. SEM of *Sipunculus nudus*, showing longitudinal muscle bundles and coelomic canals. ×158.

test for the presence of chitin in the cuticle, in both soft parts and hard parts such as hooks and shields, proved negative. This is confirming evidence for previous reports of the absence of chitin which were based on the solubility of cuticular structures in hot KOH (Andreae, 1882; Andrews, 1890).

In a series of histochemical and chemical analyses of sipunculan cuticles, Manavalaramanujam (1978a,b, 1979) reported the distribution of protein, lipid, calcium, and carbohydrate in *Sipunculus robustus, Themiste lageniformis,* and *Cloeosiphon aspergillus.* The carbohydrate component, an acid mucopolysaccharide identified as hyaluronic acid, and a protein resembling collagen were found throughout the cuticle in all regions of the body. However, in studies of *Sipunculus robustus,* the cuticle of the tentacles and midre-

gion of the trunk differed from other regions by the presence of organic sulfur in the form of sulfhydryl groups and the absence of aromatic substances, lipid, and calcium.

The cuticle serves as the barrier through which osmotic regulation is maintained and through which respiratory gases may be transported (see Respiration). In addition, it provides the structural and chemical basis for the strong contractility and extensibility of the body as well as the locomotory and burrowing activities of the animal. Along with the musculature of the body wall, the orthogonally arranged fiber layers, which are cross-helically wound around the outer body, form the support for the hydrostatic skeletal system of these worms.

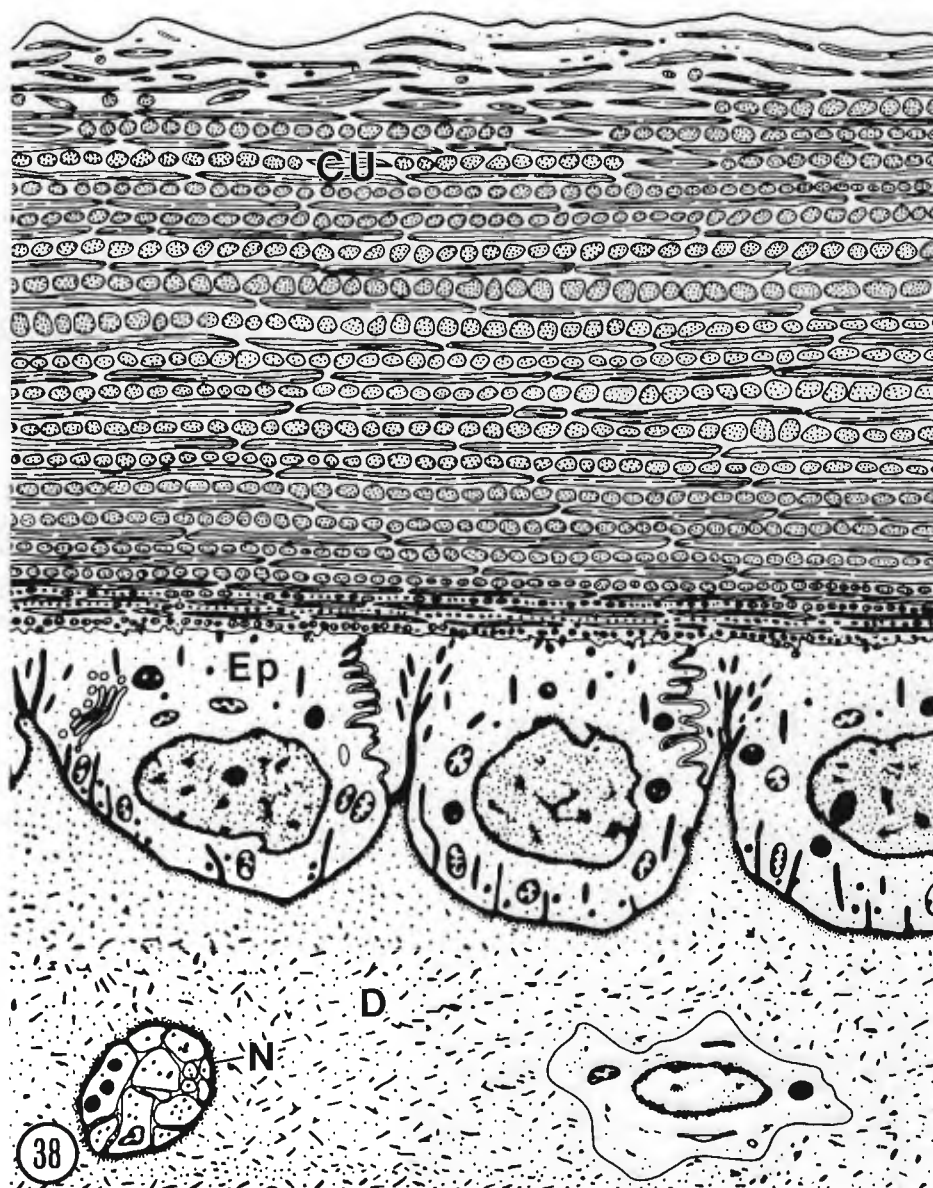Both in the field and in the laboratory, sipunculans are observed occasionally to shed

Fig. 38.   Schematic illustration of the integument of *Phascolion strombus*. Cuticle (CU) showing layered organization of fiber bundles; epidermal cells (Ep) with basal infoldings, junctional interdigitations of plasmalemma, Golgi apparatus, scattered mitochondria, and tonofilaments; dermis (D) with nerve (N) and connective tissue cell. (From Moritz and Storch, 1970.)
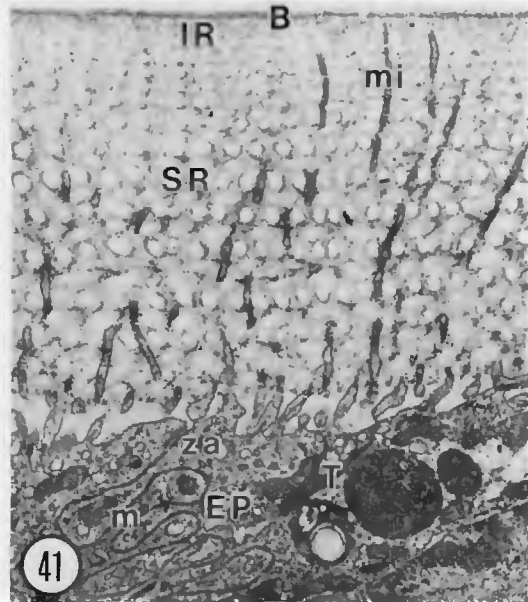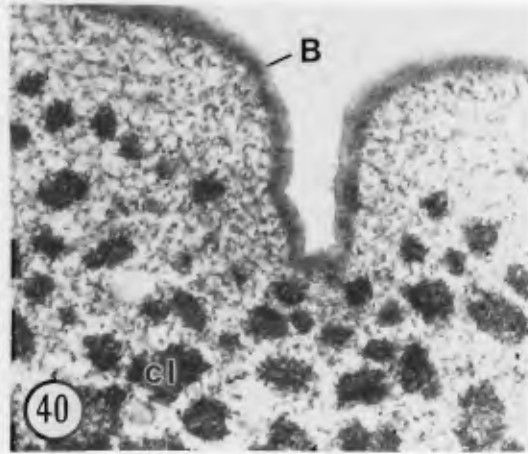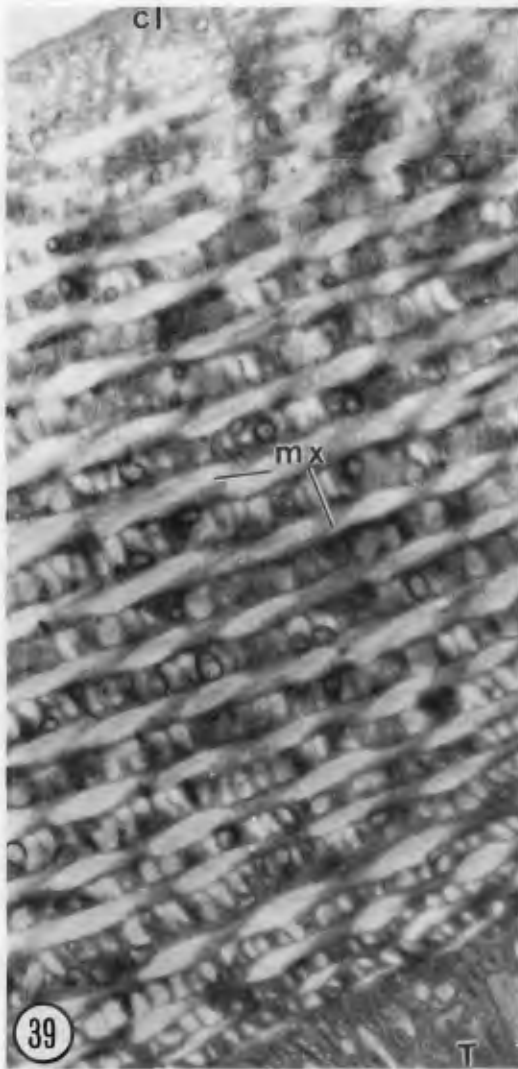
Figs. 39–41. Cuticle of *Sipunculus nudus*. TEM. B, electron-dense border; cl, clusters; EP, epidermis; m, sinuous epidermal plasma membrane; mi, microvilli; mx, matrix; IR, intermediate region; SR, stratified region; T, tonofilament bundles; za, zonula adhaerens. (From Goffinet et al., 1978.)
Fig. 39. Trunk cuticle showing typical orthogonal arrangement of fiber bundles. ×10,597.

Fig. 40. Enlargement of outer electron-dense border and intermediate region of trunk cuticle, showing clusters of fibers. ×34,667.
Fig. 41. Introvert cuticle, thinner than the trunk cuticle, showing penetration of epidermal microvilli (mi) and underlying epidermis (EP). ×9,353.

parts of their cuticle. The morphological and physiological implications of this process have not been investigated.

Epidermis

The epidermis underlying the cuticle is composed of a single layer of cuboidal cells, the relative proportions of which can vary depending on the state of contraction or extension of the body (Figs. 38, 42, 45, 47). Laterally the cells are strongly interdigitated, and cell membranes are joined near their apical borders by structures resembling zonulae adhaerentes and more basally by septate desmo-
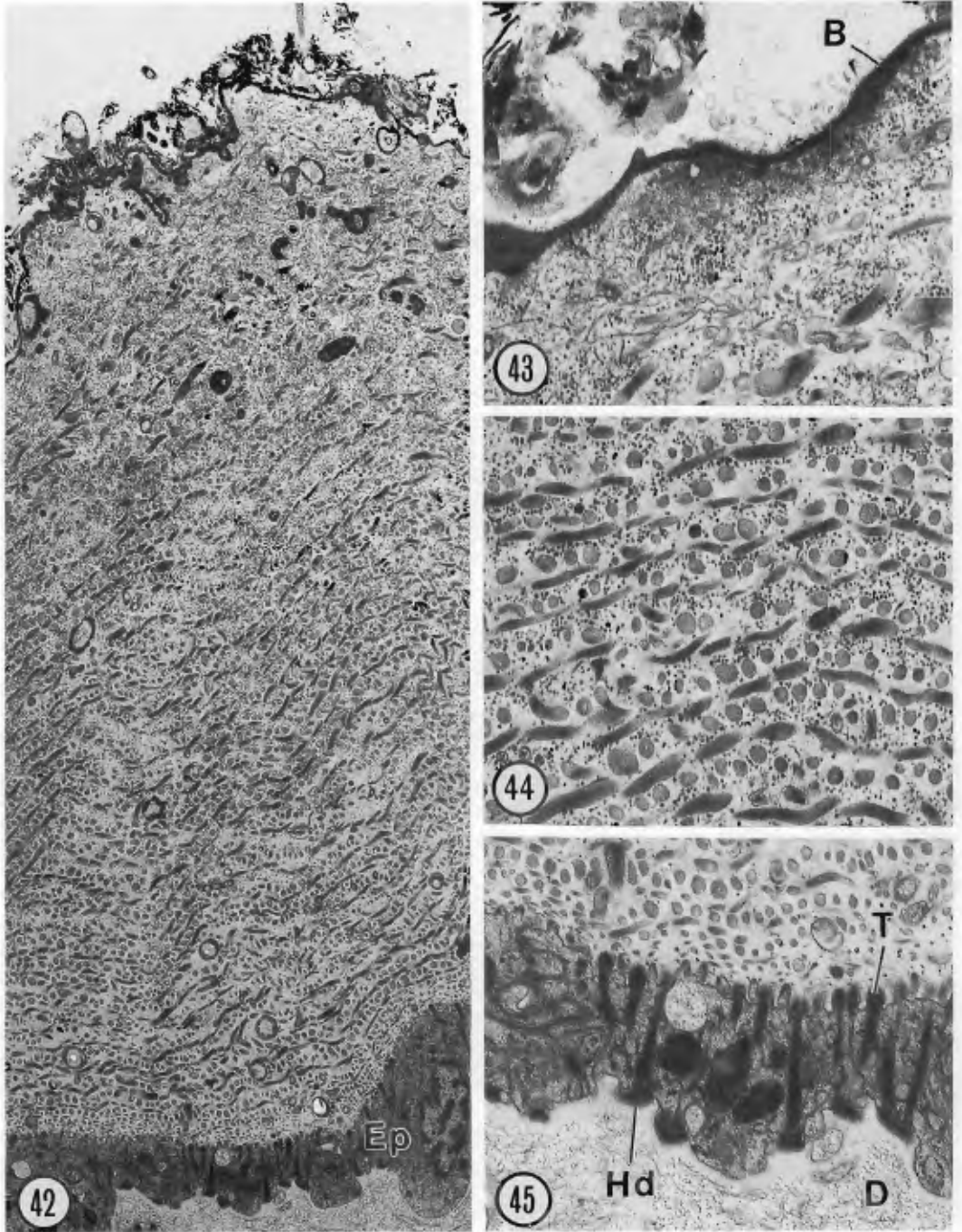
Figs. 42–45.   Cuticle of *Nephasoma pellucida*. TEM. B, outer electron-dense border; D, dermis; Ep, epidermis; Hd, hemidesmosome; T, tonofilament.
Fig. 42.   Section through entire cuticle and epidermis. ×4,800.
Fig. 43.   Outer cuticle, showing electron-dense border and irregular array of fibers. ×16,320.
Fig. 44.   Stratified region of orthogonally arranged fiber bundles in the midcuticle. ×14,880.
Fig. 45.   Inner stratified layer of cuticle with smaller fiber bundles and epidermis. ×14,160.

Fig. 46. Cuticular fibers of *Phascolion* sp., a small interstitial sipunculan. Outer cuticle has been removed to show stratified region of orthogonal layers of fiber bundles. SEM. ×7,000.

somes (Figs. 41, 42, 45, 47). Abundant tonofilaments fasten the basal plasmalemma to the cuticle, extending from hemidesmosome-like structures attached to the extracellular basal lamina to the apical surface of the cell and frequently into the microvilli at the junction with the cuticle (Figs. 45, 47). The cytoplasm of epidermal cells is characterized by numerous lysosome-like bodies, an even distribution of mitochondria, and a sparsity of endoplasmic reticulum and granules. The Golgi apparatus is lateral or apical to a chromatin-rich nucleus containing a nucleolus. In *Phascolion strombus* glycogen rosettes occur mostly in the basal folds of the cell, and at the base of the microvilli are small vesicles or bladders, suggestive of secretory activity (Moritz and Storch, 1970). Presumably the material composing the fibrous cuticle is secreted at the apical surface of the epithelial cells as fine fibers, which then self-assemble to form the orthogonal arrays of fiber bundles. This presumption is supported by the small

size of the fibers in the innermost cuticle adjoining the epidermis.

Epidermal organs, containing glandular and sensory elements, extend as bulbous protrusions into the cuticle where they open through pores to the exterior (see Glandular Organs, below).

Dermis

A basement membrane of fine filaments lies immediately beneath the epidermis, adjacent to the underlying dermis or connective tissue that separates the epidermal layer from the circular and longitudinal muscle layers. The dermis is of variable thickness throughout the body, but, as in the case of the cuticle, is several times thicker in the posterior trunk than in the introvert or midbody. It consists of scattered collagenous fibers with undetermined periodicity embedded in an electron-lucent homogeneous matrix (Fig. 47). The dermis is often protracted between the bases of the epidermal cells. A variety of cells and structures occur within the dermis, including pigment cells, pigment granules, granulocytes, nerves, proximal projections of epidermal organs, and, in some species, coelomic extensions.

Body Wall Musculature

The structure of the circular and longitudinal layers of muscles in the body wall will be considered in conjunction with the retractor muscles in the section on the muscular system.

Peritoneum

Forming the inner lining of the body wall, the peritoneum consists of a thin layer of squamous cells, generally with prominent nuclei, but sparse cytoplasmic components. The cells may be nonciliated or multiciliated (Fig. 48). Chlorogogue cells, defined by their large storage granules, are scattered in the peritoneal layer. Some cells are differentiated into fixed urns, as noted in the section on coelomic elements.
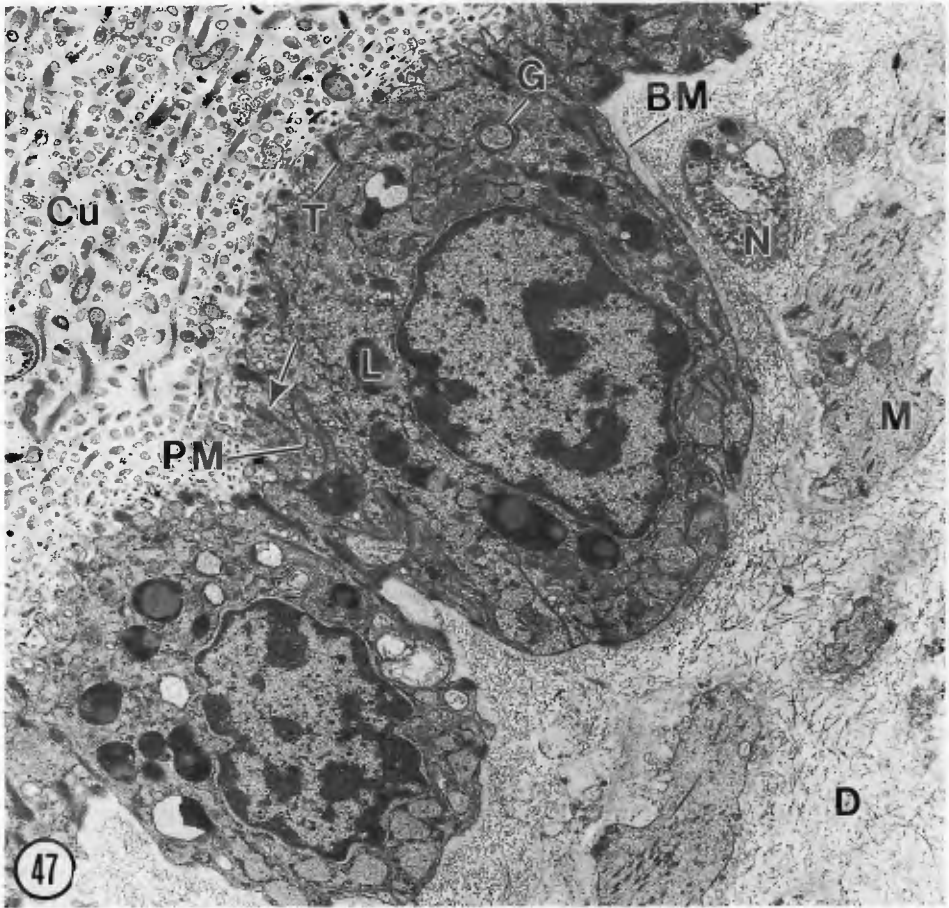
Fig. 47.   Epidermis and dermis of *Nephasoma pellucida*. TEM. BM, basement membrane; Cu, cuticle; D, dermis; G, Golgi apparatus; L, lysosome-like bodies; M, muscle cell; N, nerve; PM, folded plasma membrane; T, tonofilament. Arrow points to zonula adherens. ×10,560.

GLANDULAR ORGANS

Epidermal organs are the most numerous and prominent glandular organs of sipunculans. Commonly comprised of both glandular and sensory elements, they are distributed over the surface of the body, opening to the exterior through cuticular pores usually situated in the centers of papillary elevations or sometimes in cuticular depressions (Figs. 49–54). Because of the close association in the epidermal organ of sensory cells with gland cells, the sensory component will also be considered in this section.

The size, configuration, and number of organs vary among different species as well as in different regions of the body in the same species. Bordered laterally by epidermal cells, from which the glandular portion is differentiated, the organs extend into the cuticular layer. Large epidermal organs or those in regions of thin cuticle may bulge inward into the dermis or circular muscles. Typically, the epidermal organ is spherical or onion shaped with a central cavity that opens distally into an elongate duct leading to the surface. In the basic type, there are two kinds of gland cells
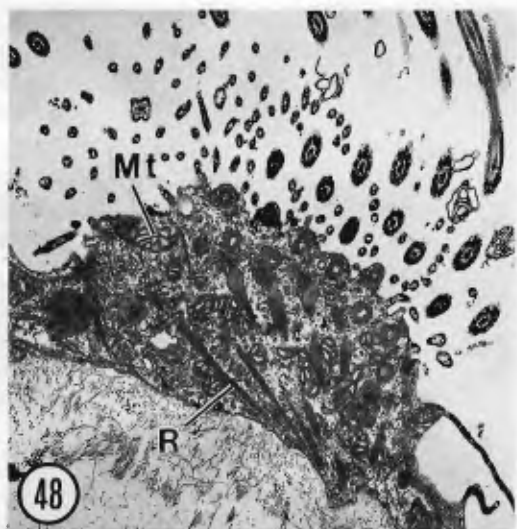
Fig. 48. Multiciliated peritoneal cell on the inner body wall of *Sipunculus nudus*. TEM. ×10,000. Note the abundance of mitochondria (Mt) and long ciliary rootlets (R). (Courtesy of E. Ruppert.)

and a cluster of sensory cells that open externally through a common pore. The gland cells are distinguished by the type of secretory granules and whether they empty directly into the central cavity or by way of intracellular cavities or receptacles. The secretory material of the former is usually in the form of large granules and is acidophilic, whereas that of the latter is amorphous and may be basophilic (Åkesson, 1958). Variations in this typical form include the presence of only one of the two types of gland cells, as is characteristic of species of *Phascolosoma* and *Aspidosiphon*, and the separation of sensory and glandular elements, as found in *Sipunculus*. The typical form of epidermal organ is characteristic of the Golfingiidae and is considered by Åkesson (1958) to be primitive.

In certain species, one epidermal organ may have several openings to the exterior. For example, in a small, meiobenthic species of *Phascolion* the gland cells have separate pores, as does the sensory component (Figs. 55–57). There are two distinct types of gland cells: one with small (0.4–0.6 μm diameter), mostly spherical, electron-dense granules of varying degrees of density and the other with large (2–3.7 μm diameter), tightly packed, polygonal granules filled with a fine, light granular material (Fig. 57). Processes of the sensory cells extend to a central pore at the apex of the papilla; the glandular pores appear to be lateral and inferior.

Epidermal organs of *Sipunculus nudus*, highly modified from the basic plan, are well-known from numerous light microscopic studies (Andreae, 1882; Ward, 1891; Metalnikoff, 1900; Åkesson, 1958). There are two types of glandular organs, multicellular and bicellular, each with characteristic secretory granules and separate sensory organs (Figs. 58–63). Sensory pores are recognized by their large size and terminal receptors (Fig. 63). Ultrastructural studies of the bicellular glands reveal an abundance of endoplasmic reticulum in the cytoplasm and an amorphous secretory product. Mitochondria are concentrated toward the periphery of the cell. The secretory material is, for the most part, not enclosed in granules. It appears to be composed of a fine granular material that is released into the central cavity or receptacle by way of numerous microvilli.

The function of the epidermal organs is not known. Hyman (1959) suggested that secretions from the glandular component may serve to protect the animals on exposure at low tide. The adherence of mud to the body surface of some species has been attributed to the epidermal secretions (Åkesson, 1958). Other authors have considered the possibility that the secretions might be involved in the dissolution of limestone and other calcareous substrates (Schmidt, 1865; Rice, 1969).

MUSCULAR SYSTEM
Body Wall Muscles

The body wall musculature consists of an outer circular layer and an inner longitudinal layer (Figs. 35–37, 64, 65). In some species oblique fibers may be found between the circular and longitudinal layers. The longitudinal muscle layer occurs in bundles in the
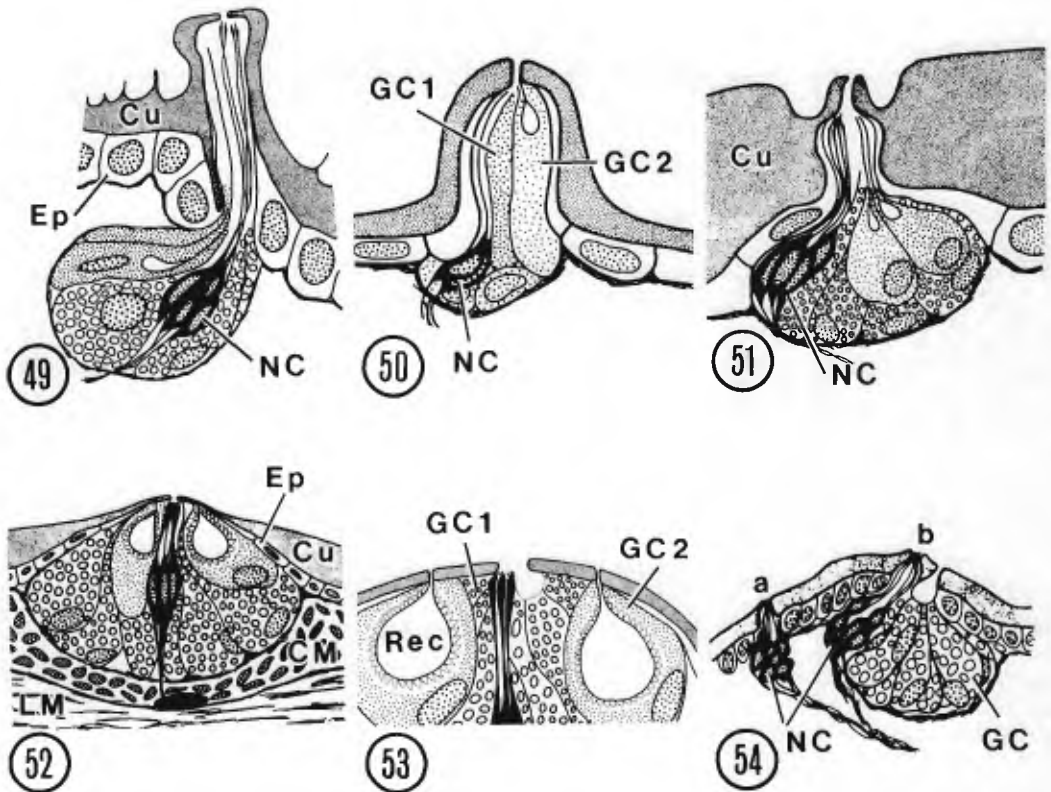
Figs. 49–54. Schematic illustrations of sections of various types of epidermal organs. Organs usually have both sensory and glandular components that open through pores on papillae or in cuticular depressions. Sensory cells have a characteristic position in organ, either to one side of or in center of gland cells; terminal receptors extend to distal pore. Gland cells may have amorphous secretory product or granules, the former secreting into receptacles and the latter directly to the pore or pore canal. CM, circular muscle; Cu, cuticle: Ep, epidermis; GC, gland cell; GC1, gland cell 1; GC2, gland cell 2; LM, longitudinal muscle; NC, nerve cell; Rec, receptacle. (Redrawn from Åkesson, 1958).
Fig. 49.   Organ opening on papilla, from trunk of *Golfingia procera*.

Fig. 50.   Organ opening on papilla of introvert of *Golfingia elongata*.
Fig. 51.   Organ opening in cuticular depression on trunk of *Golfingia elongata*.
Fig. 52.   Organ from smooth region of trunk of *Phascolion strombus*, with one opening for gland cell 1 (GC1) and sensory receptors and separate openings for receptacles of gland cell 2 (GC2).
Fig. 53.   Enlargement of Figure 52.
Fig. 54.   Separate sensory epidermal organ (a) and sensory glandular organ (b) from smooth region of introvert of *Sipunculus nudus*.

genera *Antillesoma, Aspidosiphon* (in part), *Lithacrosiphon, Phascolopsis, Phascolosoma, Siphonomecus, Siphonosoma, Sipunculus,* and *Xenosiphon*, but is continuous in others. The circular muscle layer, usually continuous, is divided into distinct bundles in the genera *Sipunculus* and *Xenosiphon* and into anastomosing bundles in *Siphonosoma* and *Siphonomecus*.

Ultrastructure, previously reported only for the longitudinal muscles of *Sipunculus nudus*, reveals constituent fibers, oval or polygonal in section, ranging in diameter from 2 to 15 μm (Fig. 66) (Eguileor and Valvassori, 1977). Nuclei are usually peripheral, and the few, small mitochondria (0.1–1 μm) are distributed just beneath the sarcolemma or centrally in the fiber. Contractile material, which
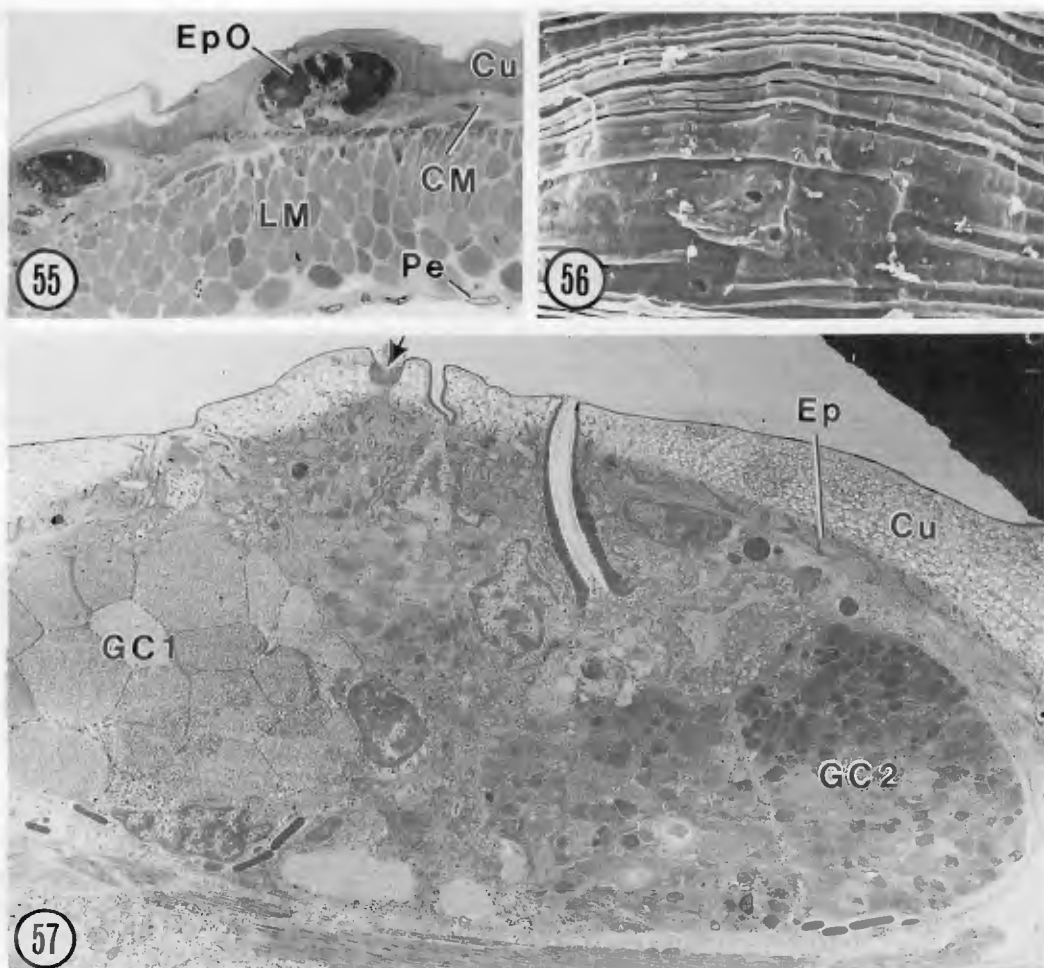
Figs. 55–57. Epidermal organs of *Phascolion* sp., a small interstitial species. Cu, cuticle; CM, circular muscle; Ep, epidermis; EpO, epidermal organ; GC1, gland cell 1; GC2, gland cell 2; LM, longitudinal muscle; Pe, peritoneum.
Fig. 55. Light micrograph of epidermal organ and body wall of posterior trunk. Transverse section. ×554.
Fig. 56. Surface pores of epidermal organs. SEM. ×1,975.
Fig. 57. Epidermal organ from posterior trunk, showing several pores (arrow to pore of sensory receptor). TEM. ×4,014.

takes up most of the non-nuclear area of the cells, consists of thick and thin filaments that show no particular geometric pattern of arrangement (Figs. 67–69). The thick filaments range in diameter from 20 to 60 nm and may be oval or in the shape of a "figure 8" in cross section. The thin filaments have a relatively constant diameter of approximately 7 nm.

Smooth reticulum is localized at the cell periphery as subsarcolemmal cisternae. The sarcolemma is well developed and may form deep invaginations into the cell, sometimes dividing the cells into lobes (Fig. 66, 67, 69). Electron-dense formations, found among the cisternae and on both the inner and outer sides of the membrane, are interpreted as hemides-
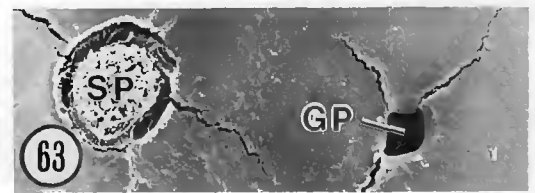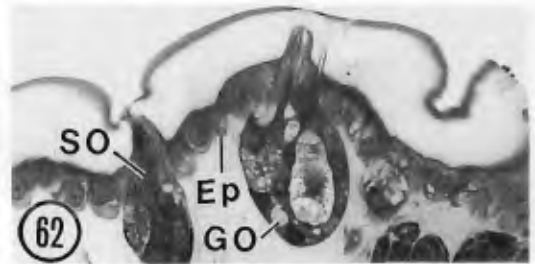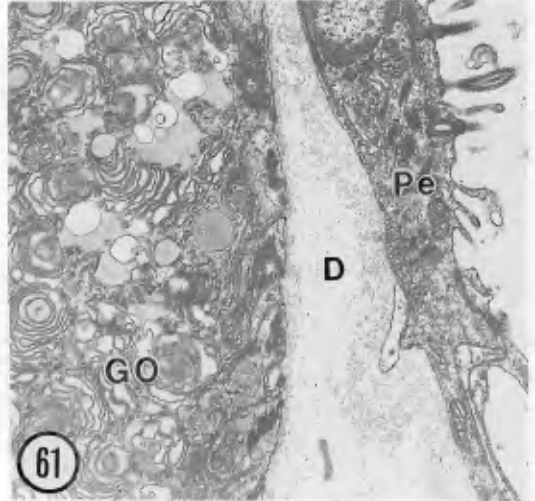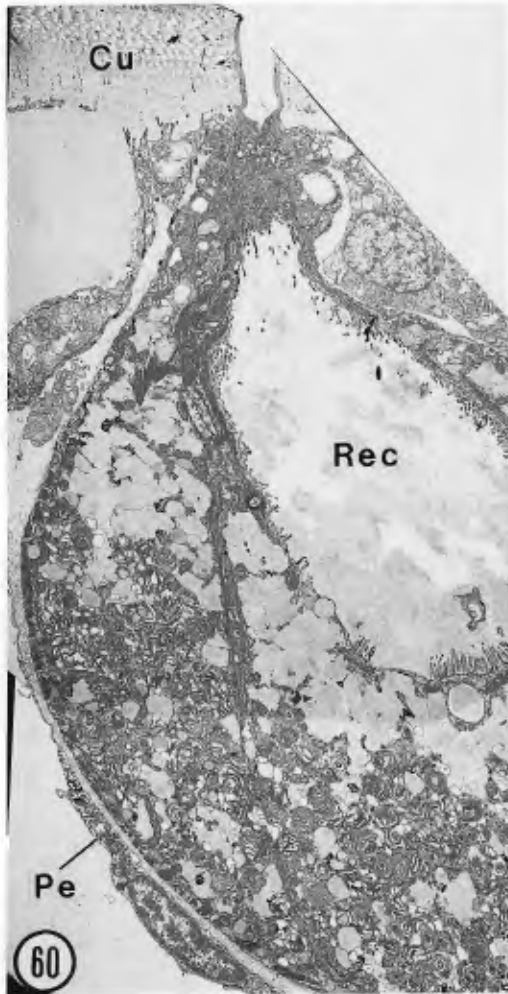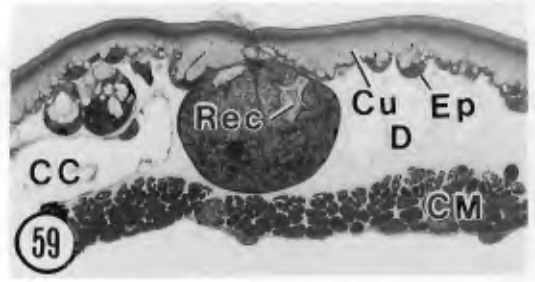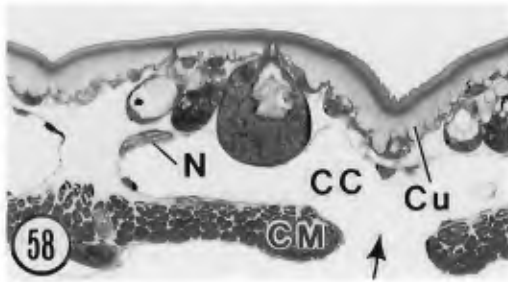
Figs. 58–63.   Epidermal organs of *Sipunculus nudus*. CC, coelomic canal; CM, circular muscle; Cu, cuticle; D, dermis; Ep, epidermis; GO, glandular organ; GP, glandular pore; N, nerve; Pe, peritoneal cell; Rec, receptacle; SO, sensory organ; SP, sensory pore. (Sections for Figs. 58–61 courtesy of E. Ruppert.)
Fig. 58.   Light micrograph of sagittal section of body wall showing multicellular glandular and sensory organs from trunk. ×352.
Fig. 59.   Light micrograph of sagittal section of large bicellular glandular organ from trunk. ×352.

Fig. 60.   Epidermal glandular organ and pore. TEM. ×2,600.
Fig. 61.   Enlargement of epidermal organ shown in Figure 60 (different section) showing cytoplasmic secretory organelles and outer dermal and peritoneal covering. ×7,500.
Fig. 62.   Light micrograph of transverse section of sensory and multicellular glandular organs from terminal region of body. ×480.
Fig. 63.   Pores on the surface of the terminal region. SEM. ×3,000.
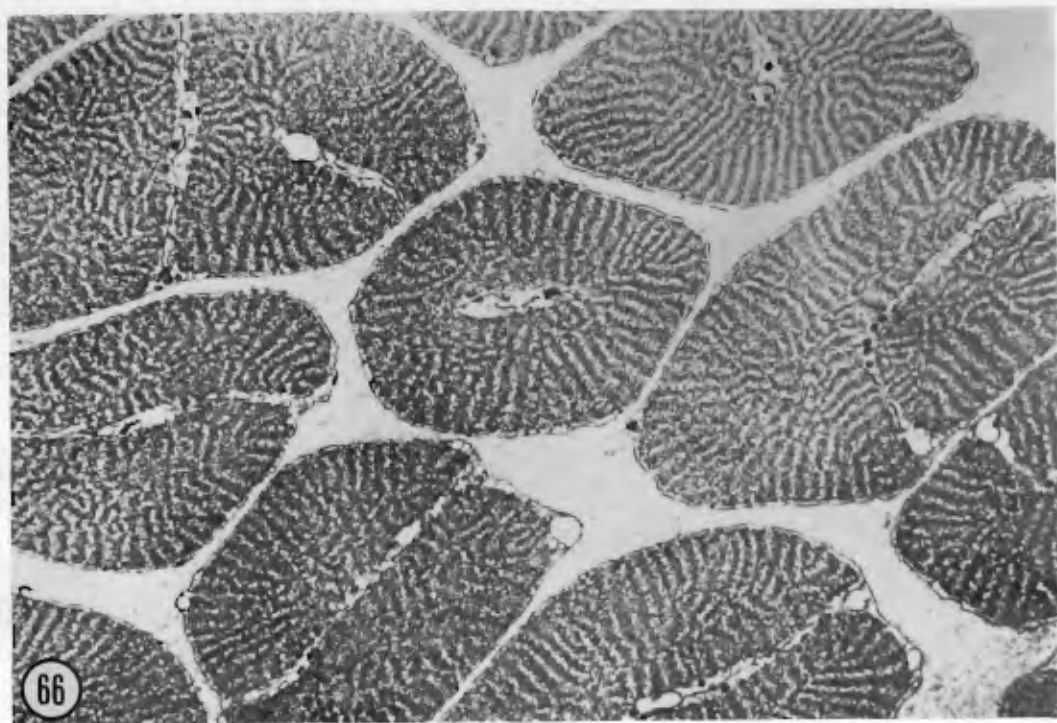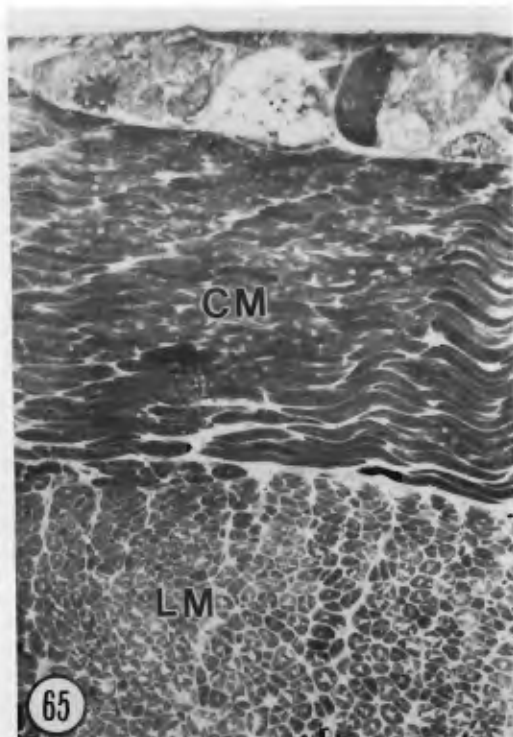
Figs. 64–66.   Body wall muscles of *Sipunculus nudus*. Transverse sections. (From Eguileor and Valvassori, 1977.)
Fig. 64.   Light micrograph of body wall. A circular muscle layer (CM) lies beneath the cuticle and to the outside of the longitudinal muscle layer (LM), which is divided into separate bundles. ×80.

Fig. 65.   Detail showing arrangement of fibers of circular muscle (CM) and longitudinal muscle (LM). ×250.
Fig. 66.   Individual fibers (cells) of longitudinal muscle. The sarcolemma forms deep invaginations into the cells. TEM. ×5,000.
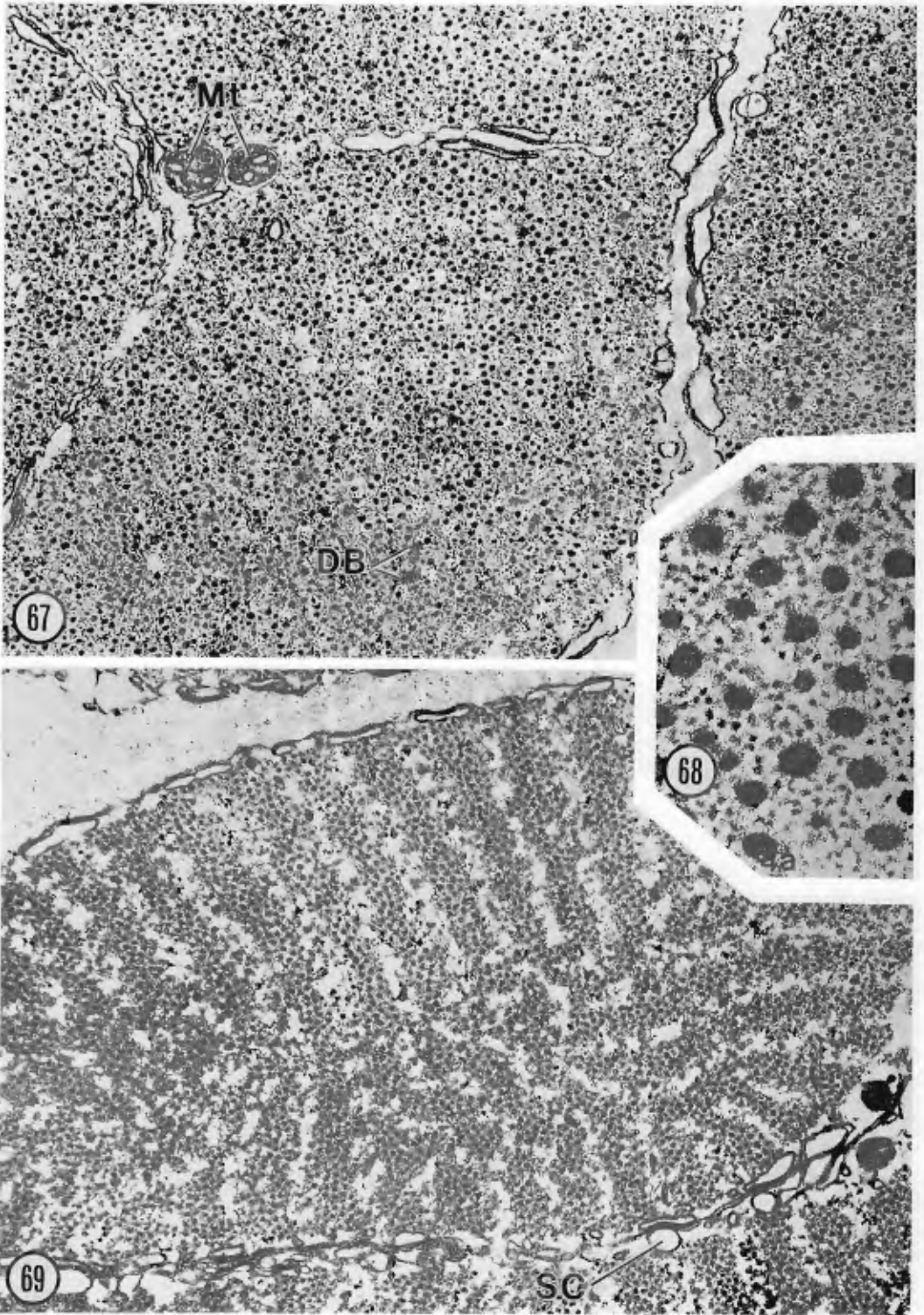
Figs. 67–69. Longitudinal body wall muscles of *Sipunculus nudus*. Transverse sections. TEM. (From Eguileor and Valvassori, 1977.) Note subsarcolemmal cisternae (SC), peripheral mitochondria (Mt), and dense bodies (DB).
Fig. 67. Contracted muscle. Thick and thin filaments are more or less uniformly distributed. ×36,000.

Fig. 68. Detail showing crowns of thin (actin) filaments around thick (myosin) filaments. ×130,000.
Fig. 69. Stretched muscle, showing two types of bands: 1) thin filaments with central row of dense bodies; 2) combined thick and thin filaments. ×21,000.

mosomes that serve for attachment of the thin filaments to the sarcolemma.

In contracted muscle, the thick and thin filaments are distributed more or less uniformly, except for a greater density of thin filaments in the vicinity of their points of attachment to the randomly distributed dense bodies, similar to the Z bands of striated muscle (Figs. 67, 68). The thick filaments in contracted muscle occur in curving rows around the dense bodies and may be surrounded by a crown of up to 17 thin filaments. In the decontracted or extended muscle the two types of filaments apparently "slide apart," resulting in two defined zones: zone I of thin filaments only and zone A of combined thick and thin filaments (Fig. 69). The zones are radial from the center, the dense bodies occurring in rows at the center of the I zone. Hemidesmosomes, points of anchor of thin filaments to the sarcolemma, are found among the sarcolemmal cisternae around the periphery of the cell.

Smaller muscle cells, 1–3 μm in diameter, have been distinguished in the center of the muscle bundle of *Sipunculus nudus*. The thick filaments of these cells are especially large, having diameters up to 100 nm. At the periphery of the longitudinal muscle bundle toward the body cavity the cells tend to be more oval and smaller in diameter and more closely packed. The fewer subsarcolemmal cisternae in these cells occupy only 35% of the membrane as contrasted with 55% in the larger cells, but the mitochondria are more dense, covering up to 7% of the cross section of the cell as opposed to 0.1% in other cells. No distinctive function has been attributed to the smaller cells. The longitudinal muscles of *Nephasoma pellucida* show a similar ultrastructure to *Sipunculus nudus* in that there are scattered dense bodies and peripheral subsarcolemmal cisternae, although the sarcolemmal invaginations are lacking (author's observations) (Fig. 70). Associated with the sarcolemma are numerous prominent hemidesmosomes. Differing from this arrangement, the muscle cells of *Apionsoma*

misakiana have conspicuous Z rods extending into the fiber from their peripheral attachments to the sarcolemma (Figs. 71, 72).

The body wall muscles of sipunculans are similar to the helical muscles described for annelids, except that no H zone (all thick filaments) has been detected in the decontracted state. Myofilaments, both thick and thin, show no pattern of arrangement, and, in at least one species, *Sipunculus nudus*, dense bodies with attached thin filaments are dispersed at random.

Retractor Muscles

Retractor muscles traverse the body cavity from a point of attachment on the inner wall of the head to an attachment in the mid- to posterior body. These exceptionally long muscles serve, upon contraction, to retract the anterior introvert into the trunk. The number of muscles varies from one to four.

The retractor muscles of sipunculans have been the subject of several physiological and biochemical investigations (cf. Prosser, 1973; Iwamoto et al., 1988), but few ultrastructural studies (Reger, 1964; Ernst, 1970; Ruppert and Rice, 1983). The most comprehensive treatment is that of Ernst (1970), who carried out light and electron microscopic investigations of the introvert retractor of *Phascolopsis* (=*Golfingia*) *gouldii* in different states of contraction. Although Reger (1964) first reported two types of muscle cells in an ultrastructural observation of the retractor of *Sipunculus nudus*, it was Ernst (1970) who described in great detail the ultrastructure of large and small muscle cells in the retractor of *Phascolopsis gouldii* and hypothesized the manner in which they functioned in contraction of the muscle. She demonstrated that the large cell folds on contraction, whereas the small cell remains straight, providing evidence for the theory advanced earlier by Matsumoto and Abbott (1968) that large fibers fold passively at contraction due to the "active shortening of small fibers."

The large muscle cell in the retractor of *Phascolopsis gouldii* is 5.3 μm in diameter

70

71

72
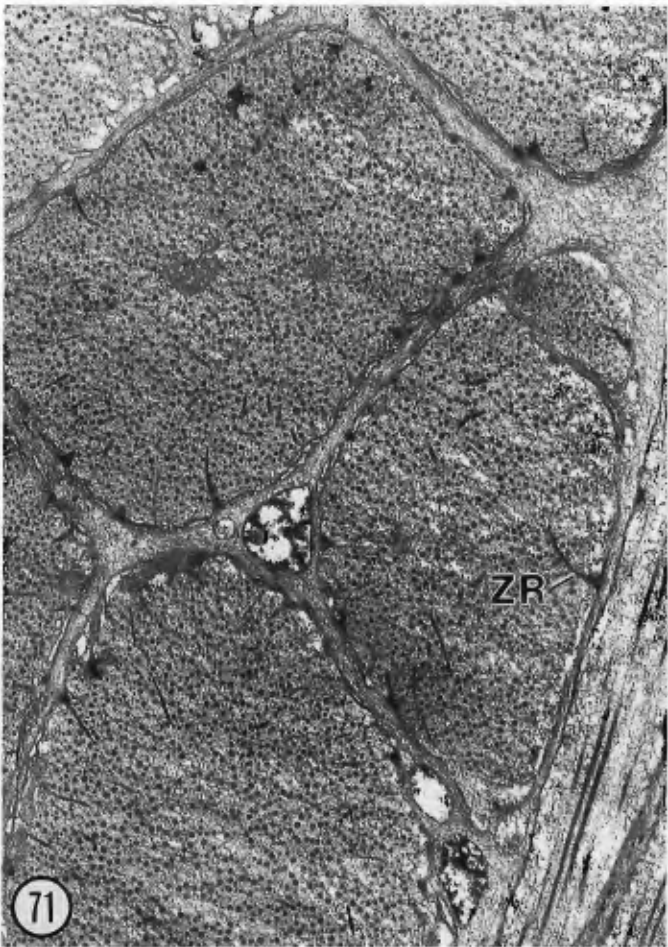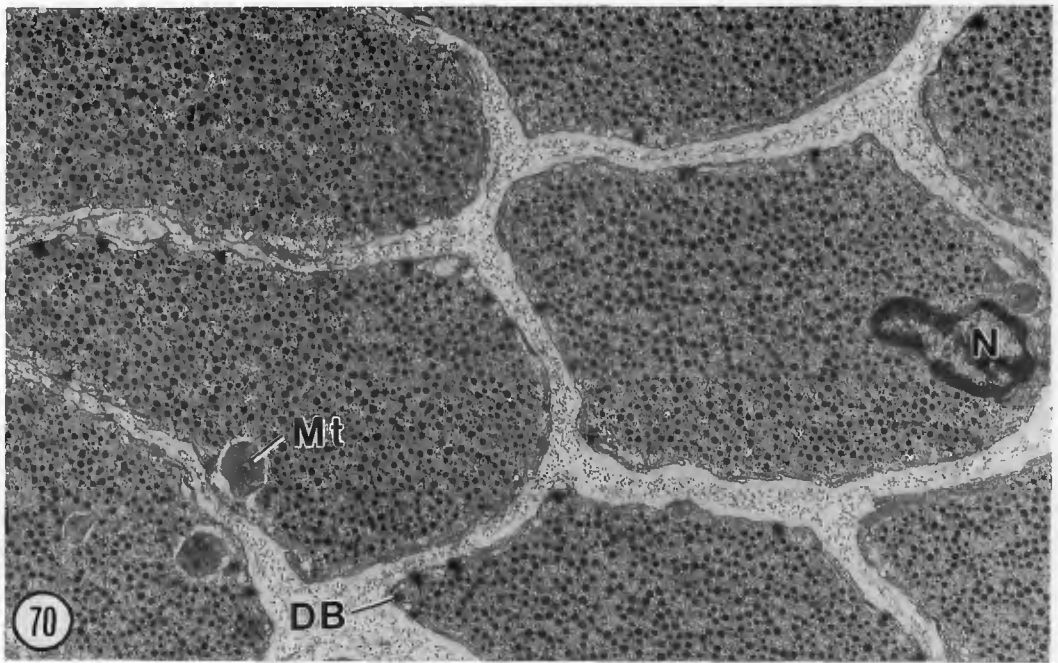
and 570 μm long, whereas the small is 2.3 μm in diameter and 440 μm long. The ratio of large to small cells is approximately 3 to 1, increasing slightly from periphery to center. Muscle cells or fibers are spindle shaped and separated from one another by connective tissue consisting of a ground substance and collagenous filaments (Ernst, 1970) (Fig. 73). When the large muscle cell folds on contraction, it "pleats in a zigzag fashion" (Ernst, 1970). The myofilaments within the large cell are arranged in segments between the angles of the pleat so that there is no breaking of filaments on folding.

The size, arrangement, ratios, and composition of myofilaments differ in the two sizes of muscle cells (Figs. 73–75). In the large cells thick filaments are about 27 nm in diameter. The ratio of thick to thin filaments is 1:6.9, with 12 actin filaments surrounding each myosin, indicating that one actin is shared by two myosin filaments (Fig. 75). Thick and thin filaments are arranged in a spiral or concentric circle around dense bodies. Similar to striated muscle, thick filaments appear to be in a hexagonal pattern. In small muscle cells the thick filaments are 34 nm in diameter and up to 30 μm in length. There are also 12 actin filaments surrounding each myosin, but the ratio of thick to thin is 1:15, suggesting a different arrangement than in the large cells. A further difference between thick filaments in the two cell sizes is the presence of both myosin and paramyosin in the small cells, but myosin only in the large cells.

In cells of both sizes, dense bodies are randomly distributed in the cytoplasm. Mitochondria are few and, along with small vesicles of sarcoplasmic reticulum, occur just under the sarcolemma. A single nucleus also lies near the periphery. Hemidesmosomes, attached randomly along the sarcolemma, serve
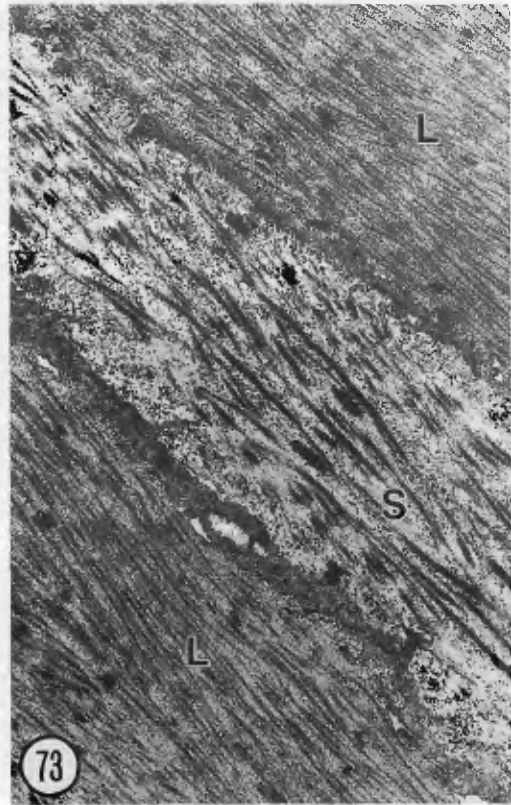
Figs. 70–72. Longitudinal body wall muscles. Transverse sections. TEM.
Fig. 70. *Nephasoma pellucida*, showing peripheral nucleus (N), mitochondria (Mt), and dense bodies (DB). ×14,640.
Figs. 71, 72. *Apionsoma misakiana* showing Z rods (ZR). Fig. 71, ×13,920. Fig. 72, ×22,325.

Fig. 73. Large (L) and small (S) muscle cells in the retractor muscle of *Phascolopsis* (=*Golfingia*) *gouldii*. The small cell has a smaller number of myofilaments per unit area than does the large cell. The thick filaments are larger in the small cell, and the glycogen appears to be in greater abundance. TEM. ×14,150. (From Ernst, 1970.)

as anchors for the thin filaments. Glycogen content is constant in the small cell, but variable in the large.

The retractor muscles of sipunculans show an extraordinary extensibility, reported for *Phascolopsis gouldii* to be five times the resting length (Matsumoto and Abbott, 1968). Contraction of the muscle cannot be accomplished solely by the increase in diameter of the large cells that would be necessary for the shortening of the muscle, because of structural limitations. The shortening, however, as noted previously, is accomplished by their folding. The sipunculan retractor muscle is
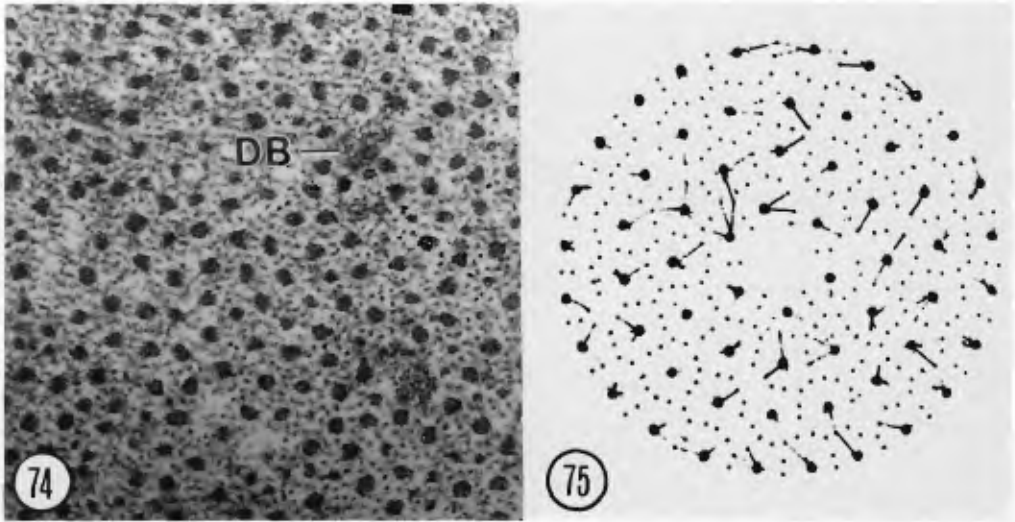
Fig. 74. A glycerinated large muscle cell in *Phascolopsis gouldii*. Transverse section. Three dense bodies (DB) are surrounded by curved rows of thick (myosin) filaments. TEM. ×40,936. (From Ernst, 1970.)
Fig. 75. Diagram of a model of thick (myosin) and thin (actin) filaments arranged in concentric circles around a dense body in *Phascolopsis gouldii*. (From Ernst, 1970.)

the only muscle in which such folding has been reported.

Physiological studies of living muscles have provided further data on the folding of fibers at relative muscle lengths (Matsumoto and Abbott, 1968; Abercrombie et al., 1984; Abercrombie and Bagby, 1984). Fold angles and fiber diameters of retractor muscles of *Phascolopsis gouldii* were measured from birefringent banding patterns in resting and contracted muscles, and a correlation was found between the extent of folding and relative muscle length. Furthermore, a bimodal length–tension curve was interpreted to indicate the presence of two populations of cells: straight fibers interspersed among folded fibers (Abercrombie et al., 1984). Configurations of the foldings were elucidated by scanning electron micrographs of glycerinated muscle cells, fixed at different lengths (Abercrombie and Bagby, 1984). The folding is seen as three-dimensional and spiraled (Figs. 76–78).

Other Musculature

Muscle layers of the digestive tract consist of an inner longitudinal layer adjacent to the epithelium and an outer circular layer covered by the peritoneum of the body cavity. Other musculature includes the spindle muscle, which runs through the center of the intestinal spiral, the dorsal muscle of the ventral nerve cord, and the muscle fibers in the nephridia. These muscles are similar in morphology in *Phascolopsis gouldii*; thick filaments of all contain paramyosin as indicated by size and banding patterns. However, they may differ in cell size and in number of mitochondria and hemidesmosomes (Ernst, 1970). The role of paramyosin in the nephridium is uncertain, but in the spindle muscle and dorsal muscle of the nerve cord it functions in sustained contraction of these organs during prolonged contractions of the body.

The musculature of sipunculans has been generally classified as smooth. However, the presence of Z rods in the body wall muscula-
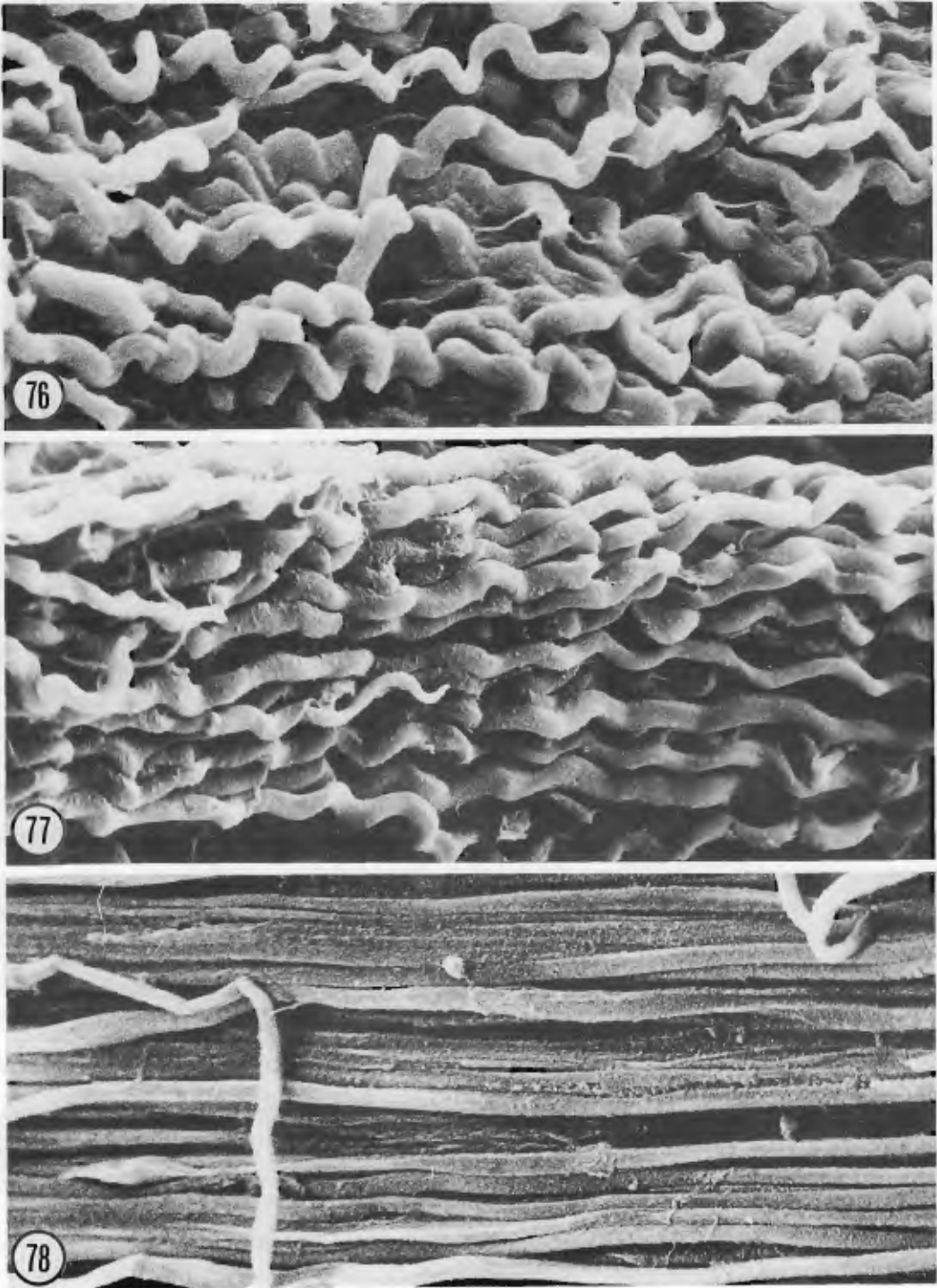
Figs. 76–78. Muscles glycerinated at different relative lengths (L/Lo) and teased to reveal individual fibers. L/Lo equals 0.5, 1.0, and 1.3, respectively. SEM. Note folding and spiral configuration of contracted fibers and decrease in folding with extension. ×1000. (From Abercrombie and Bagby, 1984.)

ture of *Apinosoma misakiana* (Figs. 71, 72) suggests that, in this species, the muscles may be obliquely striated and that further comparative investigations are required for a general classification of sipunculan muscles. In a previous study of a sipunculan larva, tentatively identified as *Apionsoma misakiana*, the retractor muscle of the terminal organ was defined as intermediate between smooth and obliquely striated (Ruppert and Rice, 1983). Z rods were found to radiate centripetally from their origin on the sarcolemma, although not in the regular order of obliquely striated muscles of many annelids. Other observations showed the ratio of thick to thin filaments to be 1:12–14, the former measuring 30–50 nm in diameter and the latter 5–6 nm. Peripheral couplings were present as subsarcolemmal cisternae of sarcoplasmic reticulum coupled with sarcolemma.

DIGESTIVE SYSTEM

The digestive tract of a sipunculan may be two to seven times the length of the body. Typically an elongate esophagus extends at least the length of the introvert into a recurved intestine with descending and ascending arms coiled around one another in a double spiral (Figs. 2, 10–15). The intestine ends in a straight rectum, which opens at a dorsal anus, usually at the base of the introvert or anterior trunk. Other regions that have been distinguished in some species are pharynx, a short muscular area at the beginning of the esophagus, and stomach, an area at the end of the esophagus marked by prominent inner longitudinal folds (cf. Stehle, 1953, for review). At its beginning the esophagus is surrounded by the retractor muscles, which unite anteriorly to form a sheath. The level of union of the retractors and the extent of the sheath vary among species. Posterior to the point of union, the retractors remain attached by mesenteries to the esophagus for most of its length. Thin filamentous muscles, usually two to five, often form attachments between the body wall and posterior esophagus and/or anterior intestine (Fig. 2). Passing through the

center of the intestinal coil is a strong spindle muscle (Fig. 79). Connected by mesenteries to the ascending and descending arms, it arises anteriorly on the body wall near the anus or on the rectum and in many species attaches posteriorly to the body wall (Figs. 10, 14, 15). Except for this posterior attachment and occasional lateral mesenteric connections, the intestinal coil hangs freely in the body cavity. Characteristic of most species is a diverticulum or cecum at the beginning of the rectum. Exceptions to this general arrangement are found in the Sipunculidae in which the descending intestine forms an additional loop (Fig. 10) and in the Phascolionidae in which the intestine forms a series of loops rather than a coil (Fig. 12).

Food is moved into the digestive tract through a heavily ciliated mouth region surrounded entirely or partly by tentacles (Figs. 80, 81). Ciliary tracts or grooves along the oral surface of the tentacles create feeding currents that move particulate matter into the mouth, while tentacular glands secrete mucus to which the particles adhere. Those species
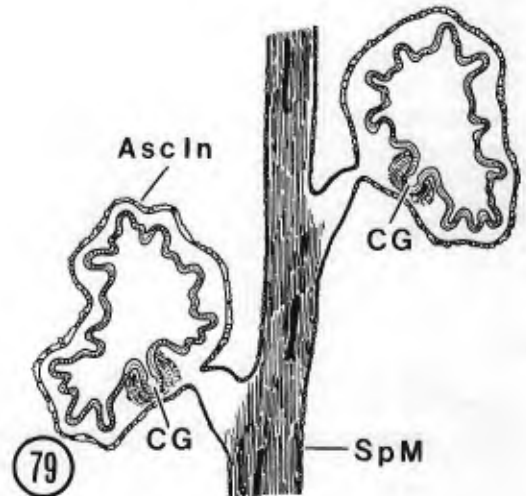

Fig. 79. Drawing of spindle muscle (SpM) in sagittal section and cross sections of coiled ascending intestine (AscIn) showing ciliated groove (CG) and mesenteric attachments to muscle. *Phascolopsis gouldii.* (Redrawn from Andrews, 1890.)
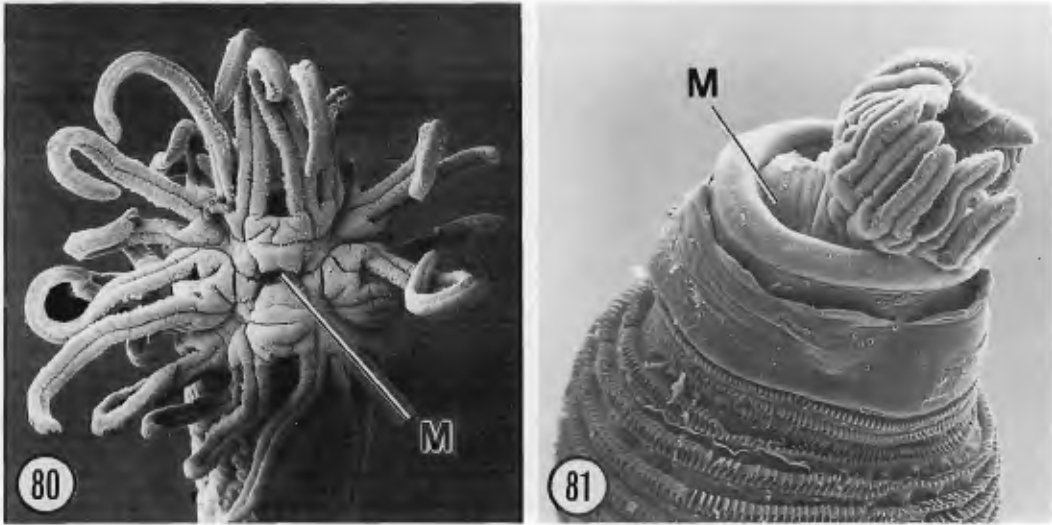
Fig. 80. Tentacular crown of *Themiste lageniformis*. Apical view. Elongate tentacles arise from the periphery of the oral disc and surround the central mouth (M). SEM. ×43.
Fig. 81. Tentacular crown of *Phascolosoma perlucens*. Lateral view. Tentacles are in an arc dorsal to the mouth. Note ciliated tentacular grooves leading to mouth region (M) in both species. SEM. ×65.

with highly developed tentacular crowns are suspension feeders, extending tentacles for long periods above the substratum. Burrowing species, such as *Sipunculus nudus* with broad, leaf-like tentacles, engulf sediment as they burrow.

The sipunculan tentacle is usually triangular in cross section, with a ciliated oral surface, often grooved, and a convex nonciliated aboral surface. Three canals pass through each tentacle as part of a tentacular system, which will be considered in the section on respiration. Ultrastructural studies of the elongate, filamentous tentacles of *Themiste lageniformis* reveal that cells of the oral epidermis, characterized as columnar epithelium, are joined laterally by zonulae adherentes (Pilger, 1982). Their apical surface is marked by numerous microvilli (1.4 μm); bifurcated tips of the microvilli protrude through the cuticular layer to the surface of the tentacle (Fig. 82). Also extending from the apical surface of each cell are several cilia. Each cilium has a single rootlet that is protracted obliquely at an angle of about 100°

from the basal body toward the midline and base of the tentacle. The apical cytoplasm contains electron-dense bodies, presumably lysosomes, and numerous mitochondria that are often in contact with the ciliary rootlets. The nucleus is in the central or basal region of the cell, which is in contact with a connective tissue or extracellular matrix, made up of collagenous fibers. Nerves and muscle cells are embedded in this matrix (Fig. 83). Mucous cells occur within the oral epidermis and in the oral and aboral transitional zones (Fig. 82). These cells are packed with mucous droplets (up to 1 μm in diameter) to the exclusion of most other organelles.

With the exception of scattered ciliary tufts (presumably sensory) the aboral epidermis is nonciliated (Fig. 83). Cells are cuboidal, becoming more columnar toward the oral surface. The apical arrangement of microvilli and cuticular matrix is similar to the oral epidermis. Projecting into the apical cytoplasm from the microvilli are parallel microfilaments, probably providing rigidity to the tentacular structure.
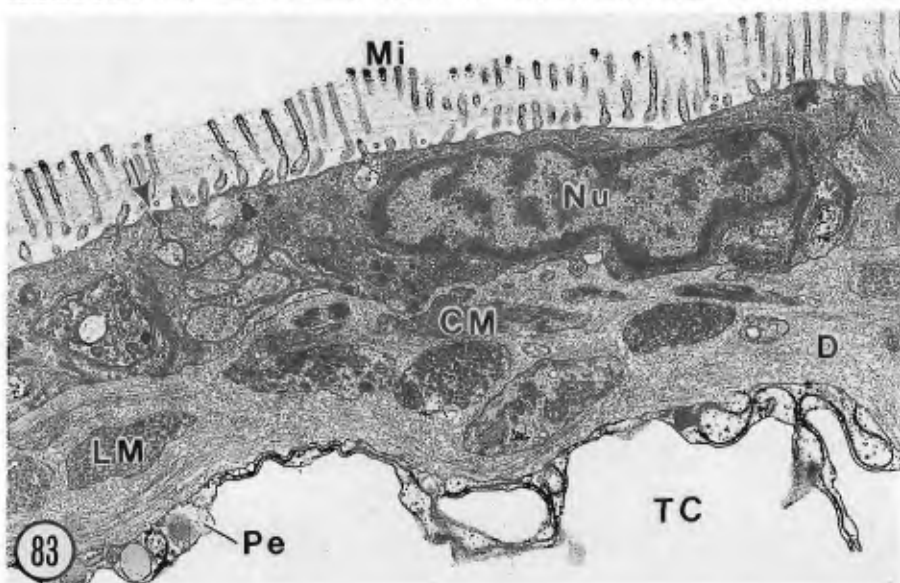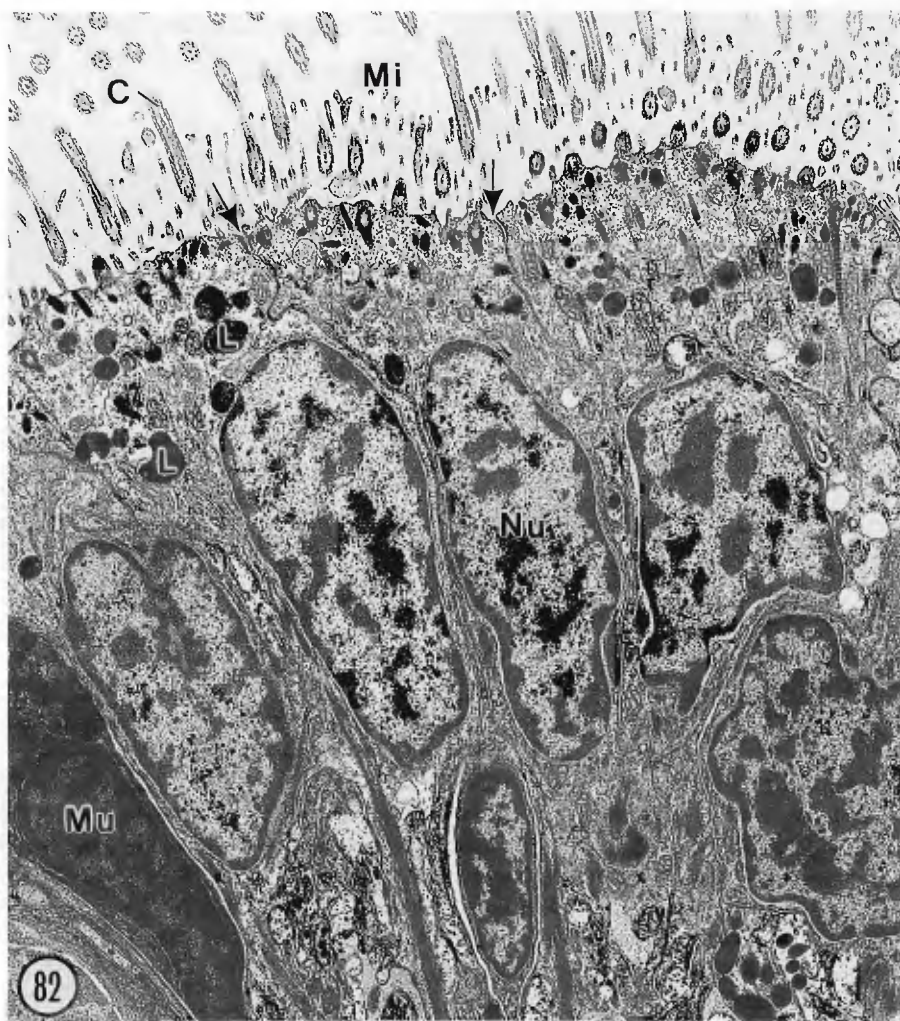
Little information has been published on the ultrastructure of the digestive tract (Brown et al., 1982), but the histology of several species is well known (Andrews, 1890, for *Phascolopsis gouldii;* Shipley, 1890, for *Phascolosoma varians;* Metalnikoff, 1900, for *Sipunculus nudus;* Awati and Pradhan, 1935, for *Themiste lageniformis;* for species of Golfingiidae: Cuénot, 1900; Paul, 1910; Stehle, 1952, 1953). The basic construction of the digestive tube consists of an inner cylindrical epithelium lining a central lumen, a layer of connective tissue with embedded muscle fibers, and a peritoneal covering. The thickness, degree of epidermal folding and ciliation vary in different regions (Figs. 84–86). Longitudinal folds are characteristic of the lining of the esophagus, descending intestine, and rectum, but not of the thinner ascending intestinal loop. Running the length of the ascending intestine in most species is a ciliated groove, which ends at the rectal diverticulum or cecum (Fig. 79); in *Sipunculus nudus* it runs from the mouth to the rectal diverticulum (Metalnikoff, 1900). The groove of *Sipunculus nudus* and *Phascolopsis gouldii* is reportedly marked by a reddish coloration. *Phascolosoma varians* lacks both the groove and rectal cecum (Shipley, 1890).

Ultrastructural descriptions to follow are based on Brown et al. (1982) for *Phascolopsis gouldii;* Dybas (personal communication) for *Phascolosoma perlucens;* Ruppert (personal communication) for *Phascolosoma varians, Aspidosiphon steenstrupi,* and *Themiste lageniformis;* and author's personal observation for *Phascolion sp.*

The epithelial lining of the esophagus is composed primarily of multiciliated columnar cells (Fig. 87) interspersed with occasional
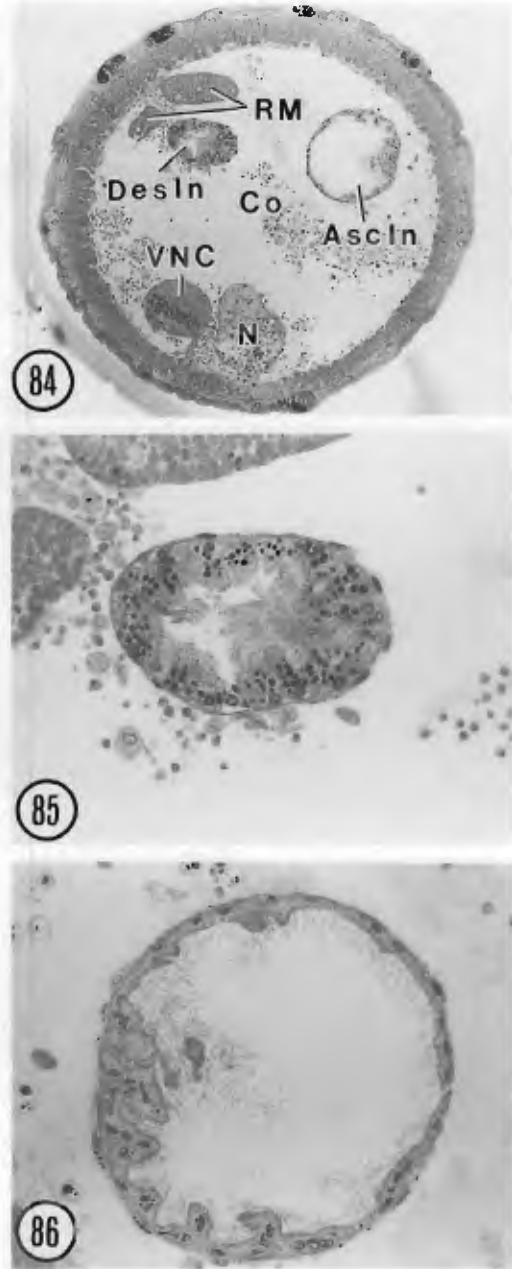
Figs. 84–86. Light micrographs of *Phascolion* sp., a small interstitial species. Transverse sections.
Fig. 84. Section through posterior body, showing ascending intestine (AscIn), coelomic cavity (Co) with coelomocytes, descending intestine (DesIn), nephridium (N), retractor muscles (RM), and ventral nerve cord (VNC). Note epidermal organs in outer cuticular layer of body wall. ×149.
Fig. 85. Section of descending intestine. Wall is thick, internally folded, and marked by characteristic dark granules in inner epithelium. ×373.
Fig. 86. Section of ascending intestine. Intestinal wall is relatively thin and inner epithelium markedly ciliated. ×373.

Figs. 82, 83. Epithelium of oral and aboral surfaces of the tentacle of *Themiste lageniformis.* TEM. C, cilium; CM, circular muscle; D, dermis; L, lysosome; LM, longitudinal muscle; Mi, microvilli; Mu, mucous cell; Nu, nucleus; Pe, peritoneum; TC, tentacular canal. Arrows point to zonulae adherentes.
Fig. 82. Ciliated columnar epithelium of the oral surface. ×9,125. (From Pilger, 1982.)
Fig. 83. Nonciliated cuboidal epithelium of the aboral wall. ×9,840. (Micrograph courtesy of J. Pilger.)
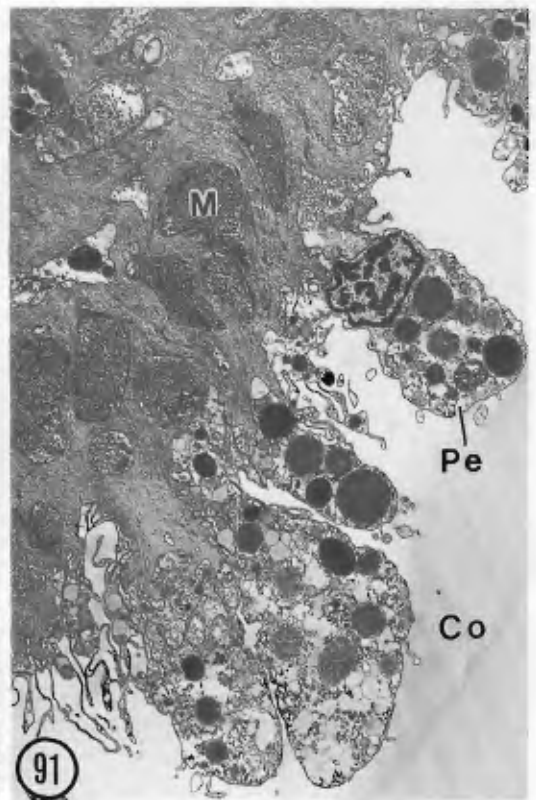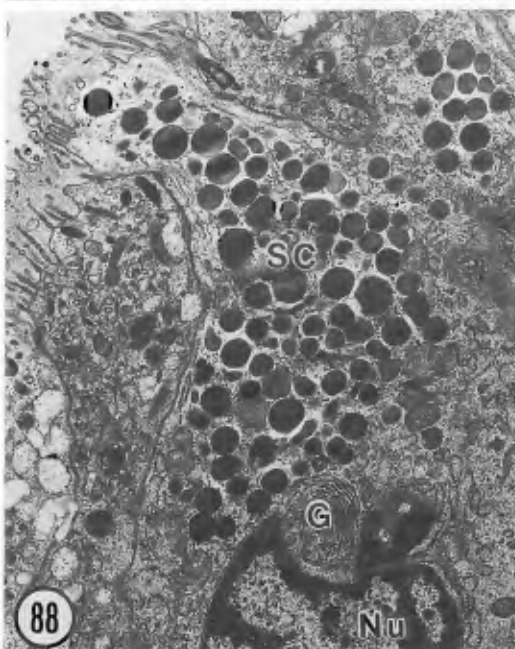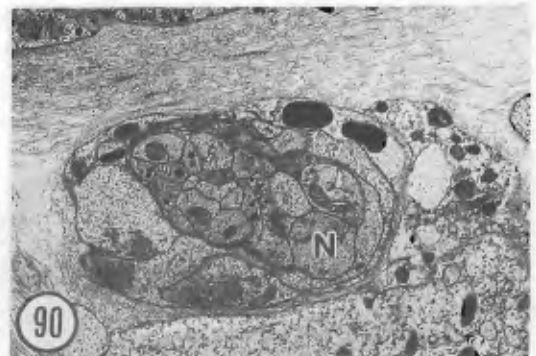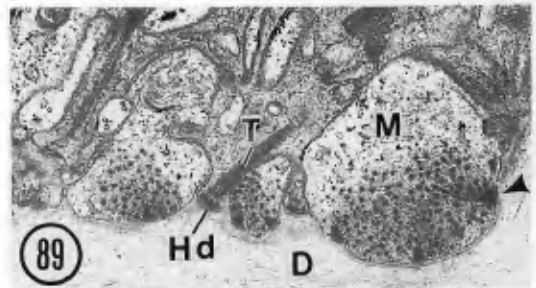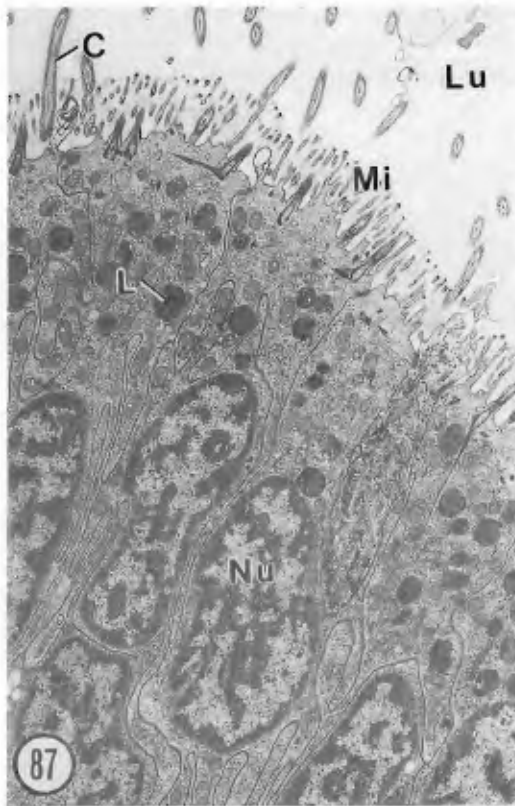
secretory cells identified by their concentration of electron-dense granules (Fig. 88). Nuclei are situated in the basal region of the cells. The apical surface of the ciliated cells bears a dense array of microvilli, parallel and of similar height, forming a characteristic brush border. Frequently bifurcating near their tips, the microvilli possess an electron-dense terminal knob and are surrounded laterally by an extraneous coat of fine granular extracellular material. The cilia are of the typical 9 + 2 pattern of microtubular arrangement. Basal bodies of the cilia are anchored by two rootlets: one short horizontal and one long vertical, which extends far into the cell. Epithelial cells are attached to one another near the apical surface by zonulae adherentes and more distally by septate junctions (Figs. 87, 88). Considerable infolding of the plasmalemma may occur in the mid- and basal portions of the cell. Cytoplasmic components include lysosome-like bodies of varying size and density and numerous mitochondria. Gland cells, scattered among the other epithelial cells, are characterized by an absence of cilia and by an abundance of electron-dense spherical granules in the proximal portion of the cell (Fig. 88). Cytoplasm in the mid- and basal regions of the cell is dominated by smooth and rough endoplasmic reticulum; a prominent Golgi apparatus lies in the vicinity of the nucleus, and mitochondria are randomly dispersed. Extending into the basal cytoplasm of the epithelial cells are tonofilaments, forming an attachment to the underlying basal lamina and connective tissue layer through hemidesmosomes (Fig. 89). At the bases of the epithelial cells, frequently within the connective tissue projecting between the infoldings of the plasmalemma, are muscle cells and small groups of neurites surrounded by glial cells (Fig. 90). The connective tissue layer, composed of collagenous fibers of undetermined periodicity, may also harbor cells resembling coelomic granulocytes and hemerythrocytes. The peritoneum covering the esophageal tract consists of both ciliated and nonciliated cells. Frequently associated with the esophagus, chlorogogue cells occur as part of the peritoneal lining (Fig. 91). These cells are characterized by large membrane-enclosed granules, possibly functioning for storage.

In a brief description of the epithelial cells in the anterior descending intestine of *Phascolopsis gouldii*, Brown et al. (1982) noted numerous granules of varying densities and some vacuoles in the apical region of the cells (Fig. 92). They further reported microvilli on the apical surface and cilia with long rootlets penetrating deeply into the cell. In the intestinal lumen, cytoplasmic blebs containing granules were interpreted as evidence for apocrine secretion. Cells emitting blebs had swollen microvilli and no cilia (Fig. 93) (see later discussion for correlation with enzymatic analyses). In an early light microscopic study, a similar phenomenon of bleb production and loss of cilia was reported in the posterior descending intestine of *Phascolopsis gouldii* (Andrews, 1890). Ultrastructural observations on the digestive tract of a small, meiofaunal species of *Phascolion* revealed the descending intestine to be distinguished by an abundance of cells having typical secretory features (author's observations) (Fig. 94). These elongate, columnar cells are packed with smooth and rough endoplasmic reticulum. There are prominent mitochondria, and, in the upper portion of the cell,

Figs. 87–91. Esophagus. TEM. C, cilium; Co, coelom; D, dermis; G, Golgi apparatus; Hd, hemidesmosomal attachment; L, lysosome-like body; Lu, lumen; M, muscle cell; Mi, microvilli; N, nerve; Nu, nucleus; Pe, peritoneal lining; Sc, secretory cell; T, tonofilaments. Arrow points to Z rod of muscle cell. (Micrographs courtesy of L. Dybas, Figs. 87, 91, and E. Ruppert, Figs. 88–90.)

Fig. 87.   Ciliated columnar epithelium lining the esophageal lumen of *Phascolosoma perlucens*. ×7,175.

Fig. 88.   Secretory cell in the esophageal epithelium of *Phascolosoma varians*. ×8,160.

Fig. 89.   Basal region of esophageal epidermal cells of *Aspidosiphon steenstrupii*, showing tonofilament with hemidesmosomal attachment and muscle cells. ×13,200.

Fig. 90.   Nerve, composed of small groups of neurites surrounded by glial cells, within the esophageal connective tissue of the dermis of *Aspidosiphon steenstrupii*. ×5,890.

Fig. 91.   Chlorogogue cells of the peritoneal lining (Pe) of the esophagus of *Phascolosoma perlucens*. ×3,895.
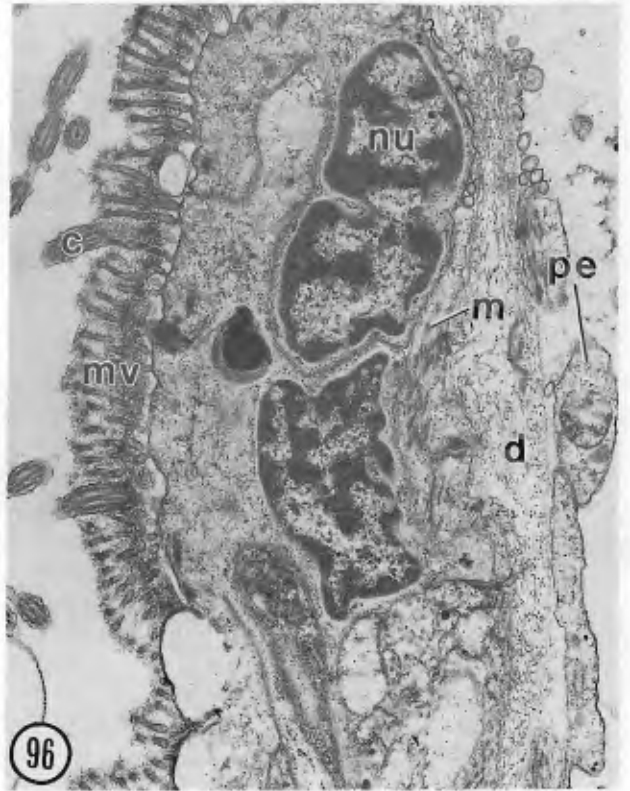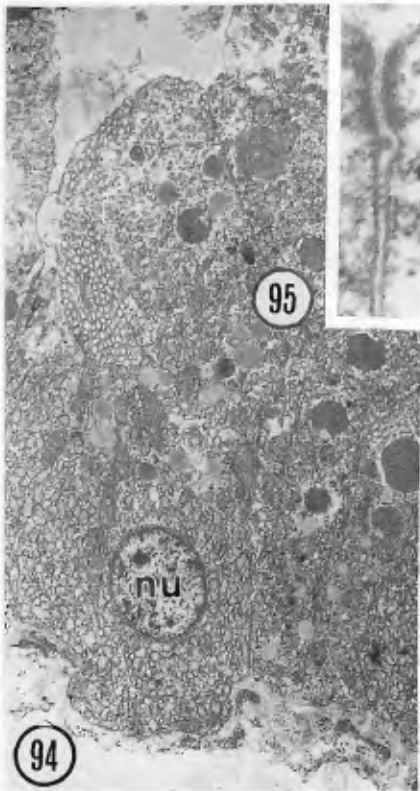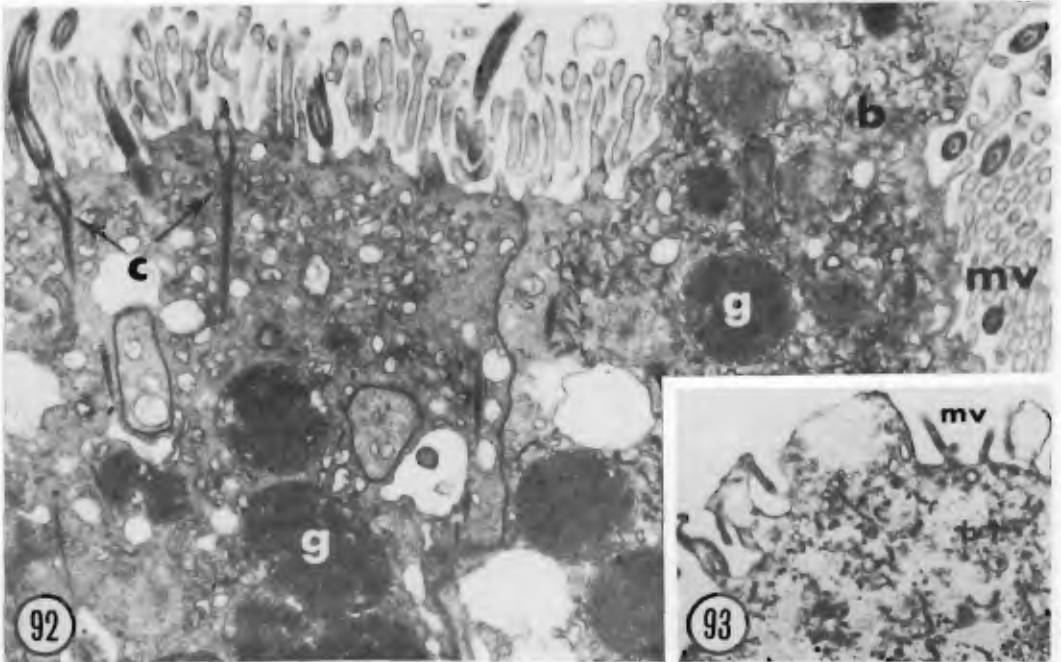
some large granules of varying density. Cytoplasmic blebbing was not documented, although blebs of various sorts occur in the lumen. Adjacent cells are joined apically by zonulae adherentes and more basally by septate junctions (Fig. 95).

Unlike the elongate epithelial cells of the esophagus and descending intestine, those of the ascending intestine are more cuboidal in shape and have fewer granules or lysosome-like bodies. As observed in the small meiofaunal species of *Phascolion*, the apical surface of the cells bears multiple cilia and a brush border, composed of numerous, closely packed microvilli, of similar height and parallel configuration, surrounded by a coat of well-developed extraneous material (Fig. 96).

The ciliated groove is marked by a dense array of exceptionally long cilia extending into the lumen of the groove. In the terminal portion of the intestine of *Themiste lageniformis* these cilia have two rootlets: one horizontal across the apical portion of the cell and another penetrating vertically deeply into the cell (Figs. 97, 98). Prominent, elongate mitochondria are closely associated with these rootlets. Microvilli project from the surface between the cilia. Extending from the desmosome-like junctions near the apical borders of the cells are fine filaments, which form the terminal web and provide mechanical support for the cell. Designed for strong ciliary beat-

ing and for moving water, the groove also shows some evidence of secretory activity. Certain cells with denser cytoplasm of fine granular material appear to extrude material into the lumen of the groove (Fig. 97).

No information is available on the ultrastructure of the rectum, but histological studies have shown it to have a thick, highly folded columnar epithelium of either simple ciliated cells (Andrews, 1890, for *Phascolopsis gouldii*) or ciliated and vacuolated cells (Shipley, 1890, for *Phascolosoma varians*). The musculature is generally more developed than in other regions of the digestive tract.

Functions of different regions of the digestive tract, inferred from what is known of the histology and ultrastructure, can be summarized as primarily absorptive for the esophagus and ascending intestine, both of which have well-developed microvillar systems that increase the epithelial surface area, and as primarily secretory for the descending intestine, which appears to have the greatest concentration of secretory cells. Cilia, present throughout the tract, serve to move particulate matter and fluids, but the prominence of ciliation in the esophagus and ascending intestine—especially the ciliated groove—denote particular significance for this activity. Ultrastructural investigations to date are at best superficial; more detailed studies, using labels and markers, are necessary to identify regions and clarify mechanisms of uptake and secretion.

Information available from physiological inquiries, including histochemical and enzymatic analyses, confirms and expands the interpretations of digestive processes based on histological and ultrastructural observations. Histochemical studies on *Phascolion strombus* (Arvy and Gabe, 1952) identified glycogen in the esophageal epithelium, but no digestive enzymes. The descending intestine, on the other hand, was designated as the main site of both digestion and absorption, because a number of food stores (carbohydrates, including glycogen, osmiophilic lipids, and ribonucleic substances) were located through-

Figs. 92, 93. Anterior descending intestine of *Phascolopsis gouldii*. TEM. (From Brown et al. 1982.) b, bleb; c, cilium; g, granule; mv, microvilli.

Fig. 92. Apical region of glandular epithelium. ×5,133.

Fig. 93. Cytoplasmic bleb of apical region of epithelium. ×12,780.

Fig. 94. Secretory cells from the descending intestine of *Phascolion* sp., a small, interstitial species, showing an abundance of cytoplasmic rough endoplasmic reticulum and a few electron-dense granules. TEM. ×4,050. nu, nucleus.

Fig. 95. Apical junction between secretory cells of descending intestine of *Phascolion* sp. Zonula adherens followed by septate junction. ×57,400.

Fig. 96. Transverse section through ascending intestine of *Phascolion* sp. Note apical cilia (c) and brush border arrangement of microvilli (mv). To the outside of the cuboidal epidermal cells lies the dermis (d) with scattered muscles covered by the peritoneum. m, muscle cell; nu, nucleus; pe, peritoneum. ×12,000.
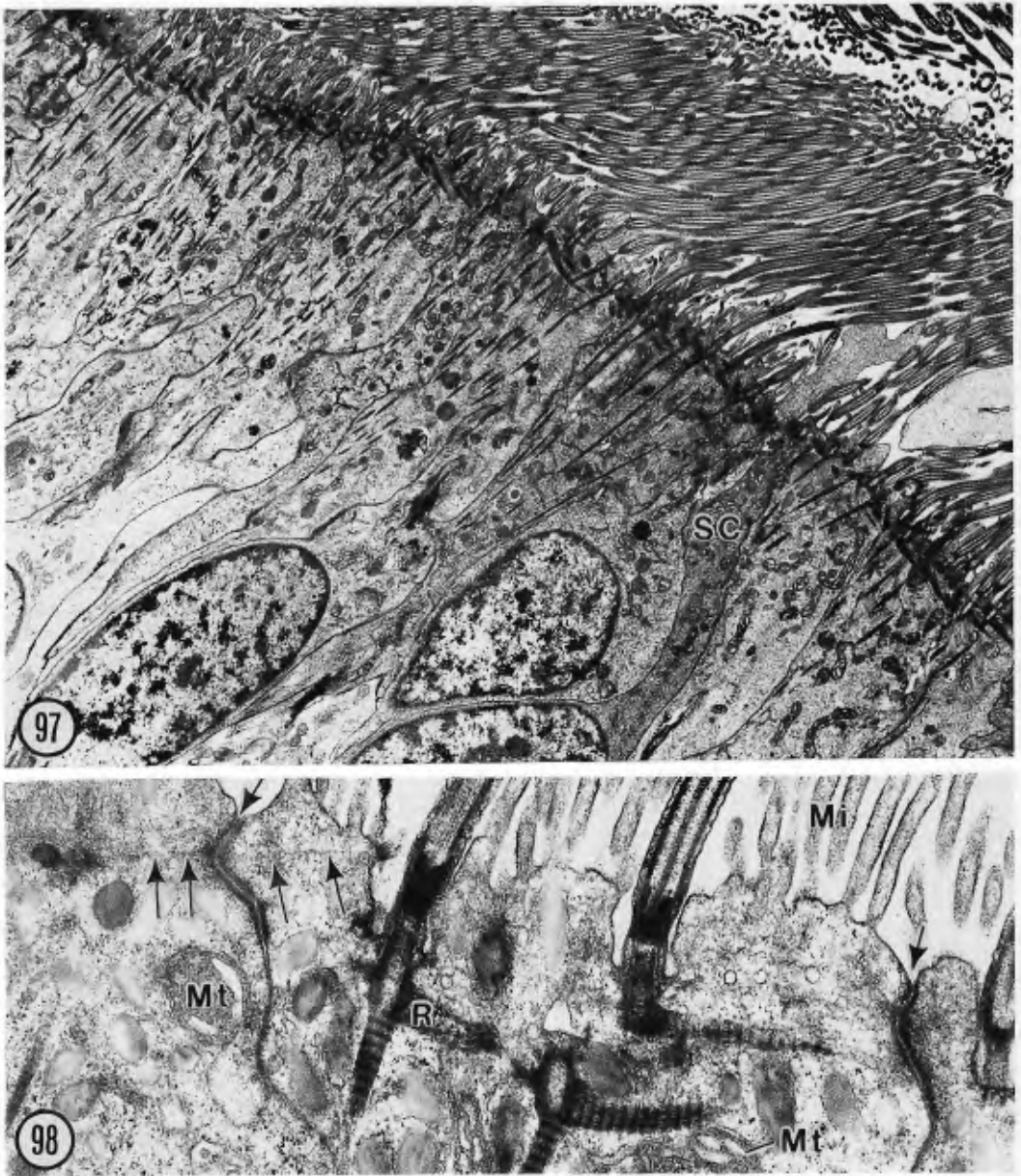
Figs. 97, 98. Ciliated groove of ascending intestine of *Themiste lageniformis*. TEM. (Micrographs courtesy of E. Ruppert.)
Fig. 97. Columnar epithelium lining ciliated groove. Note scattered secretory cells (SC). ×5,130.
Fig. 98. Enlargement of Figure 97, showing ciliary rootlets (R), microvilli (Mi), mitochondria (Mt). Short arrows point to zonulae adherentes, long arrows to terminal web. ×22,800.

out the epithelial wall, and enzymes (lipases and alkaline phosphatases) were reported in the more posterior portion. No enzymes were found in the ascending intestine, nor food stores except in the surrounding chlorogogue cells. Walter (1973), in a study of three species of sipunculans (*Golfingia elongata, Golfingia vulgaris, Phascolosoma granulatum*) demonstrated that sediment structure changed in different regions of the digestive tract, decreasing from anterior to posterior in the meiofaunal component and increasing in compactness. Both Walter and subsequently Hansen (1978) divided the gut into three regions for analyses, but unfortunately these are not correlated with histological structure or conventional morphological designations. Hansen, in the sediment-feeding *Sipunculus nudus*, showed that calorific values of gut contents decrease 70% from anterior to middle gut and another 19% in the posterior intestine. Brown et al. (1982) surveyed the distribution of cells containing zymogen granules in the digestive tract of *Phascolopsis gouldii* and found them to be concentrated in the anterior descending intestine. Electron microscopic examination of this area also revealed secretory cells (see above, Fig. 92). Activity of a chymotryptic-like enzyme, isolated from a complement of digestive enzymes, was greatest, however, in the posterior intestine, the posterior loop, and the beginning of the ascending intestine. The discrepancy in the distribution of secretory cells and enzymatic activity may be explained by the flow of the secretion produced in the anterior descending intestine in a posterior direction to accumulate in the posterior loop. Other enzymes demonstrated in the digestive tract and/or intestinal contents are chitinases in *Sipunculus nudus* and *Golfingia vulgaris* (Jeuniaux, 1969) and cellulase as well as chitinase in *Phascolosoma japonicum* (Elyakova, 1972).

CIRCULATION AND RESPIRATION

The phylum Sipuncula lacks the morphologically conventional circulatory and respiratory systems that can be identified in most large, triploblastic animals. However, the primary functions of these systems, fluid transport and gas exchange, are served by the spacious, fluid-filled coelomic cavity of the body (including trunk and introvert) and by a second smaller coelomic compartment, the tentacular system, comprised of tentacular canals and one or two continuous elongate sacs that extend posteriorly along the esophagus into the body cavity. Both the primary coelomic cavity and the tentacular system function in gas exchange and waste transport. The main coelomic cavity serves further as a hydrostatic skeleton, a repository for gametes, for internal fluid transport, and, through the functions of its numerous coelomocytes, for cellular defense and immunological responses to foreign matter. A discussion of the cellular elements of the primary coelomic cavity is presented first, followed by an account of the tentacular system.

Coelomic Elements

The coelomic cavity contains a number of different types of freely floating coelomic cells that are moved about either through the contractions of the body and introvert or by the activity of ciliated cells distributed throughout the peritoneum. Over the past 100 years, sipunculan coelomocytes have been the subject of great interest among biologists. The considerable literature that has resulted, including different classifications of cell types and various terminologies, has been reviewed by Dybas (1981a), who recognizes five categories of coelomocytes. They are hemocytes (=hemerythrocytes), granulocytes, large multinuclear cells (including cell pairs and enigmatic vesicles), ciliated urns, and immature cells. The relative frequencies of these cell types, as reported for the species *Phascolosoma agassizii*, are 90% hemerythrocytes, 4.9% ciliated urns, 4.9% granulocytes, and 0.2% large multinuclear cells (Dybas, 1975). Immature cells were not observed in this count. Ultrastructural studies of sipunculan coelomocytes, on which the discussion to follow is based, are those of Stang-Voss (1970),

Ochi and Ohnishi (1971), Ochi (1975), Dybas (1975, 1976, 1981a,b), Nemhauser et al. (1980), Valembois and Boiledieu (1980), and Terwilliger et al. (1985).

Hemerythrocytes, cells bearing the respiratory pigment hemerythrin, are the most abundant of the coelomocytes (Figs. 99–101). Ranging in diameter from 6 to 32 μm in different species, they vary in shape from biconcave or biconvex discs to circular or ellipsoid. Electron microscopic studies reveal an amorphous, electron-dense cytoplasm and a small dense, frequently eccentric nucleus

(Figs. 102–104). The density of the cytoplasm is explained as due to the iron in the respiratory pigment, hemerythrin (Valembois and Boiledieu, 1980; Terwilliger et al., 1985). Cytoplasmic organelles include small mitochondria, lysosome-like vacuoles, lipid droplets, and, in some species, glycogen rosettes. Typically, the mature hemerythrocyte has few and poorly developed organelles. Stang-Voss (1970) considers the small mitochondria and lack of Golgi apparatus to indicate a degenerative state, whereas Valembois and Boiledieu (1980) attribute the few or-
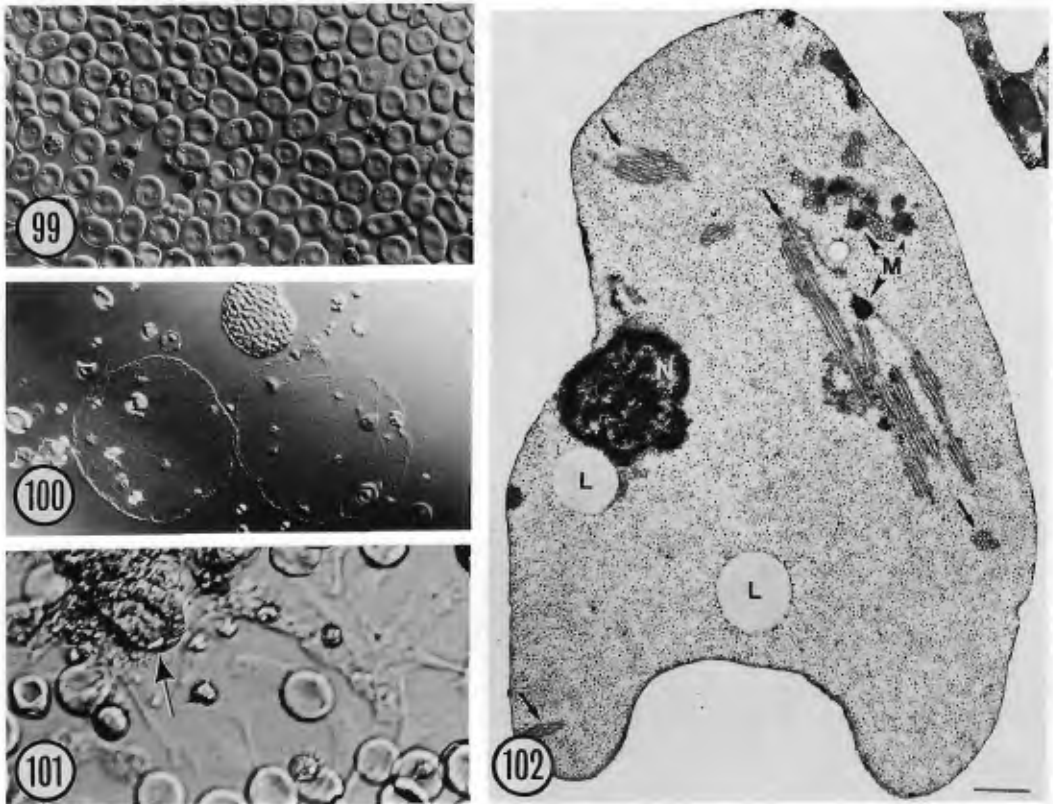

Fig. 99.   Hemerythrocytes in a sample of coelomic fluid from a living specimen of *Sipunculus nudus*. Nomarski optics. ×138.
Fig. 100.   Enigmatic vesicles, hemerythrocytes, and sperm plate from coelomic fluid of a living specimen of *Sipunculus nudus*. Nomarksi optics. ×144.
Fig. 101.   Coelomocytes from coelomic fluid of a living specimen of *Phascolosoma perlucens*, including hemerythrocytes,

granulocytes with extended pseudopods, and urns (arrow). Nomarski optics. ×350.
Fig. 102.   A coelomic hemerythrocyte of *Themiste dyscrita*. TEM. Cytoplasm contains glycogen rosettes; electron-dense background presumably corresponds with hemerythrin. L, lipid; M, mitochondria; N, eccentric nucleus. Arrows point to membranous tubules. Bar = 1 μm. (From Terwilliger et al., 1985.)

ganelles to a low metabolic requirement. A specific variation in the hemerythrocyte of *Themiste dyscrita* is the occurrence of membranous lamellae, which appear in cross section as hollow tubules (Fig. 102). No function is ascribed to these structures, but their association with mitochondria is noted as well as their similarity to the dark bands reported by Valembois and Boiledieu (1980) in the hemerythrocyte of *Sipunculus nudus* (Figs. 103, 104). Marginal band microtubules, which serve as cytoskeletal support in nonmammalian vertebrate erythrocytes and mammalian platelets, occur in the hemerythrocytes of *Phascolopsis gouldii* (Nemhauser et al., 1980). Membrane-bound vacuoles containing a dark, flocculent material, presumed to be ferritin, have been observed in *Phascolosoma scolops* (Ochi and Ohnishi, 1971), *Phascolopsis gouldii* (Stang-Voss, 1970), *Themiste minor* (Ochi, 1975), and *Phascolosoma agassizii* (Dybas, 1981a). In a survey of hemerythrocytes of five species of sipunculans, Ochi (1975) noted specific variations in lysosomal structure and glycogen content. Glycogen particles are distributed uniformly through the cytoplasm in *Golfingia nigra* and *Golfingia ikedai*, in numerous small areas in *Themiste minor*, and in a few large patches in *Phascolosoma scolops*. Glycogen particles do not appear in hemerythrocytes of *Siphonosoma cumanense*.

Immature cells in the coelomic fluid of *Sipunculus nudus*, interpreted by Valembois and Boiledieu (1980) to be hemerythroblasts or stem cells, are 8 μm in diameter with a relatively large nucleus and sparse cytoplasm containing large mitochondria, free ribosomes, and well-developed Golgi bodies (Figs. 105, 106). These cells are often in mitosis. A later transitional stage (10–20 μm diameter) has a greater proportion of cytoplasm that, similar to the mature hemerythrocyte, consists of an amorphous matrix but, unlike the mature cell, has an accumulation of numerous large mitochondria at one pole. Stang-Voss (1970) described "prohemocytes" in coelomic fluid of *Phascolopsis gouldii* and

he also noted numerous, well-developed mitochondria. As proposed by earlier authors (cf. Ohuye, 1942), Stang-Voss suggested that immature hemerythrocytes arise from a proliferation of peritoneal cells. Reports of both Stang-Voss (1970) and Valembois and Boiledieu (1980) noted small ciliated cells in the coelomic fluid that they interpreted as stem cells of either hemerythrocytes or urns. The function of hemerythrocytes is considered in the next section on the tentacular system.

Granulocytes, often termed amebocytes and/or leukocytes in the literature, are more variable in form and structure both within and among species than are hemerythrocytes. In numerous studies in the past, they have been variously classified on the basis of characters defined by the use of light microscopy: for example, behavioral characters such as amebocytic or phagocytic, size of cells, presence or absence of granules, size of granules, and staining properties of granules such as acidophilic or basophilic (cf. Dybas, 1981a, for review). More recent ultrastructural studies consider the different forms as a continuum of differentiating stages of one cell type, the granulocyte (Valembois and Boiledieu, 1980; Dybas, 1981a,b). Valembois and Boiledieu use the term small hyaline leukocyte for the early granulocyte prior to differentiation of granules and large hyaline leukocyte for the older granulocyte in which granules are degenerating.

The granulocytes of *Sipunculus nudus*, in living preparations, are 8–15 μm in diameter, oval to round, and sometimes with pseudopodial extensions. In an animal in which the volume of coelomic fluid is 12 ml, the total number of granulocytes (excluding "hyalocytes") is estimated to be from 16 to 24 million (cf. Valembois and Boiledieu, 1980). The nucleus is irregular in shape, and, as reported in ultrastructural studies by the above authors, it has aggregates of dense material concentrated toward the periphery. The cytoplasm contains cylindrical mitochondria, well-developed Golgi bodies, rough endo-
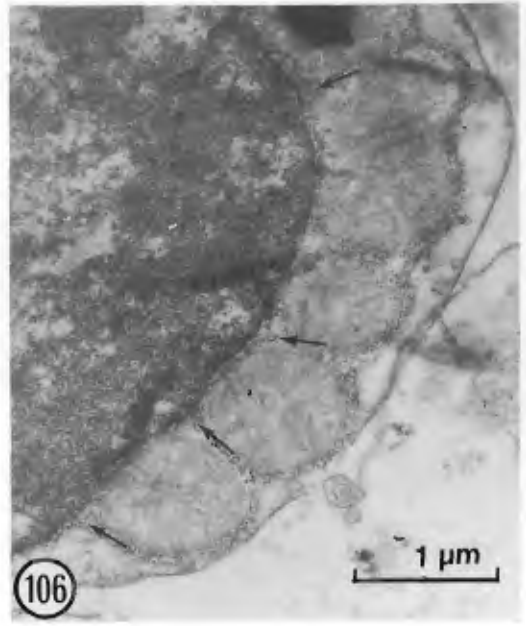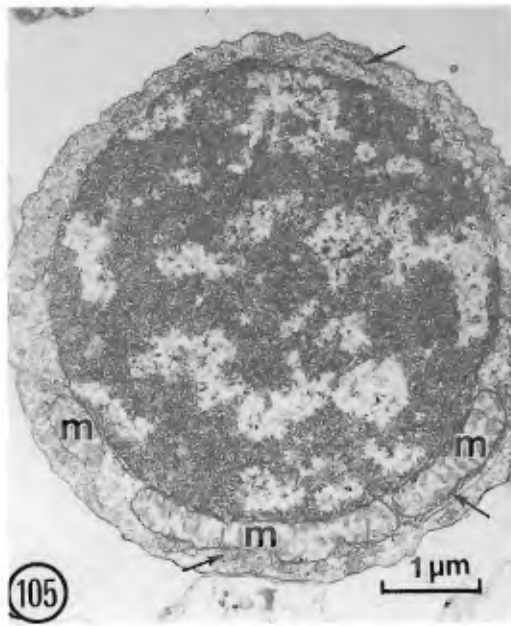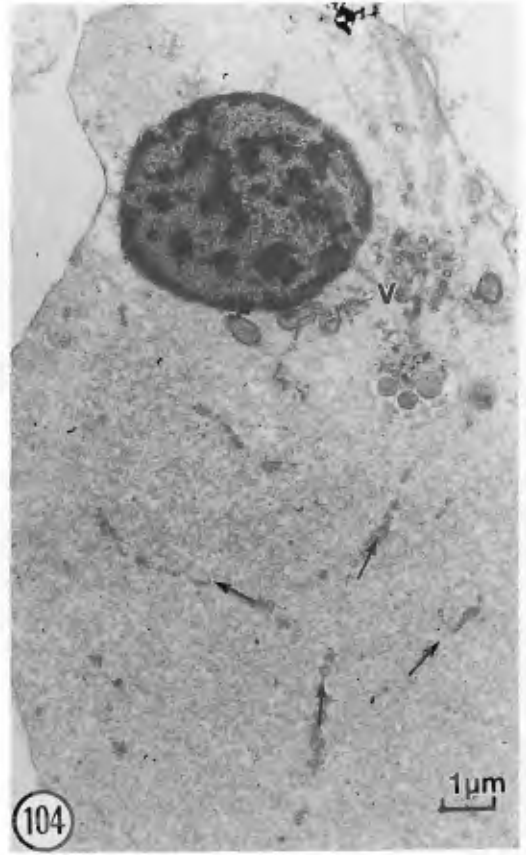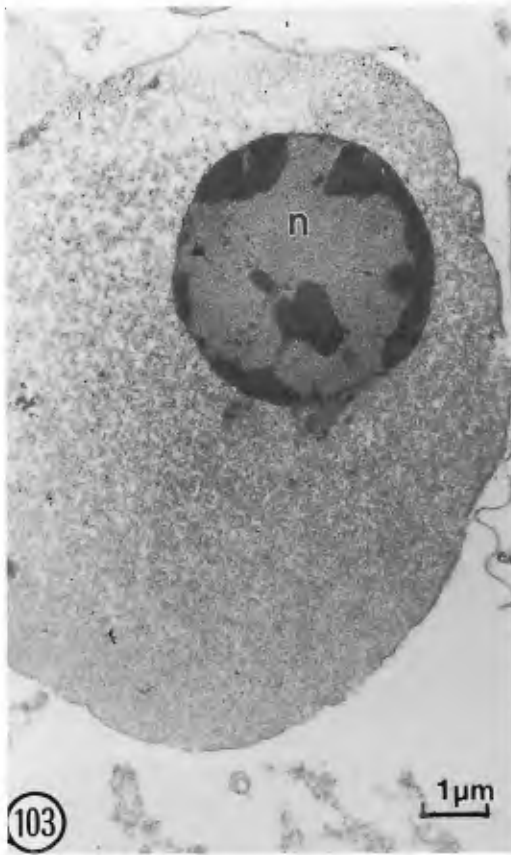
Figs. 103–106. Hemerythrocytes of *Sipunculus nudus*. TEM.
Figs. 103, 104. Mature hemerythrocytes. Cytoplasm has a
dense, amorphous matrix with scattered "ribs" of dense material
(arrows). Nucleus (n) is surrounded by small vesicles (v). (From
Valembois and Boiledieu, 1980.)

Figs. 105, 106. Immature hemerythrocytes (hemerythro-
blasts) have relatively large nuclei and little cytoplasm. Mito-
chondria (m) are large and surrounded by granular material
(arrows). (Micrographs courtesy of P. Valembois and D.
Boiledieu.)

plasmic reticulum, and membrane-bound, electron-dense granules measuring 0.01–0.1 μm (Fig. 107). Granules are enclosed by two trilaminar membranes and are observed in association with degenerating mitochondria and engulfed material (Figs. 108–110). Small granules are composed of noncrystalline and homogeneous material, becoming heterogeneous and clearer in larger granules. There is some evidence that small granules may coalesce to form larger granules. Although granules were not observed in association with Golgi in *Sipunculus nudus*, new granules have been observed to form from Golgi in granulocytes of *Phascolosoma scolops* (Ochi, 1975) and *Phascolosoma agassizii* (Dybas, 1981a,b). In *Phascolosoma agassizii* it was further noted that granules were of a homogeneous density except in the region of the Golgi where they showed an electron-dense core surrounded by a clear zone. Granules of the smaller cells generally were acidophilic in Wright's blood stain, whereas those of larger cells were basophilic (Dybas, 1981b).

Cells both larger and smaller than the granulocyte, but lacking granules (=hyalocytes), have been identified in the coelomic fluid of *Sipunculus nudus* and related to the granulocyte through intermediate stages (Valembois and Boiledieu, 1980). Large granulocytes with degenerating organelles are considered as intermediate between granulocytes and the large clear cells (Fig. 110). Cytoplasm of the latter contains vesicles, residual bodies, and a dense granular material that may be enclosed in vacuoles. Typically the nucleus is small, and mitochondria are swollen and degenerating. Small hyalocytes, on the other hand, are considered to give rise to the large granulocytes. Lacking cytoplasmic granules, they have a large, dense nucleus and a relatively small cytoplasmic component with well-developed mitochondria and numerous ribosomes and vesicles. Similar cells, but containing a few granules, are regarded as an intermediate stage (Fig. 111). In *Phascolosoma scolops*, Ochi (1975) also suggested that small "hyaline amebocytes" differentiate

into granulocytes; he further defined four types of granular cells with granules of different sizes and staining properties. Origin of the stem cells is uncertain, but earlier researchers have reported coelomocytes of various species of sipunculans to originate from the peritoneal cells lining the coelom, especially along the intestine and contractile vessel (Hérubel, 1907; Volkonsky, 1933; Ohuye, 1937, 1942).

The role of granulocytes in cellular defense has been well established by numerous in vivo and in vitro studies on the phagocytic activity of these cells (Cuénot, 1900; Hérubel, 1907; Cantacuzène, 1922; Volkonsky, 1933). An account of phagocytosis at the ultrastructural level is provided by Valembois and Boiledieu (1980) for *Sipunculus nudus* (Figs. 109, 110) and by Dybas (1981b) for *Phascolosoma agassizii*. Studies of the latter species demonstrated a breakdown or dissolution of the granules into phagocytotic vacuoles in which foreign material (e.g., polystyrene latex beads or bacteria) was trapped and at the same time a formation of new granules in the vicinity of the Golgi apparatus. Membranes of the granules fused with membranes of the phagosomes as granular contents were released into the phagosome, diffusing throughout the phagocytic vacuole. Granules reacted positively to tests for acid phosphatase, alkaline phosphatase, lipase, and peroxidase. As the same enzymes are known to be involved in digestion in vertebrates, the positive tests indicate a similar role by sipunculan granulocytes during phagocytosis.

Granulocytes are also capable of encapsulation of foreign material that is too large for phagocytosis. Studies have demonstrated encapsulation of a variety of material introduced into the coelomic cavity, e.g., autograph and homograph tentacle transplants in *Themiste zostericola* (Triplett et al., 1958) and homologous eggs treated by heating, staining, or sonification (Cushing and Boraker, 1975).

In addition to the cellular defense reactions of phagocytosis and encapsulation, numerous immunological responses have been reported
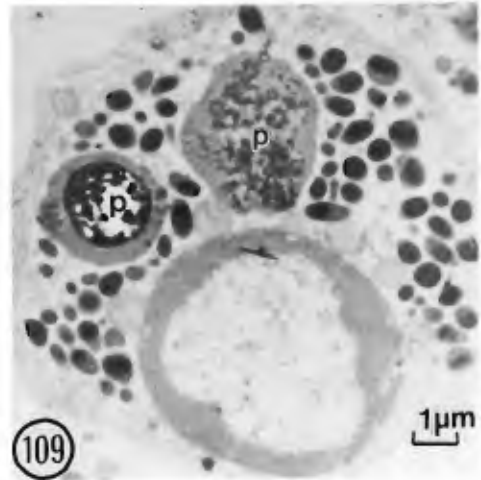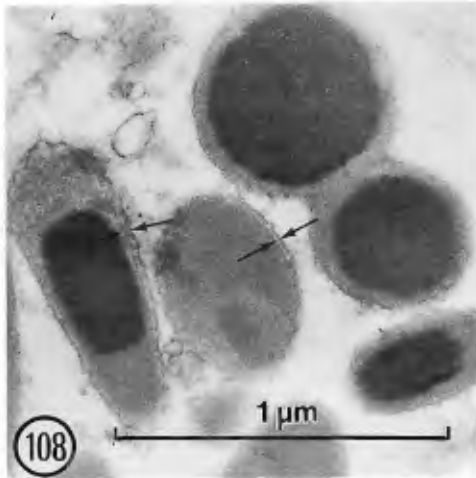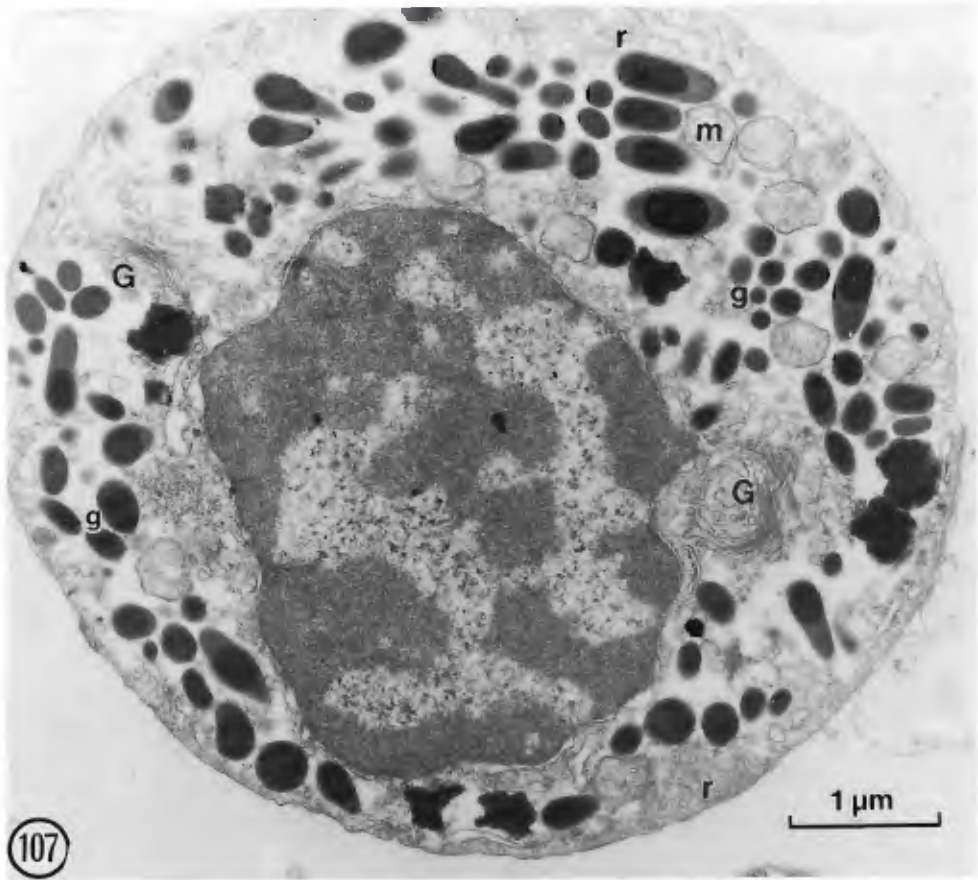
Figs. 107–109. Granulocytes of *Sipunculus nudus.* TEM.
(From Valembois and Boiledieu, 1980.)
Fig. 107.   Mature granulocyte. Characteristic dense granules
(g) may be of heterogeneous composition. Golgi bodies (G) are
surrounded by small vacuoles. m, mitochondrion; r, endoplas-
mic reticulum.
Fig. 108.   Enlargement of dense granules shows them to be
enclosed by two trilaminar membranes (arrows).
Fig. 109.   Granulocyte in process of phagocytosis of waste
particles (p).

Figs. 110, 111. Large and small "hyalocytes" of *Sipunculus nudus*. TEM. (From Valembois and Boiledieu, 1980.)
Fig. 110. Old granulocyte (large "hyalocyte") in which most dense granules and cytoplasmic organelles are absent, presumably lost. v, vesicular component; b, bacterium undergoing phagocytosis.
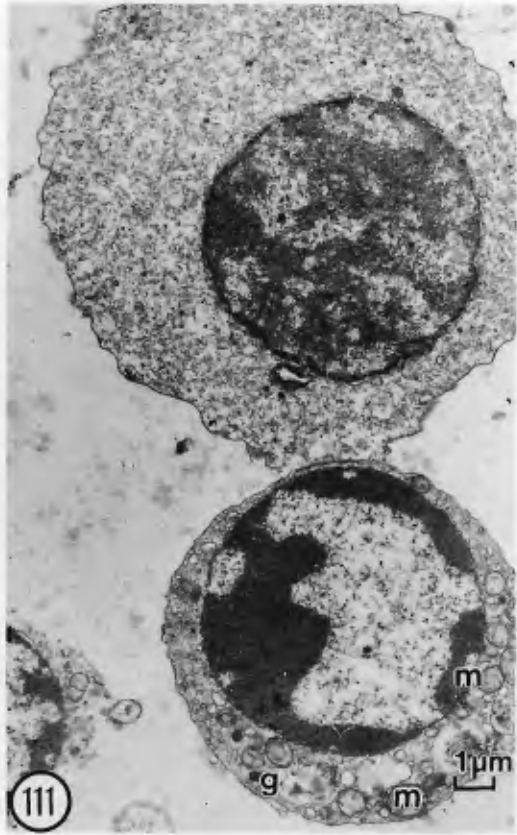
Fig. 111. Small "hyalocyte" (bottom cell), similar to transitional granulocytes but with few dense granules (g), has relatively large nucleus and numerous mitochondria (m). Cytotoxic activity follows contact with target cell, shown here as the upper cell, a xenogenic hemerythrocyte from *Sipunculus arcassonense*.

in sipunculans. These include the production, under experimental conditions, of lysins, agglutinins, and bactericidins (cf. Dybas, 1981b, for discussion). Although the cellular role in these responses is generally not known, in one instance immature granulocytes and stem cells (small hyalocytes) have been implicated in cytotoxic activities in *Sipunculus nudus*. Contact between these cells and foreign hemerythrocytes results in lysis of the latter (Boiledieu and Valembois, 1977; Valembois et al., 1980; Valembois and Boiledieu, 1980) (Fig. 111). Because of their morphology, particularly their large number of ribosomes and their "natural killing reaction," they have been compared with vertebrate lymphocytes by these authors.

In neither cellular nor humoral immune responses has an immunological memory been demonstrated. Thus the sipunculan defense system is relatively simple and as such is of interest as a model in the elucidation of the phylogeny of invertebrate and vertebrate defense mechanisms. The role of ciliated urns in defense is considered later.

Other functions that have been attributed to

granulocytes are involvement in waste removal and regeneration of musculature. Studies by Towle (1962) and Blitz (1965) have suggested that large inclusions of phagocytosed, undigested material are excreted by way of the nephridium in *Phascolosoma agassizii*. In ultrastructural studies, granulocytes are often found in the connective tissue of the nephridium (see the section on the excretory system). Moreover, in an electron microscopic investigation by Storch and Moritz (1970) on regeneration of the integument of *Phascolion strombus*, granulocytes were reported to give rise to the newly formed musculature of the body wall.

Large multinuclear cells occur in various forms among different species of sipunculans, but most usually they appear as multinucleated cell masses surrounding a vesicle (Fig. 100). Generally referred to as enigmatic vesicles, they have been reported to be as large as 540 μm in diameter for *Golfingia vulgaris*, but are more commonly 30–60 μm in diameter (Ochi, 1975). The vesicle, as revealed by ultrastructural studies, is a large, membrane-bound vacuole filled with fibrous material made up of fine, banded fibers (Ochi and Ohnishi, 1971; Ochi, 1975; Dybas, 1981a) (Fig. 112). The organelles, pushed to the periphery by the internal vacuole, consist of mitochondria, endoplasmic reticulum, glycogen particles, and a few electron-dense granules. Villi-like projections are characteristic of the surface membrane (Fig. 113). Dybas (1981a) also noted dense plaques resembling tonofilaments in enigmatic vesicles of *Phascolopsis gouldii*. The function of vesicular cells is unknown, but in preparations of living coelomocytes they are frequently in close association with clumps of other cells. Bang (1966) noted a breakdown or collapse of enigmatic vesicles in *Sipunculus nudus* associated with the appearance of lysins, induced by introducing bacteria or the parasitic ciliate *Anophrys magii* into the coelomic cavity.

Also classified with multinuclear cells are the cell pairs, described by Dybas (1975) in *Phascolosoma agassizii*. A cell pair, as shown by electron microscopy, consists of a cell within a cell, or a "granulocyte with associated cell" (GAC) (Fig. 114). Larger than other granulocytes, the cell pair measures from 26 × 15 μm to twice this length and width. Rarest of the coelomocytes in *Phascolosoma agassizii*, GACs number 1 for every 3 urns, 30 granulocytes, and 566 hemerythrocytes. The internal cell is a granulocyte in which the granules, appearing in a series of developmental stages, arise from the Golgi bodies. The outer cell consists of a thin rim of cytoplasm surrounding the inner granulocyte. Both cells have a complete array of organelles, the outer cell possessing also a few large granules similar to lipofuscin of vertebrates, as well as small granules. The extracellular space between the two cells averages 70 nm in width and is filled by a fibrous reticulum that may provide structural support for the cell complex. The particular function of this cell pair is not understood. Dybas has proposed that this unusual combination of cells might serve as a means of regulating the release of granules from the inner cell in response to certain conditions that would invoke the lysis of the outer supporting structure.

Free urns are unique multicellular ciliated bodies that move independently through the body cavity. Known to occur only in *Phascolosoma* and *Sipunculus*, they were thought at one time to be parasites (Krohn, 1851; Ohuye et al., 1961), but are now recognized as integral components of the circulating coelomocytes of these genera.

The only detailed ultrastructural study of urns is that of Dybas (1976) for *Phascolosoma agassizii*. The urn of this species is a disc-shaped complex composed of three cell types (ciliated cells, "cupola" cells, and lobe cells) arranged around a central semilunar extracellular space (Figs. 115–119). The overall size is reported to be 57 × 61 μm. The concavity of the urn is rimmed by two semicircular or crescent-shaped ciliated cells and filled by two granular cupola cells. Attached around the convex surface of the internal semilunar
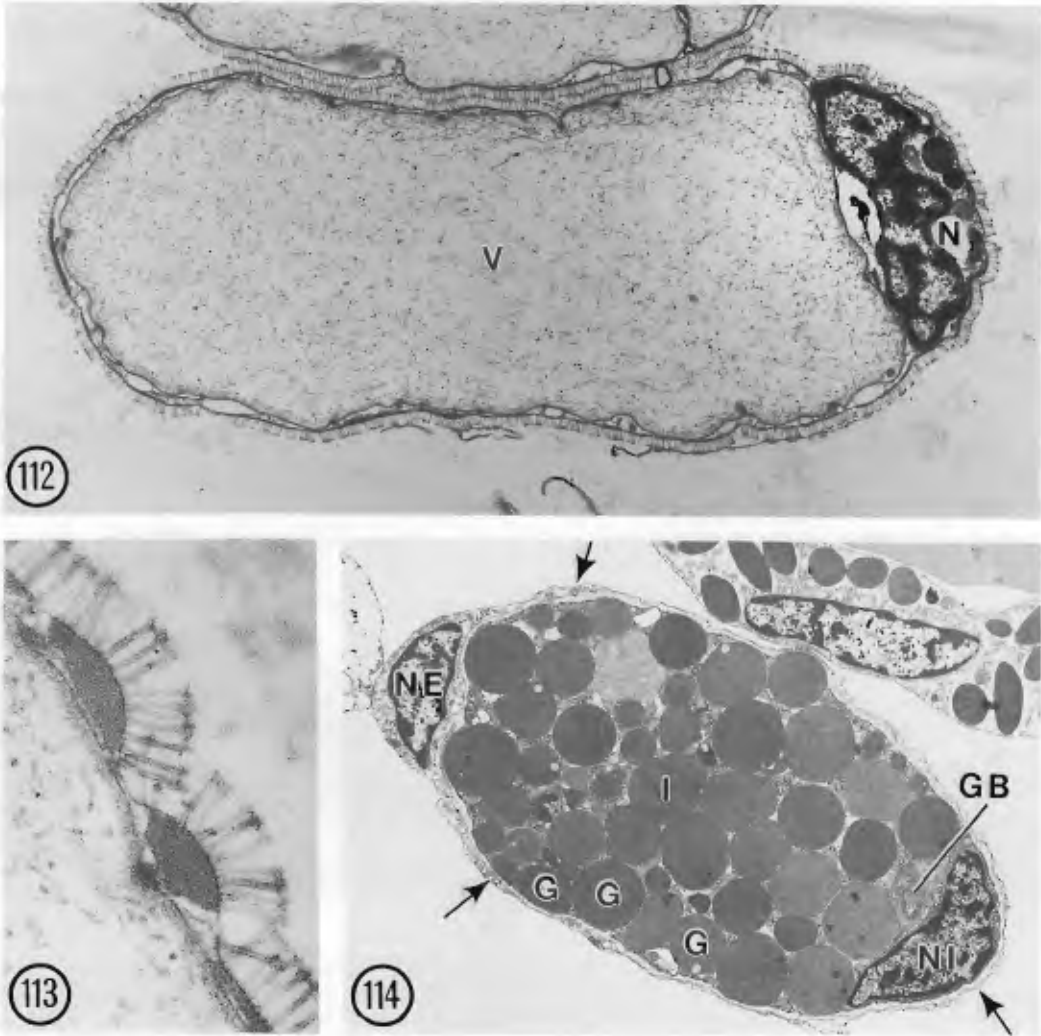
Figs. 112–114. Enigmatic vesicle of *Sipunculus nudus*. TEM. (Micrographs for Figs. 112, 113, courtesy of L. Dybas.)
Fig. 112. Note large vesicle (V) filled with fibrous material and one of probably several nuclei (N). ×7,617.
Fig. 113. Enlargement of external membrane of enigmatic vesicle showing villi-like projections. ×17,572.

Fig. 114. Cell pair (cell within a cell) or granulocyte with associated cell (=GAC) of *Phascolosoma agassizii*, ×9,660. G, granules of inner cell; GB, Golgi body of internal cell; I, internal cell; NE, nucleus of external cell; NI, nucleus of internal cell. Arrows point to cytoplasm of external cell. (From Dybas, 1975.)

space are 6–12 lobe cells. The extracellular space is occupied by a meshwork of collagen-like fibrils that have a regular periodicity but no banding (Fig. 118). Presumably this arrangement provides structural support for the urn. The two cupola cells that fit side by side into the semilunar depression are separated from the semilunar extracellular cavity as well as from each other and the surrounding ciliated cells by a narrow space, lacking desmosomal or junctional connections. Each cupola cell has a prominent nucleus, mitochon-
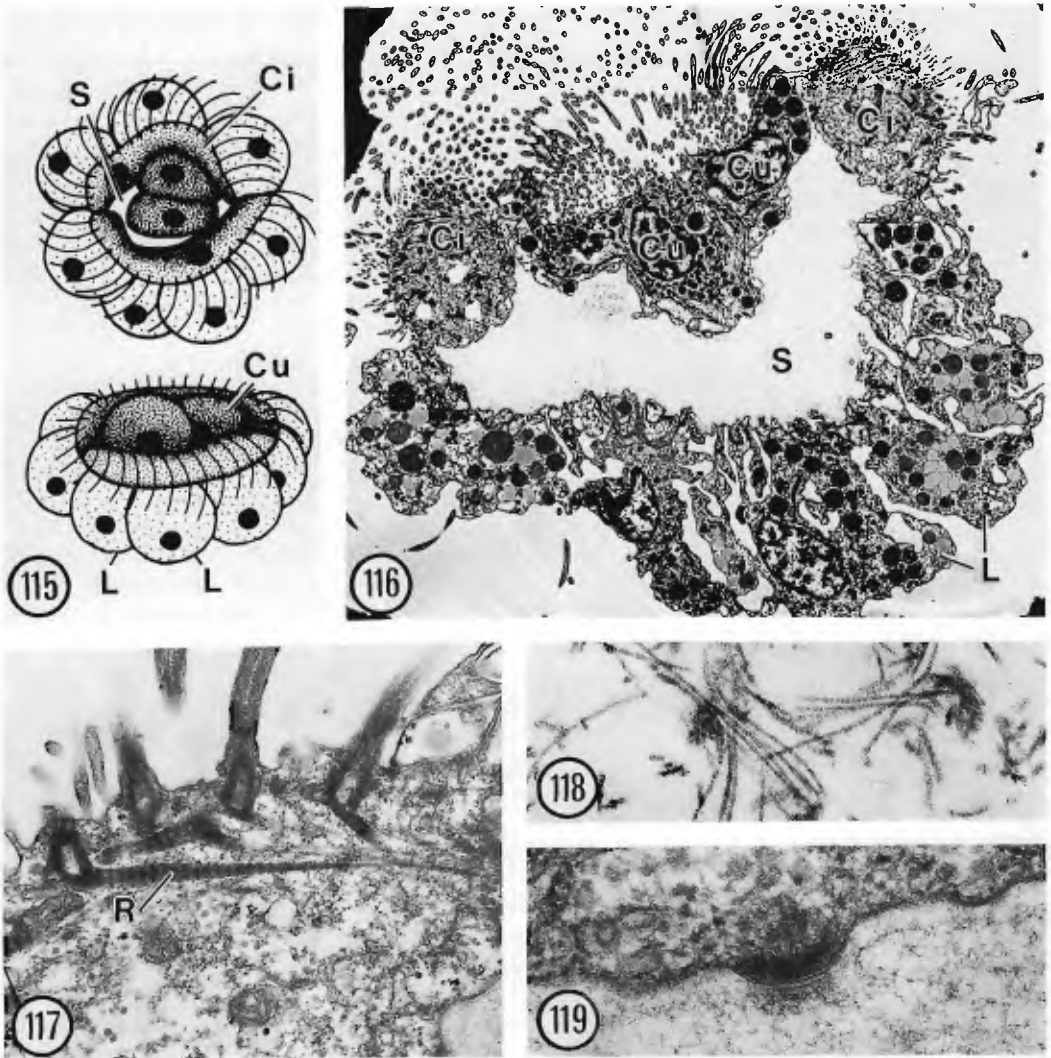
Figs. 115–119.   Coelomic urns of *Phascolosoma agassizii*. Ci, ciliated cell; Cu, cupola cell; L, lobe cells; R, rootlet; S, semilunar extracellular area. (Fig. 115, redrawn from Dybas, 1981a; Figs. 116–119, TEMs, from Dybas, 1976.)
Fig. 115.   Diagrammatic representation. Upper, apical view; lower, lateral view.
Fig. 116.   Sagittal section through entire urn. ×6,500.

Fig. 117.   Enlargement of ciliary cell, showing ciliary rootlet traversing cell to point of hemidesmosomal attachment in semilunar cavity. ×22,750.
Fig. 118.   Fibrillar material in extracellular semilunar area. ×44,571.
Fig. 119.   Hemidesmosome attaching ciliated cell and semilunar area. ×66,461.

dria, lipid droplets, glycogen, and numerous, mostly rod-shaped granules. The ciliated cells contain many mitochondria, rough endoplasmic reticulum, lipofuscin-like bodies, and glycogen (Fig. 116). The base of these cells has a strong hemidesmosomal attachment to the semilunar area (Fig. 119). Characteristic

of the hemidesmosome is a trilaminar membrane with an inner dense plate for tonofilament insertion and an extracellular plate of fine filaments. Tonofilaments run from the apical ciliary basal bodies toward the hemidesmosomes (Fig. 117). The bases of the lobe cells, on the opposite side of the semilu-

nar area, are attached to the extracellular material by numerous hemidesmosomes. Variable in size and number, the lobe cells contain lipid droplets, granules, Golgi bodies, mitochondria, glycogen, rough endoplasmic reticulum, smooth endoplasmic reticulum, and numerous vesicles (Fig. 116). These have been considered as chlorogogue cells by some authors (Selensky, 1908; Hyman, 1959).

The urn of *Phascolosoma agassizii* functions to keep the coelomic cavity free of foreign particulate matter and cellular debris. Each part of the structure plays a specific role in this process. Whereas the fibrous extracellular semilunar area provides structural integrity, the ciliated cells provide motility within the coelomic fluid and the capacity for trapping particles. Both the cupola cells and the lobe cells are phagocytic and, as Dybas (1976) demonstrated, they may have phagocytic specializations. For example, Dybas showed that cupola cells phagocytized latex particles, while lobe cells phagocytized carbon particles.

The free urn of *Sipunculus nudus* has attracted considerable interest among biologists over the last century and has been the subject of numerous morphological and physiological studies. The term urn was, in fact, derived from the shape it assumes in this species. The urn of *Sipunculus nudus* consists of a large vesicular cell with a short neck attached to a basal, saucer-shaped cell, the outer convexity of which is ciliated (Figs. 120–122). The diameter is 65–75 μm (Bang and Bang, 1980). In brief preliminary reports on ultrastructure, without photographic documentation, a third cell type was described as a small, secretory cell, designated as the *R cell*. Occurring in clusters, these cells are attached by junctional complexes to the central nonciliated area of the external membrane of the base cell (Reissig et al., 1979; Bang and Bang, 1980). The vesicle cell is thinly stretched to form a hollow dome, the base of which is attached by junctional complexes to the upper rim of the mucociliated base cell of the urn complex (Fig. 122).

In the coelomic cavity the urns swim with anterior vesicle forward, propelled by the posterior ciliated cell, and trailing a strand of mucus with entrapped debris. There are two separate secretory systems: One is located at four to five loci around the inside of the ciliated rim of the base cell, and the other is the central secretory R cells. The latter secrete a mucus that traps cell debris and foreign particulate matter, whereas the former secrete a more rapid and copious flow of mucus, producing a mucous tail six to seven times the length of the urn, in response to specific stimuli such as bacteria or other substances. Further evidence for the two types of mucus is found in the differing responses to vital stains, the R cells staining with neutral red and the basal cell foci staining with Janus green B (Bang and Bang, 1980). Experimental induction of hypersecretion of urns of *Sipunculus nudus* by exposure to various bacteria or foreign sera in vivo and in vitro has been reported in a series of papers by Bang and Bang (1962, 1965, 1972, 1975, 1980) and Nicosia (1979). These authors point to the advantages of using this relatively simple, noninnervated system to investigate fundamental processes in regulation and production of mucus and to establish a model for analysis of invertebrate and vertebrate mucus-stimulating substances in human health and disease.

Although free urns have been reported in only two genera (*Phascolosoma* and *Sipunculus*), fixed urns are known to occur in the peritoneal lining of many genera, including those with free urns (Hyman, 1959). Fixed urns have the same basic cellular components as free urns, but remain attached to the peritoneum (Figs. 123, 124). It is generally thought that fixed urns, at least in *Sipunculus nudus* in which they have been most studied, give rise to free urns (Metalnikoff, 1900; Selensky, 1908).

Tentacular System

Sipunculans do not possess a blood vascular system; however, the tentacular system, a second coelomic compartment, serves an analogous function. In contrast to a blood vascular system in which vessels are character-
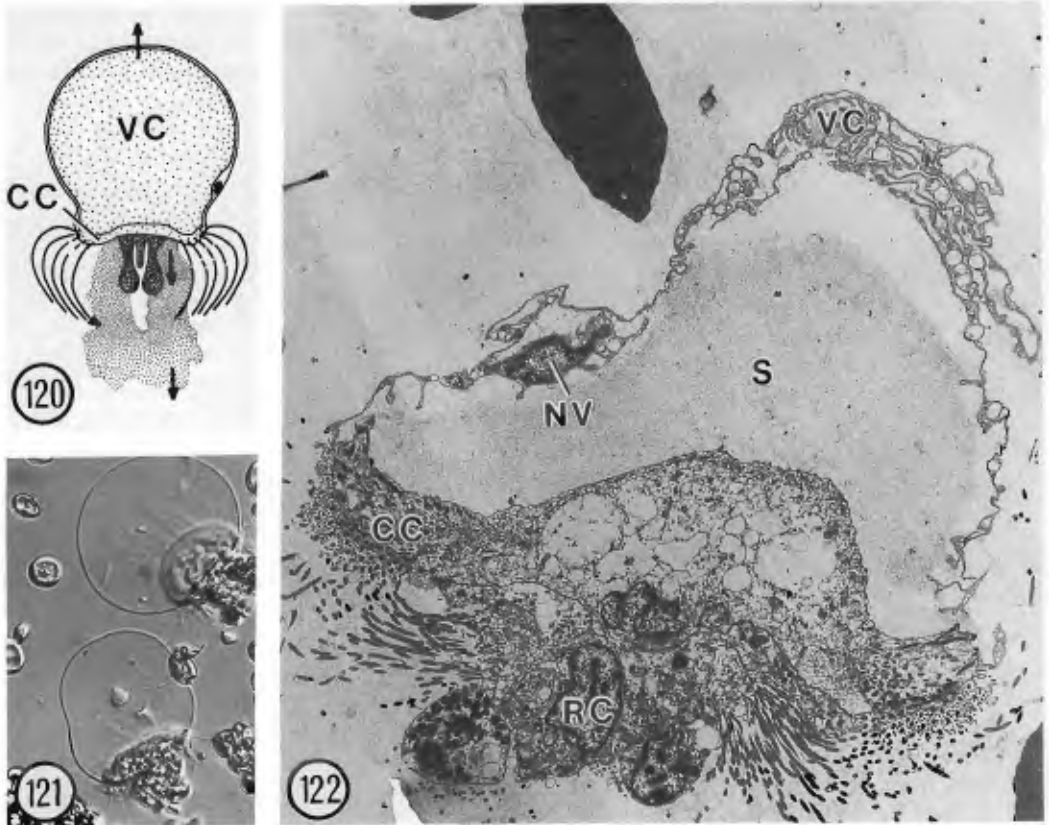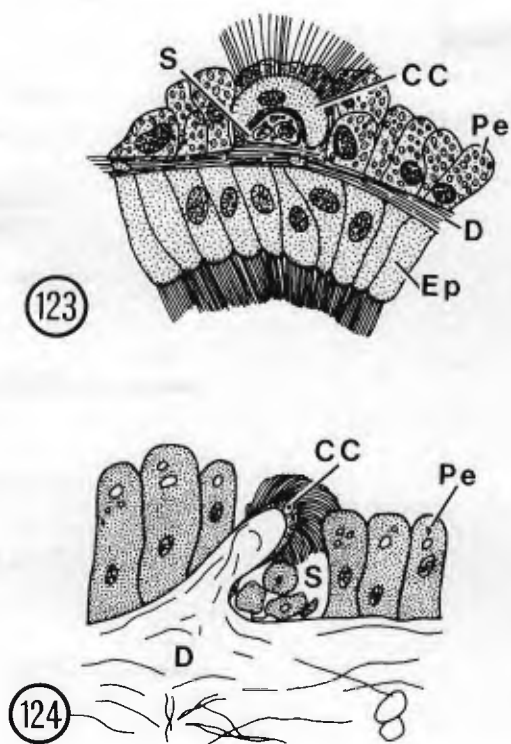
Figs. 120–122. Coelomic urns of *Sipunculus nudus*.
Fig. 120. Diagrammatic representation of urn of *Sipunculus nudus*. The urn is comprised of a large vesicular cell (VC) joined to a basal ciliated cell (CC). Upper arrow indicates direction in which urn swims; lower arrows point to direction of flow of mucus secretion. Outer and more voluminous mucous flow is from foci on rim of ciliated cell; central, less extensive mucous flow is from small "R" cells (see Fig. 122) at center of basal cell. (Modified from Bang and Bang, 1980.)

Fig. 121. Urns in coelomic fluid from living *Sipunculus nudus*. Nomarski optics. ×255.
Fig. 122. Sagittal section through urn of *Sipunculus nudus*. TEM. Note nucleus (NV) of vesicular cell (VC) and thin cytoplasmic layer surrounding a large extracellular space (S). Attached to the external membrane of the basal ciliated cell (CC) is a cluster of small secretory "R" cells (RC). ×2,000. (Courtesy of L. Dybas.)

ized by noncellular lining, the tentacular system is lined by peritoneum. The system consists of tentacular canals that are continuous with a circumesophageal ring from which one or two elongate sacs, frequently with smaller diverticula, project posteriorly into the body cavity (Fig. 125). Unlike a blood vascular system, the tentacular system does not extend throughout the body, but is generally limited to the tentacles and the length of the esophagus to which the sac-like append-

ages are attached. These sacs have been reported to expand with fluid when the tentacles contract and to pass fluid back into the tentacles when the latter expand (Hyman, 1959). They have also been reported to contract independently. Various terms used in the literature for these appendages include compensation sacs (Hyman, 1959), contractile vessels (Stephen and Edmonds, 1972), blood vessels (Metalnikoff, 1900; Awati and Pradhan, 1936), and polian vessels (Tetry, 1959).

Fig. 125.   Tentacular system of *Phascolosoma granulatum*.
Compare with Figure 81, SEM of head of *Phascolosoma per-
lucens*. CV, contractile vessel; D, dorsal side; M, mouth region;
RC, ring canal; TC, tentacular canals; V, ventral. (Redrawn
from Selenka et al., 1883, and reconstructed from cross and
longitudinal sections.)

Figs. 123, 124.   Illustrations of fixed urns. CC, ciliated cell;
D, dermis or connective tissue; Ep, epidermis of intestine; Pe,
chlorogogue cells of peritoneum; S, extracellular space or cavity
of urn with enclosed cellular debris.
Fig. 123.   Frontal section of fixed urn in intestinal wall of
Aspidosiphon muelleri. (Redrawn from Selensky, 1908.)
Fig. 124.   Sagittal section of fixed urn in the peritoneum of
Golfingia vulgaris. (Redrawn from Hérubel, 1907.)

Pilger (1982) provided the first definitive
ultrastructural evidence for the coelomic na-
ture of sipunculan tentacular canals. He de-
scribed three canals in the tentacle of
Themiste lageniformis: two smaller oral ca-
nals and one larger aboral canal (Fig. 126).
The oral canals connect with the aboral canal
at the tip of the tentacle. As reported, the
canals are completely lined by squamous peri-
toneal cells, joined to one another by zonulae
adherentes. In some areas the lining may be
only the width of two membranes of a single
cell, and in other areas it is thicker, consisting
of several layers of membranes of overlap-
ping cells. In areas of nuclei or cytoplasmic
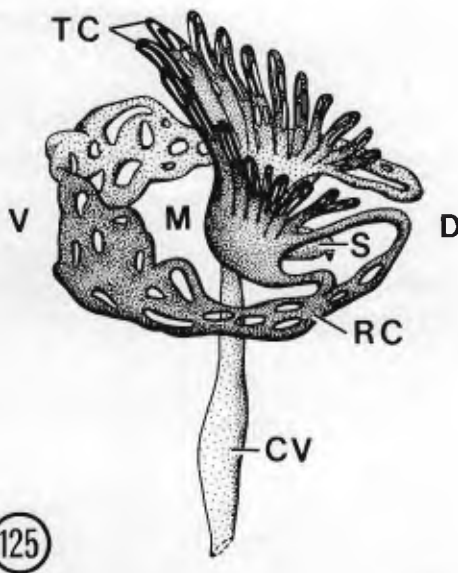organelles, the peritoneum widens to accom-

modate these structures. Intermittent perito-
neal cells bear tufts of cilia and microvilli
(Fig. 127). Hemerythrocytes and occasion-
ally granulocytes are present within the lu-
men. The direction of flow in the tentacular
canals has been described by Awati and Prad-
han (1936) as anterior from the esophageal
ring in the oral canals and posterior back to
the esophageal ring in the aboral canal
(Fig. 126).

The contractile vessel extends along the
dorsal surface of most of the length of the
esophagus and is connected to it by a continu-
ity of the connective tissue layers of the two
organs (Figs. 128–130). (In a few species
there is also a ventral vessel). In certain
genera (e.g., *Antillesoma*, *Siphonosoma*,
Themiste) the contractile vessel is distin-
guished by numerous extensions or villi that
may be elaborately developed, particularly in
the genus *Themiste* (Figs. 2, 13, 128, 130).
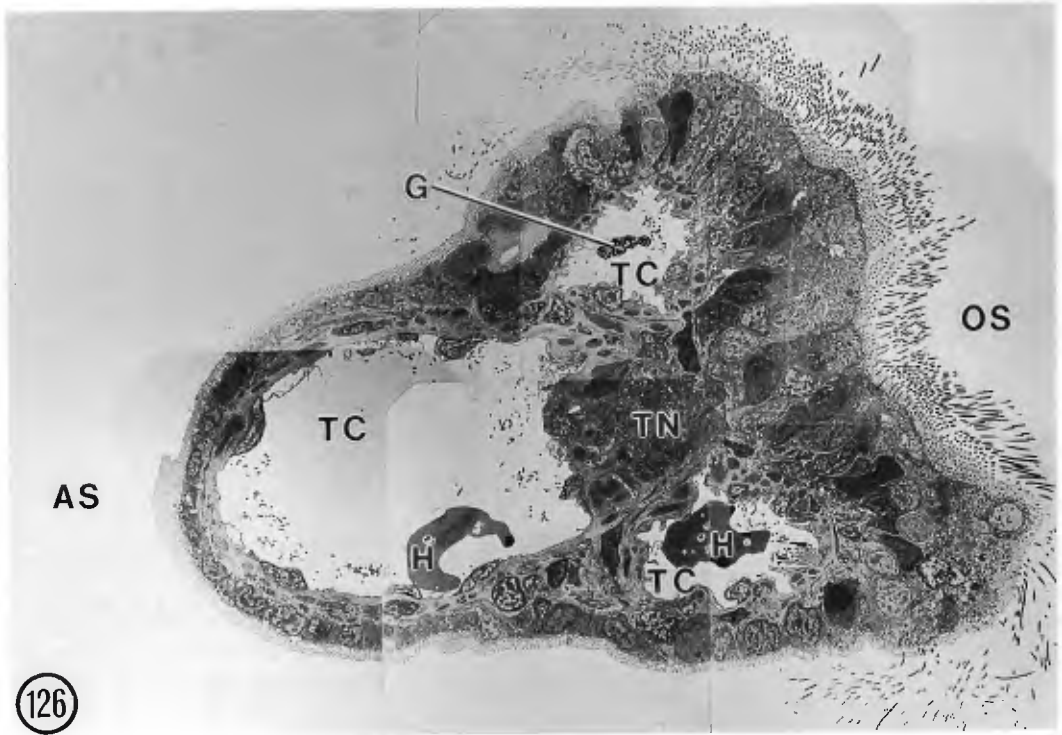Both the contractile vessel and its extended

Fig. 126.   Electron microscopic montage of a cross section of the tentacle of *Themiste lageniformis*. Oral surface (OS) has a ciliated epidermis in contrast to the nonciliated epidermis of the aboral surface (AS). Granulocytes (G) and hemerythrocytes (H) occur within the coelomic space of the three tentacular canals (TC). Note tentacular nerve (TN). ×1,165. (From Pilger, 1982.)

villi are commonly packed with hemerythrocytes (Fig. 130). The wall of the contractile vessel consists of connective tissue bordered by outer and inner epithelial layers of typical peritoneal configuration (Figs. 131, 132). Cytoplasmic processes of the outer peritoneal covering may stretch out and interdigitate over the surface of the contractile vessel (Fig. 129). The resemblance of these cells to podocytes has been noted in *Themiste lageniformis* (Pilger and Rice, 1987) and *Themiste alutacea* (Pinson, 1990). Ciliated cells are scattered throughout both inner and outer peritoneal layers. Cytoplasmic organelles are sparse except in the inner ventral peritoneum near the attachment to the esophagus, where the cells are thicker and have numerous lysosome-like granules, vesicles, mitochondria,

and ribosomes. The connective tissue layer is made up of fine, striated fibers of undetermined periodicity, among which are scattered muscle cells (Figs. 131, 132).

Size and structural differences have been reported between hemerythrocytes in the main coelomic cavity and those in the tentacular system. Smaller size has been noted for hemerythrocytes in the contractile vessel of *Themiste zostericola* (Manwell, 1960). In *Themiste dyscrita* the diameters of hemerythrocytes in the contractile vessel have been recorded as 16–21 µm and in the primary coelom as 19–32 µm (Terwilliger et al., 1983, 1985). Ultrastructural distinctions in hemerythrocytes of the contractile vessel in *Themiste dyscrita* are the presence of more small vacuoles and a darker, more electron-
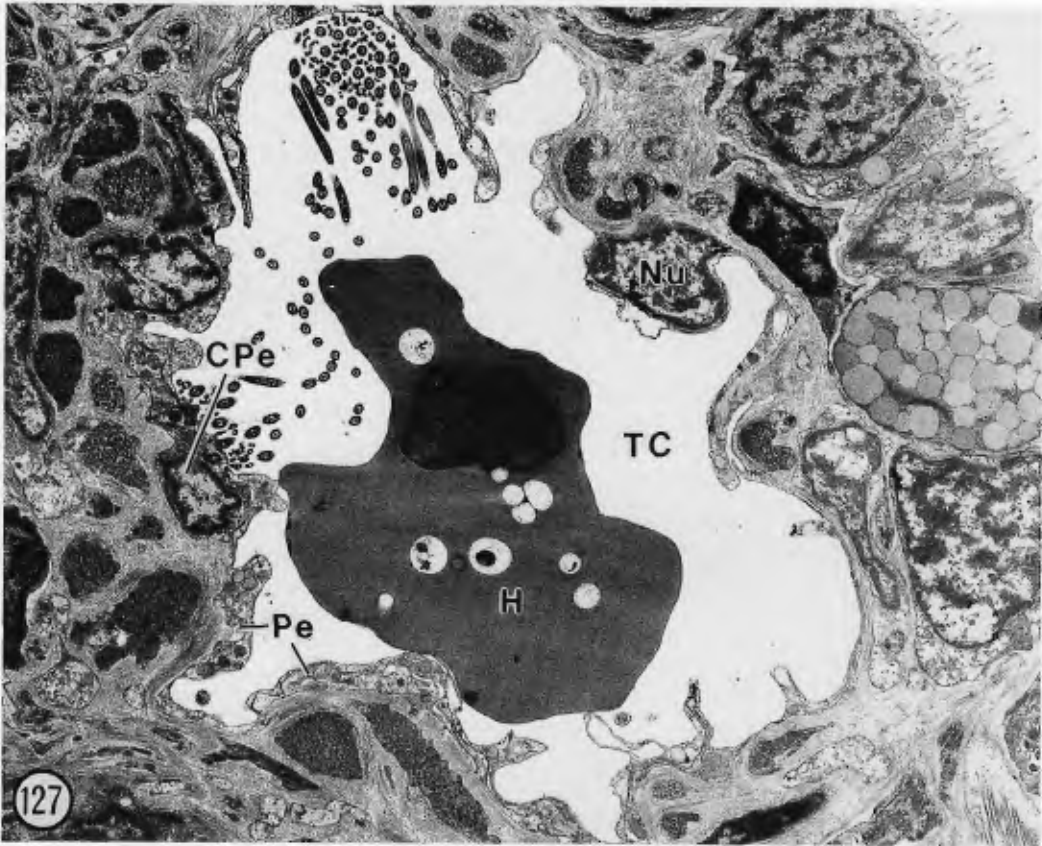
Fig. 127.   Cross section of tentacular coelomic canal (TC) of *Themiste lageniformis*, showing ciliated (CPe) and nonciliated peritoneal cells (Pe) lining the canal, nucleus (Nu) of peritoneal cell, and enclosed hemerythrocyte (H). TEM. ×5,225. (From Pilger, 1982.)

dense cytoplasm that lacks membranous lamellae and tubules (cf. Fig. 102). Differences have also been reported in the hemerythrins, as noted below. Whether these differences between cells in the two sites represent independent populations of hemerythrocytes or different stages in differentiation remains to be determined. The possibility of hemerythrocytes passing through the wall of the contractile vessel, as observed by Metalnikoff (1900) and the author (unpublished), must also be considered. In *Phascolosoma varians* somewhat similar ultrastructural variation has been found among hemerythrocytes within the contractile vessel (Fig. 131). The more typical hemerythrocytes have the greatest cytoplasmic electron density, degenerating mitochondria, and few other organelles, while others have a less dense and more granular cytoplasm that contains normal mitochondria and lysosome-like granules.

The primary function of the hemerythrocytes of both the tentacular system and the main coelomic cavity is oxygen transport. The respiratory pigment contained within these cells is hemerythrin, an iron-containing protein that lacks a heme group, but has instead a diiron site that reversibly binds one molecule of oxygen (=one subunit). It is found in four invertebrate phyla: Sipuncula,
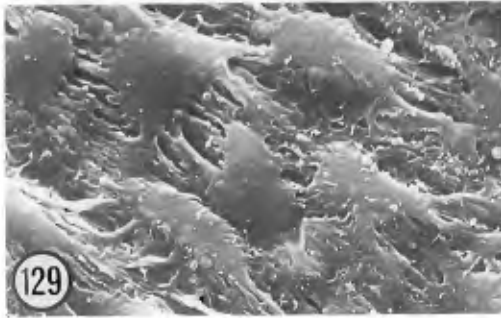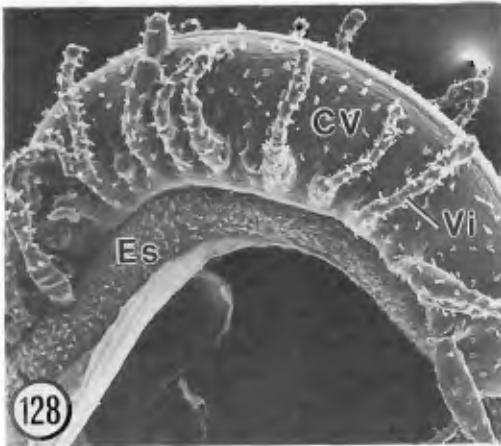
Figs. 128–130. The contractile vessel of *Themiste lageni-formis.* SEM.
Fig. 128. Contractile vessel (CV) with villi (Vi) and underlying esophagus (Es). ×70.
Fig. 129. Enlargement of surface showing podocyte-like cells with interdigitating cytoplasmic extensions. ×1,975.
Fig. 130. Enlargement of tips of villi (Vi), broken off to expose enclosed hemerythrocytes (H). ×474.

Priapula, Brachiopoda, and one genus, *Magelona*, of polychaetous annelids (for recent reviews of the large literature on molecular

structure and function of hemerythrin, see Wilkins and Wilkins, 1987, and Kurtz, 1992.) Although most sipunculan hemerythrins are octomers (eight subunits), with a molecular weight of 100,000–110,000, some may have monomeric, trimeric, or tetrameric structural subunits and molecular weights of 14,000, 40,000, and 56,000 (Klippenstein, 1980; Terwilliger et al., 1985). There are altogether three kinds of hemerythrins in sipunculans: myohemerythrin in the muscle (a monomer with a molecular weight of 14,000), coelomic hemerythrin in hemerythrocytes of the main coelomic cavity, and so-called vascular or tentacular hemerythrin in the hemerythrocytes of the tentacular system. They are distinguishable by electrophoresis, peptide mapping, and amino acid composition and sequence (Klippenstein, 1980). Coelomic and tentacular hemerythrins differ also in oxygen affinity (Manwell, 1960). In *Themiste zostericola*, a species with highly developed tentacles that afford a large surface area for respiration, the coelomic hemerythrin has a higher oxygen affinity than the tentacular. This suggests that the path of oxygen transport is from the external seawater through the tentacular system to the coelom. In *Siphonosoma ingens*, on the other hand, the reverse is the case: the coelomic hemerythrin has a lower oxygen affinity than the tentacular, suggesting a transfer from seawater through the body wall to the coelom and from the coelom to the tentacular system. This is a species that has coelomic outpocketings into the body wall, resulting in a thin integumental barrier between coelom and the external environment. Moreover, *Siphonosoma* lives burrowed in the sand, whereas *Themiste* lives under rocks, frequently extending the tentacular crown for protracted periods. Thus oxygen transfer can be correlated with the molecular characteristic of hemerythrin, body structure, habitat, and behavior. Other functions that have been proposed for hemerythrocytes include glycogen storage and transport and steroid hormone synthesis (cf. Terwilliger et al., 1985).
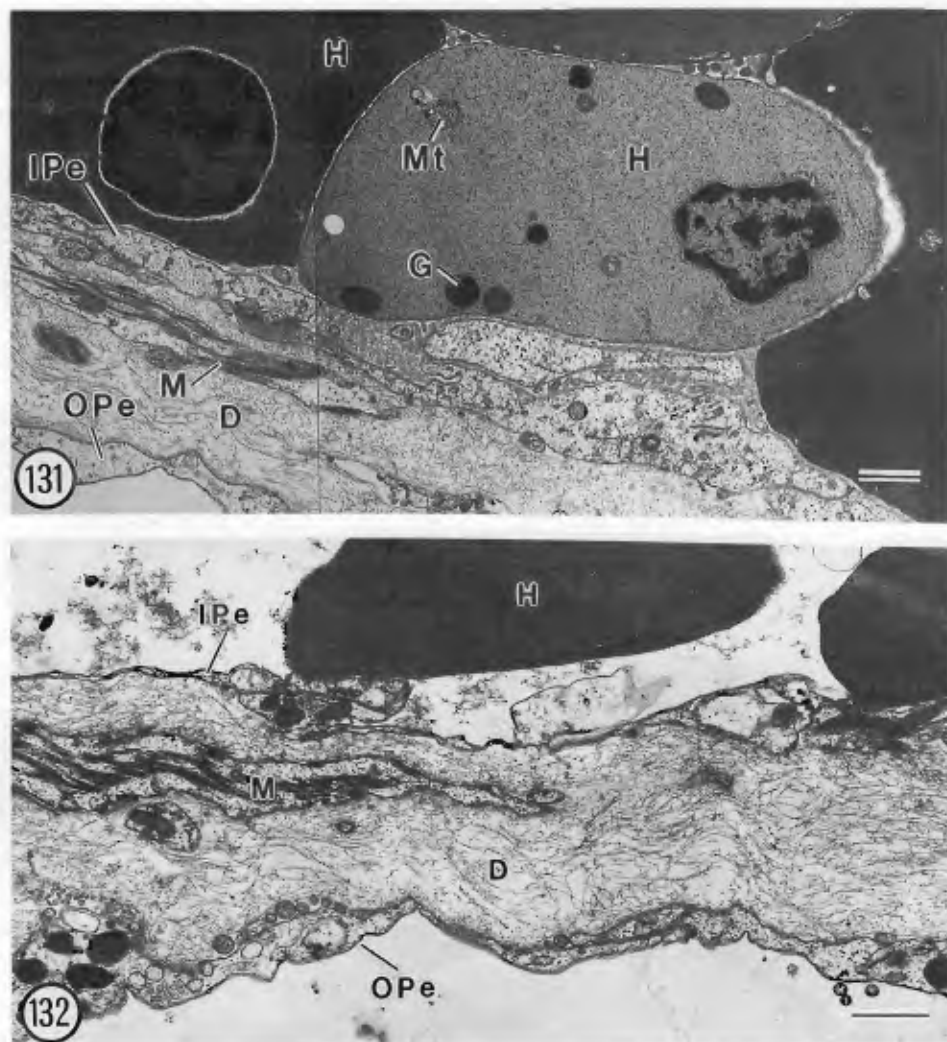
Figs. 131, 132.   Contractile vessels of *Phascolosoma varians* (Fig. 131) and *Aspidosiphon steenstrupii* (Fig. 132). TEM. Note similarity in construction. The wall of the vessel is composed of outer (OPE) and inner (IPe) peritoneal layers on either side of a dermis (D) of fine fibrillar material traversed by muscle fibers (M). Hemerythrocytes (H) occur within the lumen of the vessel. In Figure 131 there are two types of hemerythrocytes: one with dense matrix and degenerating organelles and the other with less dense cytoplasm, mitochondria (Mt), and dense granules (G). Bars = 1 μm.

EXCRETORY SYSTEM

The nephridia are the primary excretory organs of sipunculans, but, in addition, they serve in the uptake and storage of gametes prior to spawning and they have been implicated in the production of hormones and in osmoregulation. Structurally sipunculan nephridia conform to the definition of metanephridia. They are elongate sacs with two anterior openings: a ventrolateral nephridiopore opening to the exterior and a nephrostome, an internal ciliated funnel, opening to the coelomic cavity (Fig. 133). The anterior portion of the nephridium is often dis-
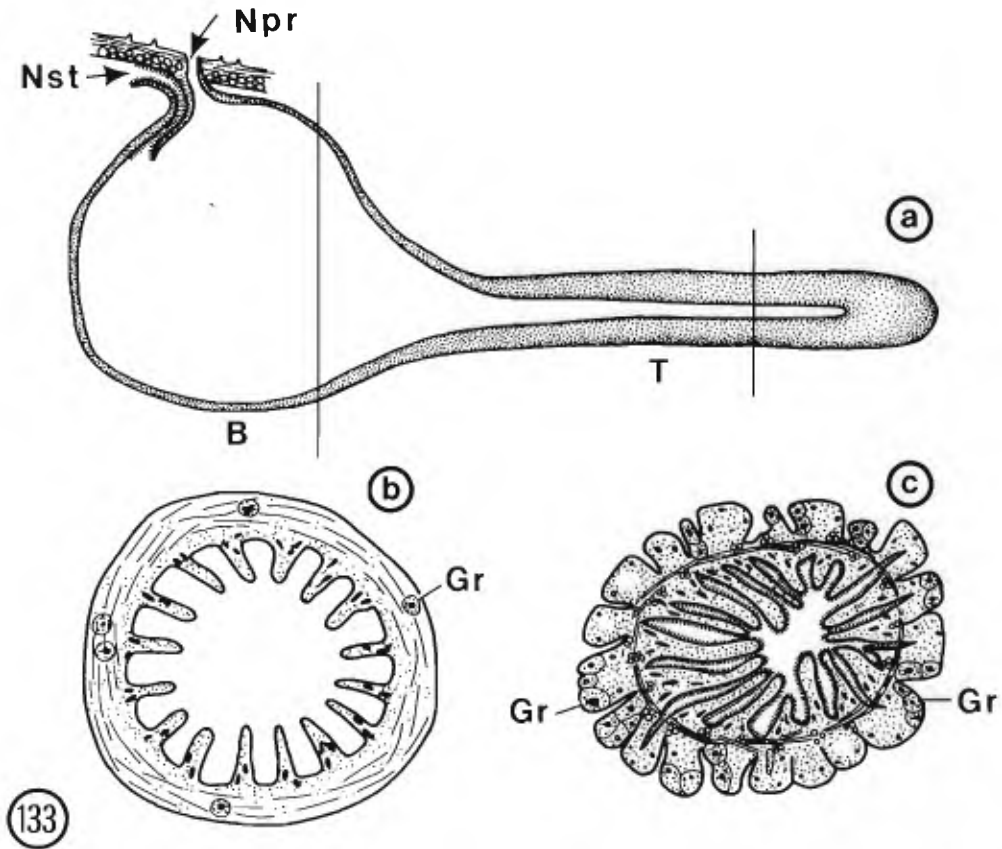
Fig. 133. Diagrammatic representation of the nephridium of *Phascolosoma granulatum*. **a:** Sagittal section shows two regions: a distended anterior region or "bladder" (B) and an elongate posterior tubular region (T). A nephrostome (Nst) opens to the coelom and a nephridiopore (Npr) opens to the exterior. **b,c:** Cross sections through the bladder region and tubular regions, respectively, showing an increased folding in outer and inner walls of the latter. Granulocytes (Gr) are common throughout the wall of the nephridium. (Redrawn from Serrano et al., 1990–91.)

tended by fluid and is sometimes designated as the bladder. The inner and outer walls of the bladder do not show the deep folds that are frequently present in the more posterior nephridial tube (Fig. 133). With the exception of species of *Phascolion* and *Onchnesoma*, which possess only a single nephridium, nephridia occur as one pair (Figs. 2, 10–15).

Information is available on the ultrastructure of three species: *Phascolosoma arcuatum* (=*Phascolosoma lurco*) (Storch and Welsch, 1972), *Phascolosoma granulatum* (Ocharan, 1974; Serrano and Moya, 1982; Serrano, 1987; Serrano et al., 1989, 1990, 1990–91),

and *Themiste alutacea* (Pinson, 1990). The nephridial wall consists of three layers: an inner epithelial lining, a middle connective tissue layer with muscles, and an outer peritoneal epithelium (Figs. 134, 135). The inner lining of the nephridial tube is a simple epithelium with apical and basal specializations (Figs. 135–139). It is columnar in *Phascolosoma granulatum* and *Themiste alutacea* and cuboidal in *Phascolosoma arcuatum*. Apically the plasmalemma bears cilia and microvilli, and basally it exhibits extensive infoldings (Fig. 134a). In *Themiste alutacea*, coated and uncoated pits occur on cell sur-
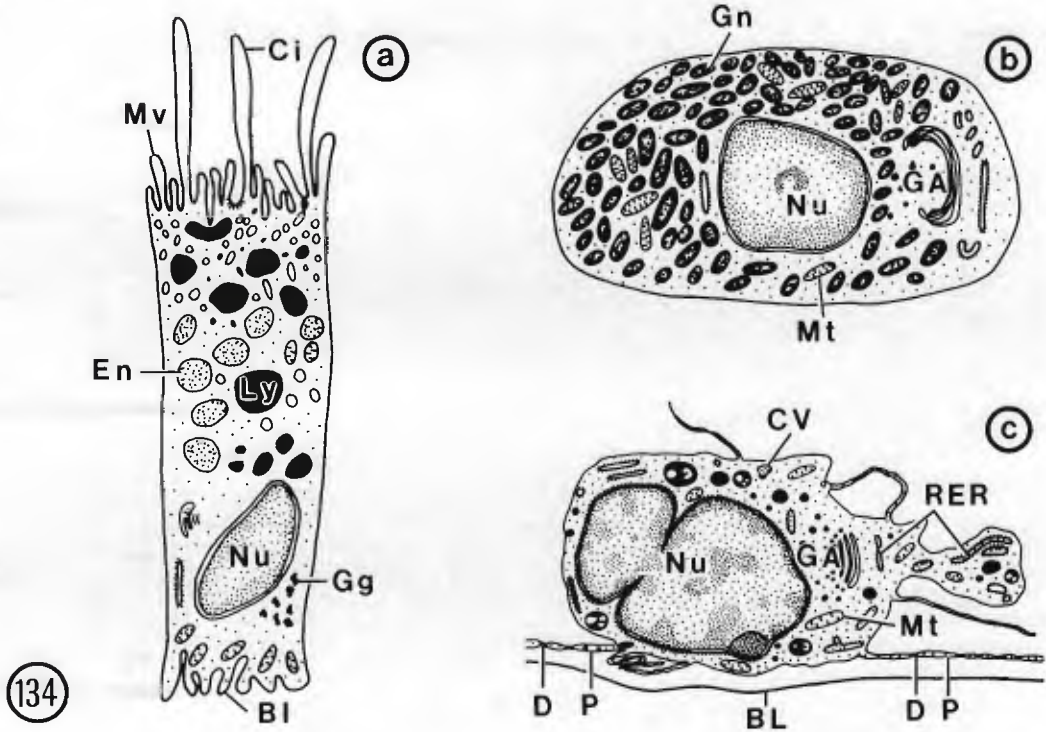
Fig. 134. Diagrams of individual cell types in the nephridial wall of *Phascolosoma granulatum*. **a**: Epidermal cell from the inner lining. **b**: Granulocyte from connective tissue layer. **c**: Podocyte from outer peritoneal layer. Bl, basal infoldings; BL, basal lamina; Ci, cilium; CV, coated vesicle; D, diaphragm; En, endosome; GA, Golgi apparatus; Gg, glycogen; Gn, granule; Ly, lysosome; Mt, mitochondria; Mv, microvilli; Nu, nucleus; P, pedicel; RER, rough endoplasmic reticulum. (a and b, redrawn from Serrano, 1987; as reproduced by Pinson, 1990; c, redrawn from Serrano et al., 1989.)

faces, and within the apical cytoplasm are both coated and uncoated vesicles, tubules, endosomes, mitochondria, and putative lysosomes (Figs. 135–139). Characteristic of transporting epithelia, apical cellular junctions are zonulae adherentes, followed by septate desmosomes. Within the numerous folds of basal cytoplasm are many mitochondria and some neurite-like processes. Glycogen is abundant in *Phascolosoma arcuatum* and *Phascolosoma granulatum*, but less prominent in *Themiste alutacea*.

Enmeshed within the loosely arranged fibrous material of the connective tissue layer is a conspicuous array of smooth muscle cells and a number of granulocytes (Figs. 134b, 135, 140). The muscle fibers of *Themiste alu-*

tacea are described by Pinson (1990) as composed of thick and thin filaments in a ratio of about 1:15, the former 35–55 nm in diameter and the latter 5–9 nm. Dense bodies are distributed among the filaments and along the sarcolemma. T-shaped junctions are seen in the sarcolemmal membrane, but no neuromuscular junctions. In a separate study of nephridial muscles of *Phascolosoma granulatum*, Serrano et al., (1990) reported thick myofilaments of three different diameters: about 28, 42, and 58 nm. Based on the distributions of these filaments of differing sizes, three classes of muscle cells were identified: 1) cells with all three sizes of myofilaments; 2) cells with filaments of 28 and 42 nm diameters; and 3) cells with filaments of an average

Lu

Nu

Bl

Mu

Mu

CT

Pe

2 µm

TC

135
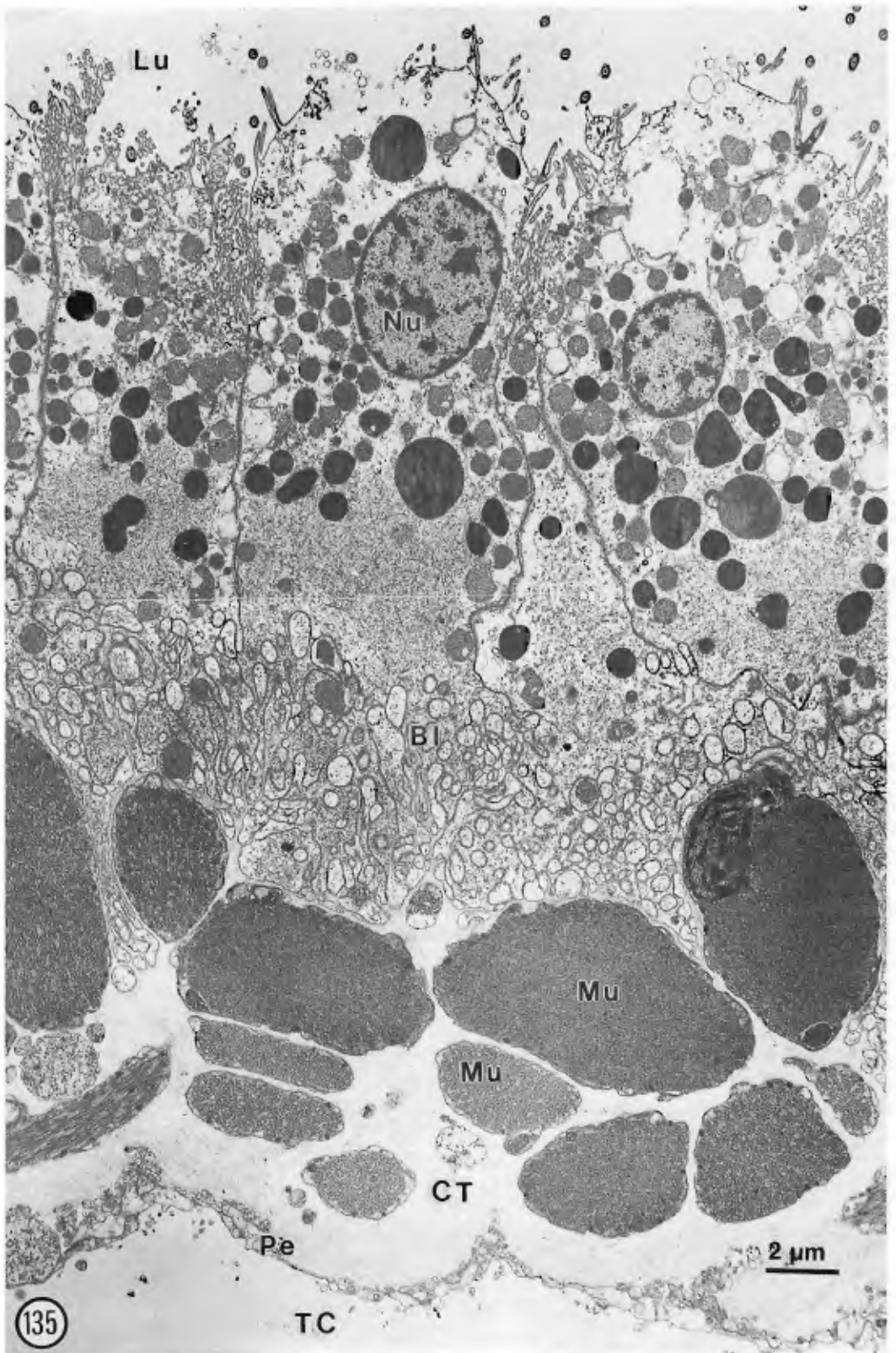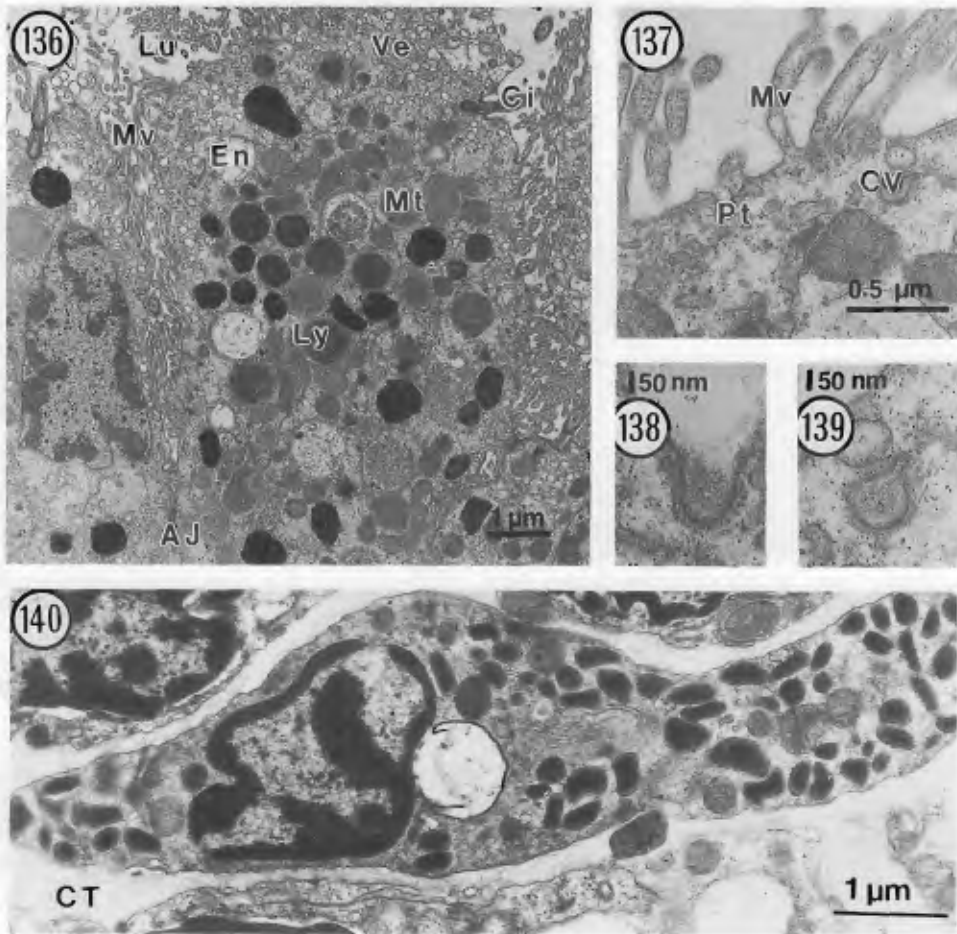
Figs. 136–139. The epithelium lining the nephridial tube of *Themiste alutacea*. TEM. (From Pinson, 1990.)
Fig. 136. Apical region of the epithelium showing adherens junction (AJ), cilia (Ci), endosomes (En), lumen of the nephridium (Lu), putative lysosomes (Ly), mitochondria (Mt), microvilli (Mv), and vesicles (Ve).

Fig. 137. Apical plasmalemma with coated vesicles (CV), microvilli (Mv), and coated pit (Pt).
Fig. 138. Detail of coated pit.
Fig. 139. Detail of coated vesicle.
Fig. 140. Granulocyte from connective tissue (CT) of *Themiste alutacea* near nephrostome. (From Pinson, 1990.)

diameter of 28 nm. The functional significance of this distribution is not clear.

Granulocytes, similar to free granulocytes of the coelomic cavity, have been reported in the nephridial connective tissue of both

Fig. 135. The nephridial wall of *Themiste alutacea*. Transverse section. TEM. BI, basal infoldings of inner epithelial cells; CT, connective tissue; Lu, lumen of nephridium; Mu, muscle; Nu, nucleus; Pe, peritoneum; TC, trunk coelom. (From Pinson, 1990.)

Themiste alutacea (Pinson, 1990) and *Phascolosoma granulatum* (Serrano et al., 1990–91) (Figs. 134b, 140). In the former species they are found near the nephrostome, and in the latter they occur most commonly close to the peritoneal covering, often grouped at the inpocketings of the outer nephridial surface (Fig. 133c). Having been found in all layers of the nephridial wall, they are assumed to be capable of migrating from the coelom into the nephridial lumen. The granulocytes vary in

cytoplasmic density, size of granules, and development of organelles, the differences being interpreted as stages of differentiation or maturity. Residual bodies and cytoplasmic enclosures of foreign material suggest that these cells may transport phagocytosed material from the coelom into the nephridium for disposal.

The outer lining of the nephridium consists of a thin layer of peritoneal cells, joined apically to one another by adherens junctions and intermediate and septate desmosomes and to the underlying connective tissue layer by hemidesmosomes. Cytoplasmic processes of the peritoneal cells cover the surface of the organ (Figs. 134c, 135, 141–144). The processes are connected by a double diaphragm (Serrano et al., 1989; Pinson, 1990) and the cells have been interpreted as podocytes (Moya and Serrano, 1984; Serrano et al., 1989) (Figs. 145–148). In *Phascolosoma granulatum*, Serrano et al. (1989) described the processes as pedicels, or small foot-like processes, extending out from the peritoneal cells. The pedicels (200 × 90 nm) are in contact with an underlying basal lamina (30 nm thick). They are separated by slits that are bridged by a double-layered membrane or diaphragm of 30 nm in length and 10 nm in thickness. Within the cytoplasm of the pedicels are rough endoplasmic reticulum, coated vesicles, mitochondria, large dense granules ("residual bodies"), and glycogen rosettes. In the region of the nucleus, the podocyte cells are 3 μm high. The perinuclear region has well-developed Golgi complexes that give rise to the dense bodies or granules of varying size (Figs. 142, 143). Otherwise, the cytoplasmic organelles are much the same as those of the pedicels.

The presence of podocytes in the peritoneal epithelium of the nephridium is evidence that the surface functions in ultrafiltration. However, since the nephridial duct is open to the coelom through the nephrostome, there is a question of how the necessary pressure gradient across the nephridial wall could be achieved. Serrano (1987) suggests that filtra-

tion is accomplished by a lowering of pressure in the duct by a contraction of nephridial musculature and by the beating of cilia within the duct. The primary urine would then be produced by filtration and the final urine by reabsorption and secretion of the epithelium of the inner nephridial lining. Cytoplasmic components indicative of reabsorption and secretion are present in the inner lining: e.g., apical microvilli, coated pits, coated and uncoated vesicles. Further experimental evidence substantiates these nephridial functions. Pinson (1990), using macromolecular tracers (iron dextran and ferritin), found in *Themiste alutacea* that, after injection of these substances into the coelom, they appeared in the nephridial lumen and within vesicles in the apical cytoplasm of the duct. It is probable that larger bodies of waste products may be taken into the nephridium directly though the nephrostome and later excreted. Clumps of dead cells and foreign material, known as "brown bodies" and thought to be accumulated and released by the urns, commonly occur in the coelomic fluid of sipunculans and have been observed in the duct of the nephridium (Hyman, 1959).

There is some evidence that ultrafiltration may also take place at the outer peritoneal epithelium of the contractile vessel. Cells resembling podocytes have been found in the outer epithelium of the contractile vessel of *Themiste lageniformis* (Pilger and Rice, 1987) and *Themiste alutacea* (Pinson, 1990). In the former species podocytes have not been identified on the nephridial surface as they have in the latter species. It is possible that

Figs. 141–144. The peritoneal covering of the nephridium of *Phascolosoma granulatum*. TEM. (From Serrano et al., 1989.)
Fig. 141. Peritoneum or coelomic epithelium (CE) with podocytes (arrowheads). ×19,200.
Fig. 142. Nuclear region of a podocyte. GA, Golgi apparatus; Nu, nucleus. ×22,800.
Fig. 143. Perinuclear cytoplasm of podocytes. Basal lamina (arrows) underlies the pedicels (PE); apically the cells are joined by zonula adherens (arrowhead). Granular endoplasmic reticulum (GER) is seen within the cytoplasm. ×42,000.
Fig. 144. Cell junctions of podocytes. Apically cells are joined by zonulae adherentes (ZA) and more basally by septate desmosomes (SD). ×63,600.
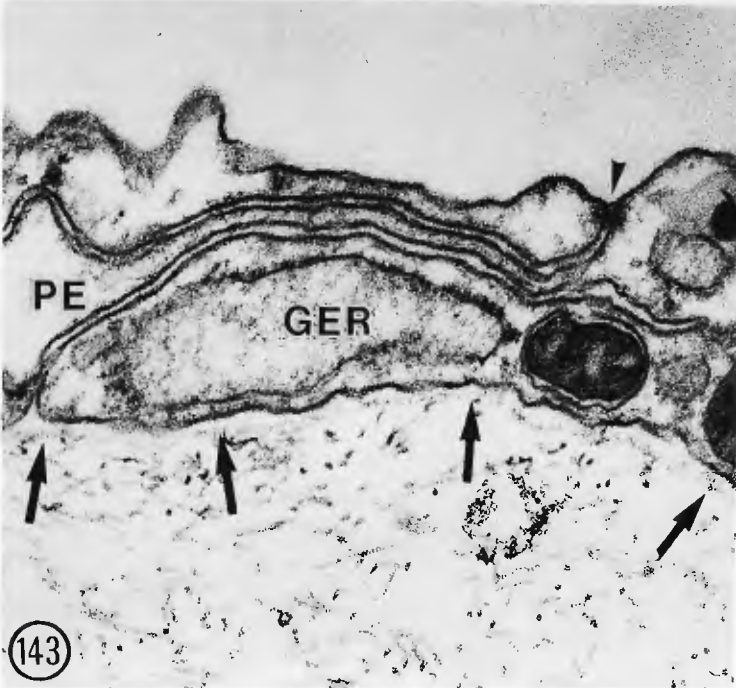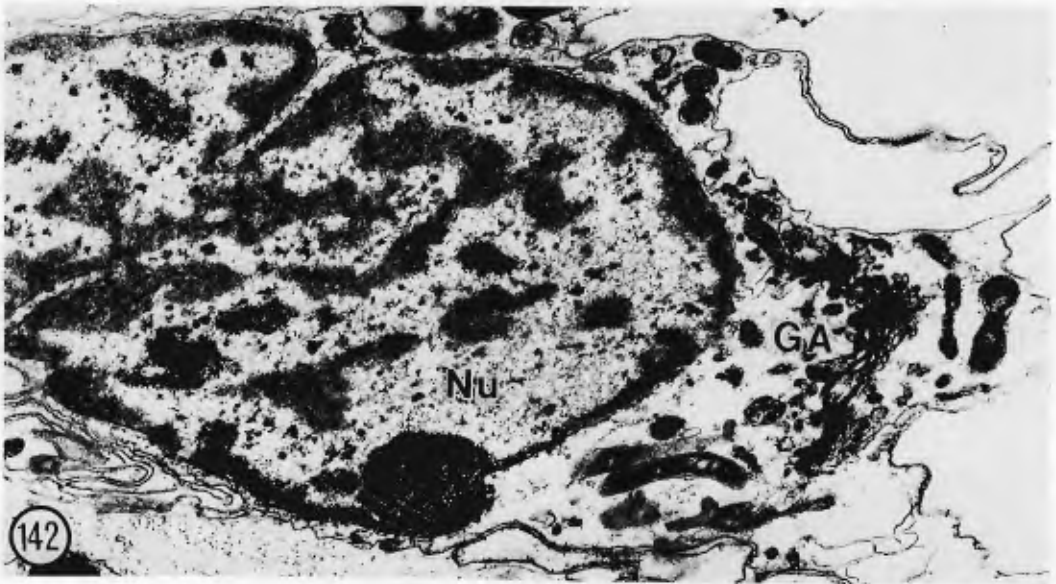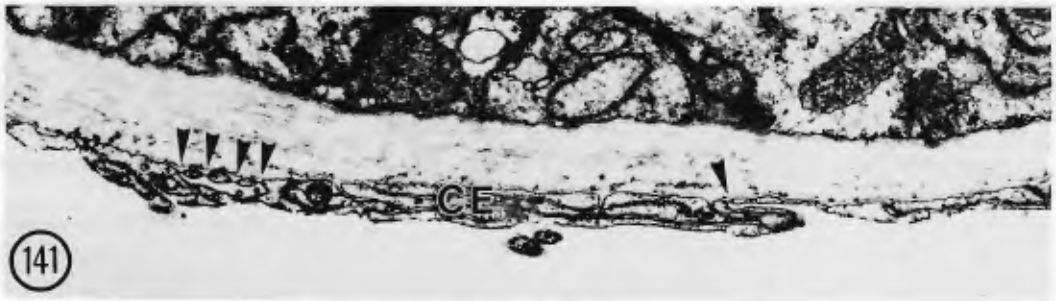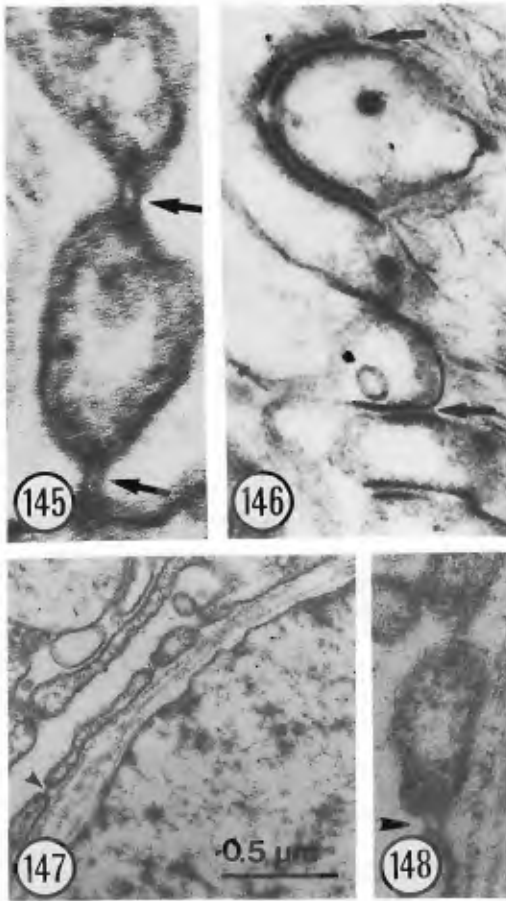
Figs. 145–148.   Pedicels of nephridial podocytes. TEM.
Fig. 145.   Pedicels of *Phascolosoma granulatum*. Pedicels are joined by double diaphragms (arrows). (From Serrano et al., 1989). ×209,000.
Fig. 146.   Longitudinal section of pedicels showing double diaphragms (arrows). *Phascolosoma granulatum*. (From Serrano et al., 1989). ×48,900.
Fig. 147.   Cytoplasmic processes of podocyte-like peritoneal cells overlying nephridium of *Themiste alutacea*. Processes are joined by a double diaphragm (arrowhead). (From Pinson, 1990.)
Fig. 148.   Detail of Figure 147. ×240,000.

ultrafiltration might be accomplished at the site of the contractile vessel by an increase in hydrostatic pressure within the contractile vessel when the tentacles and introvert are withdrawn, forcing fluid through the vessel wall into the coelom to form the primary filtrate. This could then be taken into the nephridial duct through the nephrostome and modified to form the final urine or filtrate. In

view of the theory of Ruppert and Smith (1988) a system of filtration across the contractile vessel to the nephrostome would conform to a functional metanephridial system, whereas filtration across the nephridial wall would conform to a protonephridial system. An understanding of the role of the nephridium in filtration will require additional investigations of the coelomic epithelium in both the contractile vessel and nephridium of the same species and of osmotic and hydrostatic pressures in the various ducts and cavities.

Sipunculans have long been considered as osmoconformers or simple osmometers. However, limited volume regulation has been demonstrated in *Phascolopsis gouldii*, which is able to recover partially the weight lost in dilute solutions (cf. Oglesby, 1982). This limited regulation has been attributed to mediation by gut and nephridial salt losses. Through histochemical analyses, Serrano (1987) found Na^+, and K^+-ATPases to be localized in the basal infoldings of the duct epithelium. However, Pinson (1990), in measurements of Na^+, K^+-ATPase activity in *Themiste alutacea*, was unable to demonstrate activity at reduced salinities.

NERVOUS SYSTEM

The central nervous system of sipunculans consists of a bilobed supraesophageal ganglion or brain, circumesophageal connectives, and a ventral nerve cord (Fig. 149). Circumesophageal connectives emerge from either side of the brain, encircling the esophagus to join the ventral nerve cord which is unsegmented and unpaired. Extending the length of the body, the nerve cord gives off lateral nerves that may be opposite, alternate, or of irregular arrangement and that supply the body wall, digestive system, and a subepidermal plexus. Nerves from the esophageal connectives supply the tentacles, esophagus, and retractor muscles. The brain also gives off nerves to the tentacles and surrounding musculature, as well as to the sensory organs of the head (e.g., nuchal organ). Although there have been many anatomical and histological
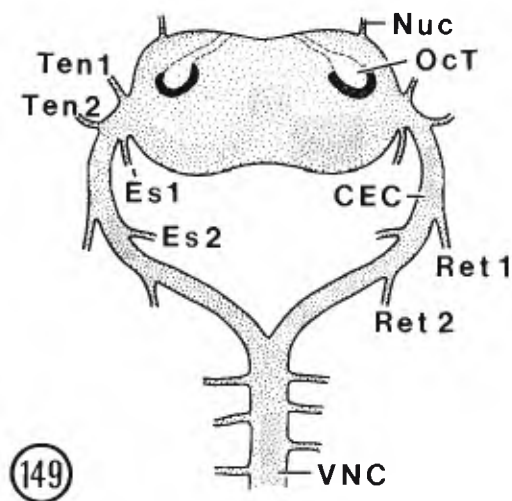
Fig. 149. Diagram of the brain (cerebral organ) and anterior ventral nerve cord of a sipunculan. CEC, circumesophageal connective; Es1, Es2, esophageal nerves; Nuc, nuchal organ nerves; OcT, ocular tube; Ret1, Ret2, retractor muscle nerves; Ten1, Ten2, tentacle nerves; VNC, ventral nerve cord and lateral nerves. (Modified from Åkesson, 1958.)

accounts of the sipunculan nervous system in the older literature (for review, see Hyman, 1959; Tetry, 1959; Bullock and Horridge, 1965), the most recent comprehensive treatment is that of Åkesson (1958). In a comparative study of the nervous systems of 14 species, representing eight genera, Åkesson describes morphology and histology of the cerebral ganglion and connected sensory organs and of the peripheral nervous system, including epidermal organs and nerve plexuses of the intestine and retractor muscles. The only ultrastructural information is that on the "regeneration cells" of the ventral nerve cord (Storch and Moritz, 1970), sensory eye spots with notations on the brain capsule and cerebral organ (Hermans and Eakin, 1969, 1975), ventral nerve cord and various peripheral nerves (author's observations), and the larval terminal organ with sensory components (Ruppert and Rice, 1983).

Ventral Nerve Cord

Covered by peritoneum and connected to the body wall by its lateral nerves and occa-sionally by an anterior mesentery, the ventral nerve cord varies in form and size throughout its length, being best developed anteriorly, often becoming thinner and somewhat flattened posteriorly (Figs. 2, 10–15). In *Sipunculus nudus* it has a posterior swelling, sometimes referred to as a terminal ganglion, associated with a terminal secretory organ. The anterior nerve cord is accompanied on either side by longitudinal paraneural muscles that are reduced posteriorly and fused to form a weak dorsomedian muscular sheath (Figs. 150, 151) or, in the case of *Sipunculus nudus*, incorporated into the longitudinal muscle bundles at the level of the nephridium. The nerve cord is encompassed by a connective tissue sheath underlying the peritoneal covering. Fiber tracts are dorsal and nerve cells are ventral, being evenly distributed along the length of the cord with no evidence of ganglionic aggregations (Figs. 150, 151). Electron microscopy reveals an elaborately developed peritoneal covering, consisting of multiple layers of membranes of peritoneal cells (Figs. 152–154). In *Nephasoma pellucida*, the number of membrane layers can vary from 7 to 12; they appear more closely compressed on the ventral than the dorsal side (Fig. 152). To the inside of the peritoneum is a densely fibrillar, narrow sheath that expands laterally to surround the cells of the paraneural muscles. Fiber-containing glial cells occur within and at the periphery of the cord. They are distinguished from the neurons by denser nuclei and large cytoplasmic granules. Their cytoplasmic extensions course throughout the cord, dividing it into what may be functional areas or tracts of axons. Less dense than other bodies within the cord, the axons possess some mitochondria, granules of varying size, small fibers, and microtubules that could function in the transport of synaptic vesicles. Cytoplasm of the neurons, located in the ventral and ventrolateral regions of the cord, contains abundant organelles, including Golgi bodies and numerous mitochondria in the vicinity of the nucleus. The sheath of the nerve cord of *Themiste lageniformis* is broader than

Pe
M
NF
NC
(150)

Pe
M
NF
NC
(151)

Pe
GC
M
NC
NC
(152)

Pe
GC
CT
(153)

NC
CT
Pe
(154)
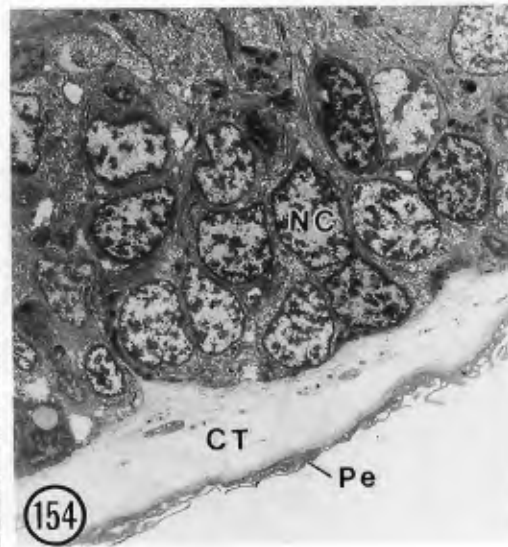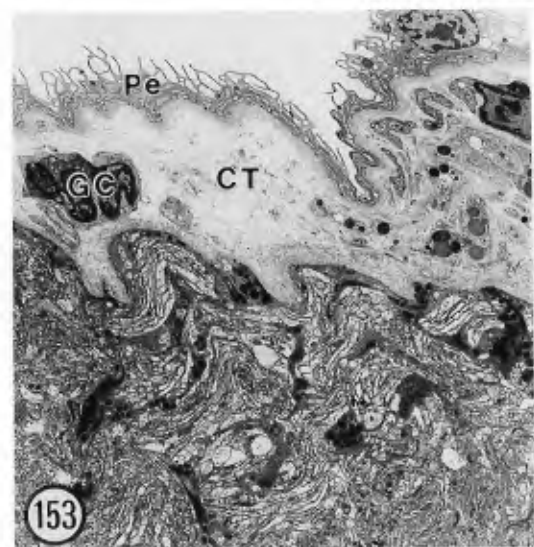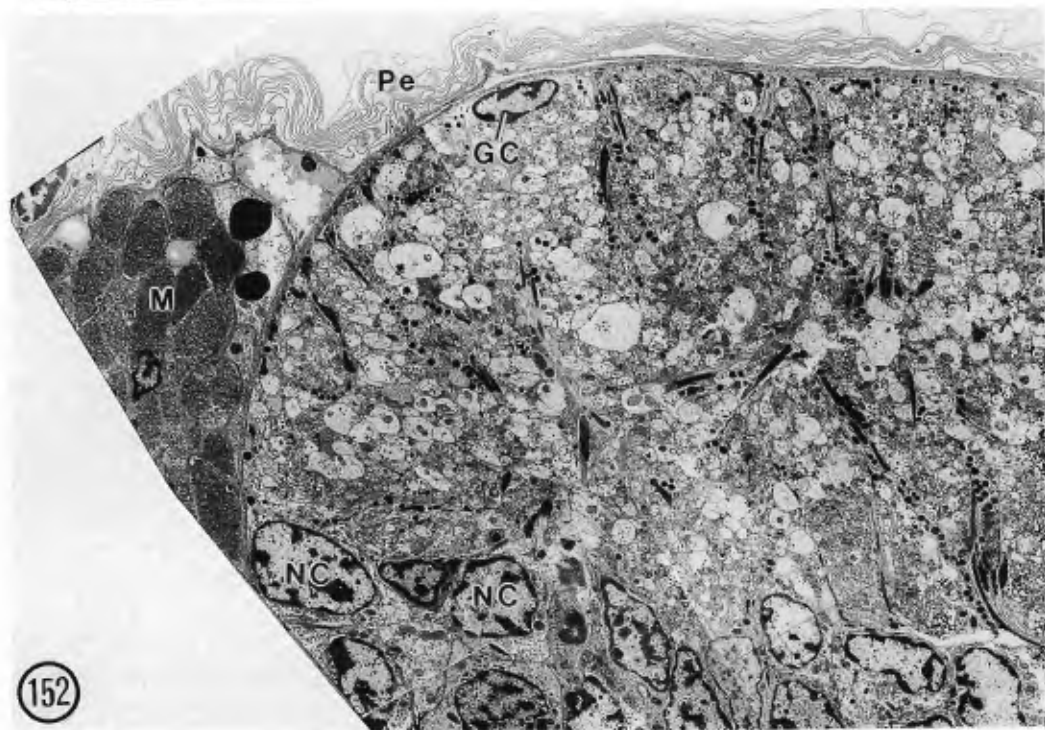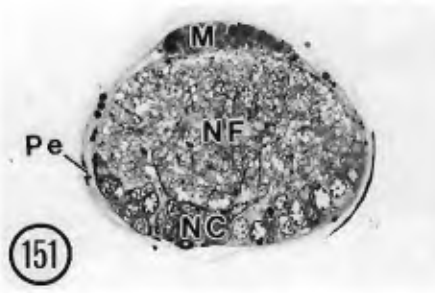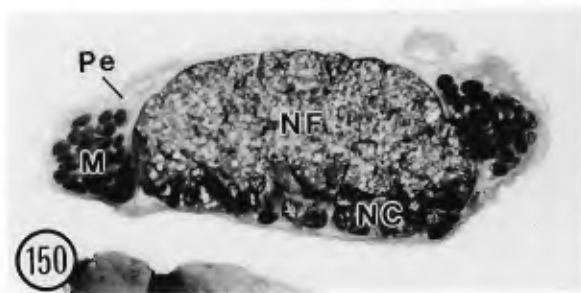
that of *Nephasoma pellucida*, the fibrous material less compact, and the overlying peritoneal membranes in more meandering and irregular layers (Figs. 153, 154). Glial cells are scattered in the sheath, as well as within the cord.

Although not distinguished in the ultrastructural observations of *Nephasoma pellucida* and *Themiste lageniformis*, a ventromedial string of cells, designated as regeneration cells, has been identified in histological preparations of the nerve cord of many species (cf. Åkesson, 1958). An ultrastructural study of regeneration in *Phascolion strombus* (Storch and Moritz, 1970) described the regeneration cells of the nerve cord as being filled with characteristic dense membrane-bound granules of 500 nm in diameter with large, relatively chromatin-rich nuclei (Fig. 155). Among the granules are glycogen rosettes and fiber bundles, other organelles being reduced. After amputation of the introvert these cells migrate from the nerve cord to the site of the wound. Migrating cells are characterized by a cytoplasm rich in glycogen and lipids. At regeneration, the granular inclusions undergo dissolution, forming fibrils that are released into the extracellular space. The more distal cells in the regenerating clump eventually de-
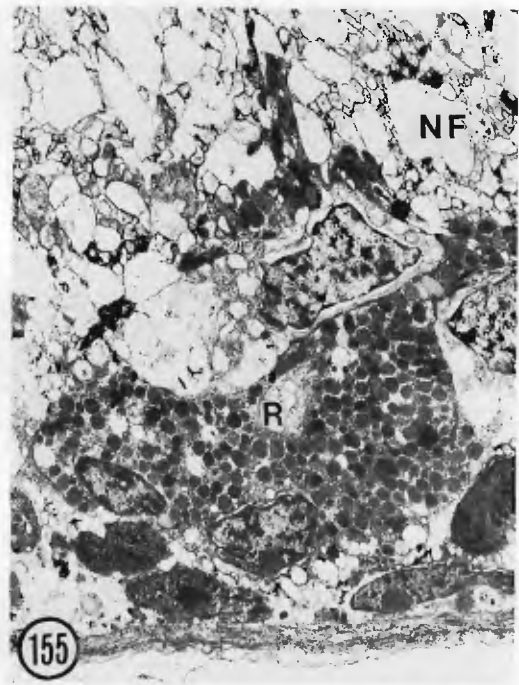

Fig. 155.   Regeneration cells (R) in the ventral nerve cord of *Phascolion strombus*. Characteristically packed with electron-dense granules, regeneration cells occur in a ventromedial string in the cord, beneath the fibrous portion (NF). They migrate from the nerve cord to the site of regenerating tissue. TEM. ×5,760. (From Storch and Moritz, 1970.)
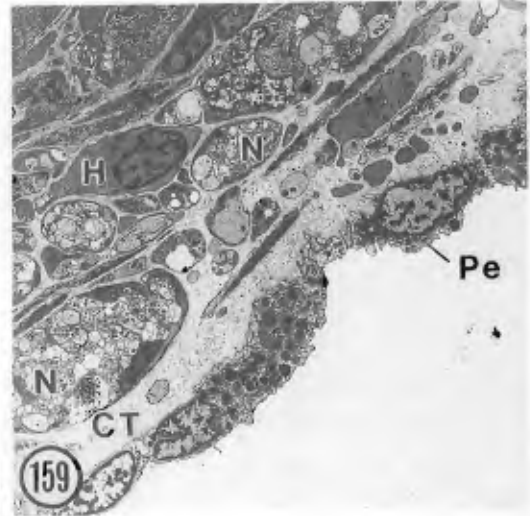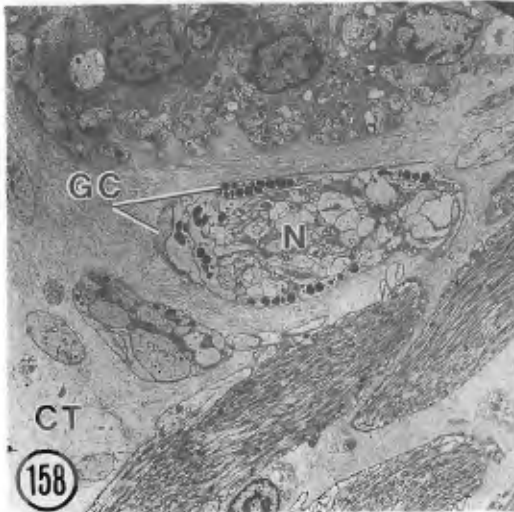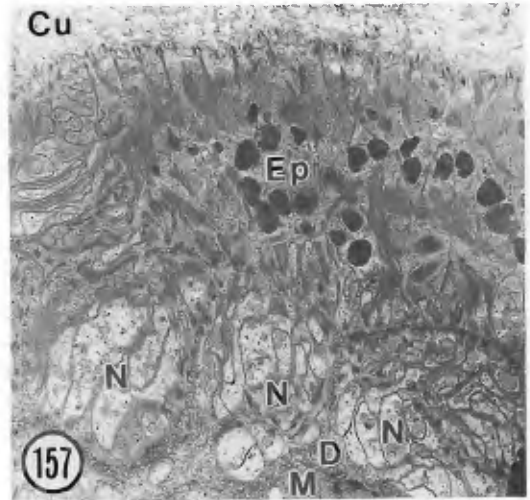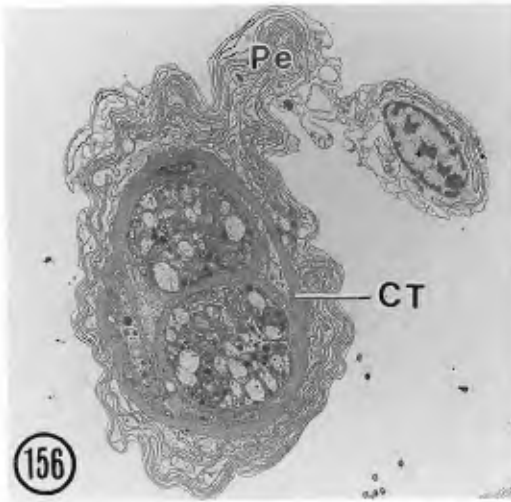
Figs. 150, 151.   Light micrographs of ventral nerve cord of *Nephasoma pellucida*. Transverse sections from introvert (Fig. 150) and trunk (Fig. 151). Nerve cell bodies (NC) are situated on the ventral side of the ventral cord; nerve fibers (NF) are dorsal. The cord, covered by peritoneum (Pe), is ellipsoid in the introvert, becoming more spherical posteriorly. Paraneural muscles (M) are lateral to the cord in the introvert, but join dorsally in the trunk. ×512.
Fig. 152.   Electron micrographic montage of partial transverse section of upper region of ventral nerve cord of *Nephasoma pellucida*. GC, glial cell nucleus; M, paraneural muscles; NC, nerve cell bodies; Pe, peritoneal covering (layers of membranes of peritoneal cells). Cytoplasmic processes of the glial cells appear as dense bands throughout the fibrous portion of the cord. Axons of the fibrous portion are less dense spherical bodies containing small fibers, some dense granules and mitochondria. ×2,940.
Figs. 153, 154.   Ventral nerve cord of *Themiste lageniformis*. TEM.
Fig. 153.   Section through dorsal fibrous portion of cord, showing connective tissue sheath (CT) with glial cells (GC) and peritoneal covering (Pe). ×2,375.
Fig. 154.   Section through ventral portion of cord at site of nerve cell bodies (NC), connective tissue sheath (CT), and overlying peritoneum (Pe). ×2,625.

velop apical microvilli and become connected by desmosomes as the cuticle develops from the extruded fibrils at the apical border of the cells.

Nerves

Lateral nerves arise from the nerve cord as several roots that join and pass into the body wall through the longitudinal musculature to the inner side of the circular muscle layer where, between the two muscle layers, they form a plexus of large nerves. These nerves radiate inwardly to form a plexus beneath the peritoneum and outwardly to form a subepidermal plexus. Epidermal organs are innervated from the latter plexus. There is a similar plexus in the connective tissue layer of the digestive tract as well as on the surface of the retractors and all other structures covered with peritoneum.

Figs. 156–159.   Sipunculan nerves. TEM.
Fig. 156.   Cross section of lateral nerves in the coelom of *Nephasoma pellucida*. Nerves are ensheathed by connective tissue (CT) covered by layers of peritoneal membranes (Pe). ×3,040.
Fig. 157.   Nerves within dermis in body wall of *Phascolion cryptus*. Cu, cuticle; D, dermis; Ep, epidermis; N, nerve; M, muscle. ×5,760.

Fig. 158.   Nerve (N), ensheathed by glial cells (GC), in basal connective tissue (CT) of esophagus of *Phascolosoma varians*. ×2,280.
Fig. 159.   Nerves (N) within connective tissue (CT) of esophagus of *Themiste lageniformis*. Note hemerthrocyte-like cells (H) and peritoneum (Pe). ×2,280.

Extending out from the nerve cord to enclose the lateral nerves are the connective tissue sheath and its many-layered peritoneal covering (Fig. 156). This investment may serve to minimize disturbance and provide support for the nerve within the coelomic cavity. When the nerves enter the body wall this covering is lost, probably fusing with the peritoneum lining the inner body wall. Nerves within the extracellular connective tissue compartments of any organs, e.g., body wall or digestive tract, are surrounded by support-

ive glial cells (Figs. 157–159). These cells have large electron-dense granules, as in the glial cells of the nerve cord, and lipid-like cytoplasmic inclusions (Figs. 157, 158). The axons composing the nerves have smaller electron-dense granules and small vesicles. Along the length of the spindle muscle, which runs through the center of the intestinal coil, there is a prominent longitudinal nerve accompanied by many smaller nerves. The latter send branches to the mesentery that joins the muscle and intestine.

Brain

Also known as the cerebral ganglion or the supraesophageal ganglion, the brain is situated at the base of the tentacles dorsal to the anterior esophagus or pharynx (Fig. 160). It is embedded in a capsule of connective tissue that is enclosed ventrolaterally by the anterior attachments of the retractor muscles (Fig. 161). Within the connective tissue ventral to the brain is the subcerebral sinus (ring canal), which is part of the tentacular system and continuous with the tentacular canals. At the anterior margin of the brain and separated from it by the connective tissue capsule is the cerebral organ of uncertain function (Figs. 149, 160, 162). Ocular tubes, in many species, extend inward into the cerebral ganglion from either side of a transverse furrow located dorsal and lateral to the cerebral organ. In species of *Sipunculus* the brain and cerebral organ are sunken posteriorly from the tentacles and connected to the exterior by a cerebral pit or tube (Fig. 163). The extent of this separation varies among different species and genera. Dorsal to the cerebral organ or cerebral pit is the nuchal organ, a specialized sensory structure.

Although there is considerable variation among species in the histological structure of the brain, from simple to complex organization, the basic structure consists of a central core of fibers, termed the neuropil, surrounded by neurons. The size and cytology of the neurons vary with the degree of differentiation in the species. Common to all, there are
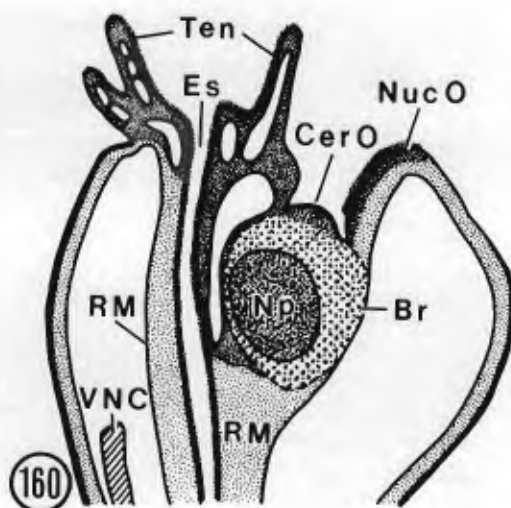

Fig. 160. Diagram of the sipunculan head in sagittal section. Br, brain or cerebral ganglion; CerO, cerebral organ; Es, esophagus; Np, neuropil of brain; NucO, nuchal organ; RM, retractor muscle; Ten, tentacles; VNC, ventral nerve cord. (Redrawn from Åkesson, 1958.)

unipolar, bipolar, and multipolar cells. Bipolar cells are associated with sensory organs and multipolar cells, usually in the posteromedian brain, are reported to be neurosecretory. In the more highly differentiated brain of *Sipunculus nudus* (Fig. 163), the unipolar cells are further differentiated into small cells with little nuclei, forming cell masses that are concentrated as dorsolateral globuli. Axons of the globuli are fused in bundles of glomerulus structure, resembling the corpora pedunculata of polychaetes. Bipolar cells are found at the base of the digitate processes, a papillate organ described by Åkesson (1958) as neurosecretory. Occurring in species of *Sipunculus* and *Xenosiphon*, it is situated at the anterodorsal part of the brain and extends into the coelom.

No information is available on the ultrastructure of the brain, except for notations on the brain capsule and cerebral organ made by Hermans and Eakin (1975) in their electron microscopic study of photoreceptors in the brain. They reported banded connective tissue fibers and muscle fibers in the brain capsule
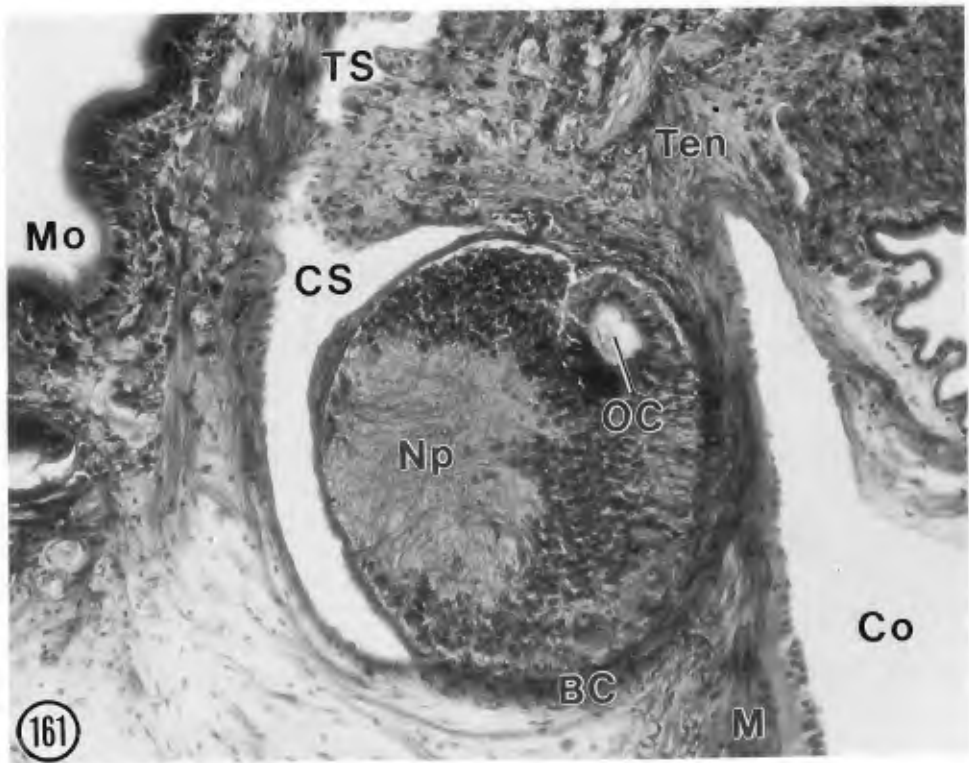
Fig. 161. Light micrograph of parasagittal section (1 μm thick) of brain of *Phascolosoma agassizii*. BC, brain capsule; Co, coelom; CS, subcerebral sinus; M, muscle; Mo, mouth; Np, neuropil of brain; OC, ocellus; Ten, tentacle; TS, tentacular system. ×240. (From Hermans and Eakin, 1975.)
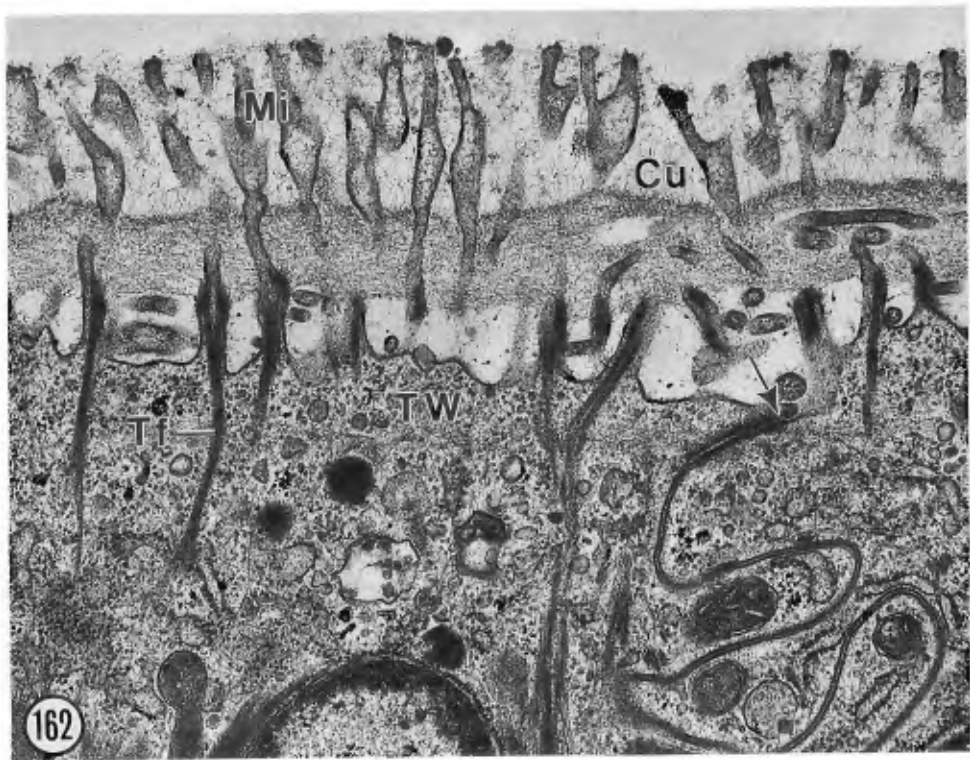
Fig. 162. Cerebral organ of *Phascolosoma agassizii*. Cu, cuticle; Mi, microvilli; Tf, tonofilament; TW, terminal web. Arrow indicates zonula adherens. TEM, ×22,000. (From Hermans and Eakin, 1969.)
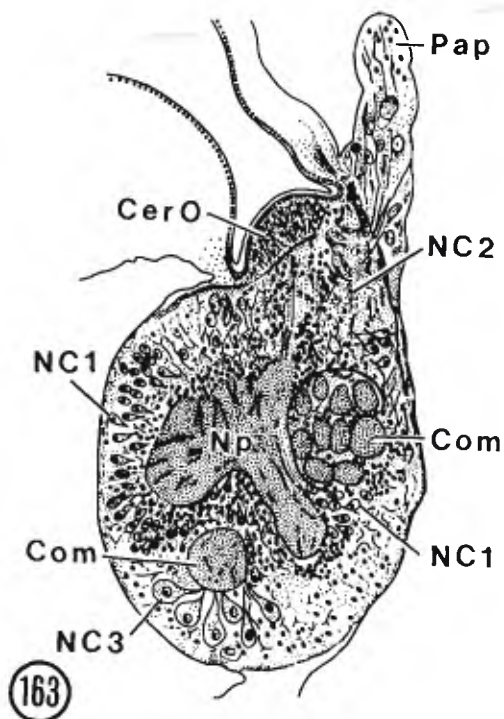
Fig. 163.   Drawing of longitudinal section of the brain of *Sipunculus nudus*. CerO, cerebral organ; Com, commissure; NC1, small unipolar nerve cells; NC2, bipolar nerve cells; NC3, giant multipolar cells; Np, neuropil; Pap, papillate organ or "digitate processes." (Redrawn from Metalnikoff, 1900.)

and tonofilaments of glial cells in nerves traversing the capsule (Fig. 164). They further described the cells of the columnar epithelium of the cerebral organ as having highly convoluted cell membranes joined apically by zonula adherens, forming a terminal web across the apex of the cell, followed by septate junctions in the more convoluted area (Fig. 162). Cell surfaces bear microvilli, often bifurcated; tonofilaments extend into the microvilli and end in hemidesmosomes. Åkesson (1958) considers the cerebral organ as a rudiment of a larval secretory organ of unknown function in the adult.

Sensory Organs

Prominent sensory organs associated with the head region in most sipunculans are the nuchal organ and photoreceptors. Tentacles are well innervated in all sipunculans, and those in *Sipunculus nudus* have been reported to have eyespots (Åkesson, 1958). Other sensory organs, associated with the epidermal organs, have been discussed in the previous section on the body wall.

Nuchal organs are lobulated, ciliated epithelial thickenings situated dorsal to the cerebral organ or pit (Figs. 165–170). Composed of secretory columnar epithelial cells and underlying loose connective tissue, they are innervated by nerves from the anterior brain. Small clusters of cells or ganglia along the branches of the nerves may represent bipolar sensory cells. In some species the bipolar cells are incorporated into the brain, the terminal receptors of the cells ending in the nuchal organ (Åkesson, 1958). The function is presumed to be chemoreception, but ultrastructural and physiological studies are lacking. Form, size, and lobulation of nuchal organs vary among different species (Figs. 165–170). In Aspidosiphonidae and Phascolosomatidae the organ fills the dorsal space within the U-shaped tentacular crown, appearing in *Aspidosiphon brocki* and *Phascolosoma perlucens* as a simple ciliated pad (Figs. 165, 166). The nuchal organs of Golfingiidae, Phascolionidae, and Themistidae are outside and slightly below the circle of tentacles. The organ of *Nephasoma pellucida* is distinctly bilobed, each lobe with a median furrow (Fig. 167), and that of *Themiste lageniformis* multilobed, divided by numerous furrows (Figs. 169, 170). In contrast, the nuchal organ of *Phascolion cryptus* is a ciliated band with numerous longitudinal furrows that encircles the dorsal half of the head between the primary and secondary tentacles (Fig. 168).

A morphological series of photoreceptors or ocelli, from simple invaginations into the cerebral ganglion to a more complex optic tube with vesicular swelling and refractive body have been described and compared by Åkesson (1958) in comparative light microscopic studies of 14 species (Fig. 149). Inter-
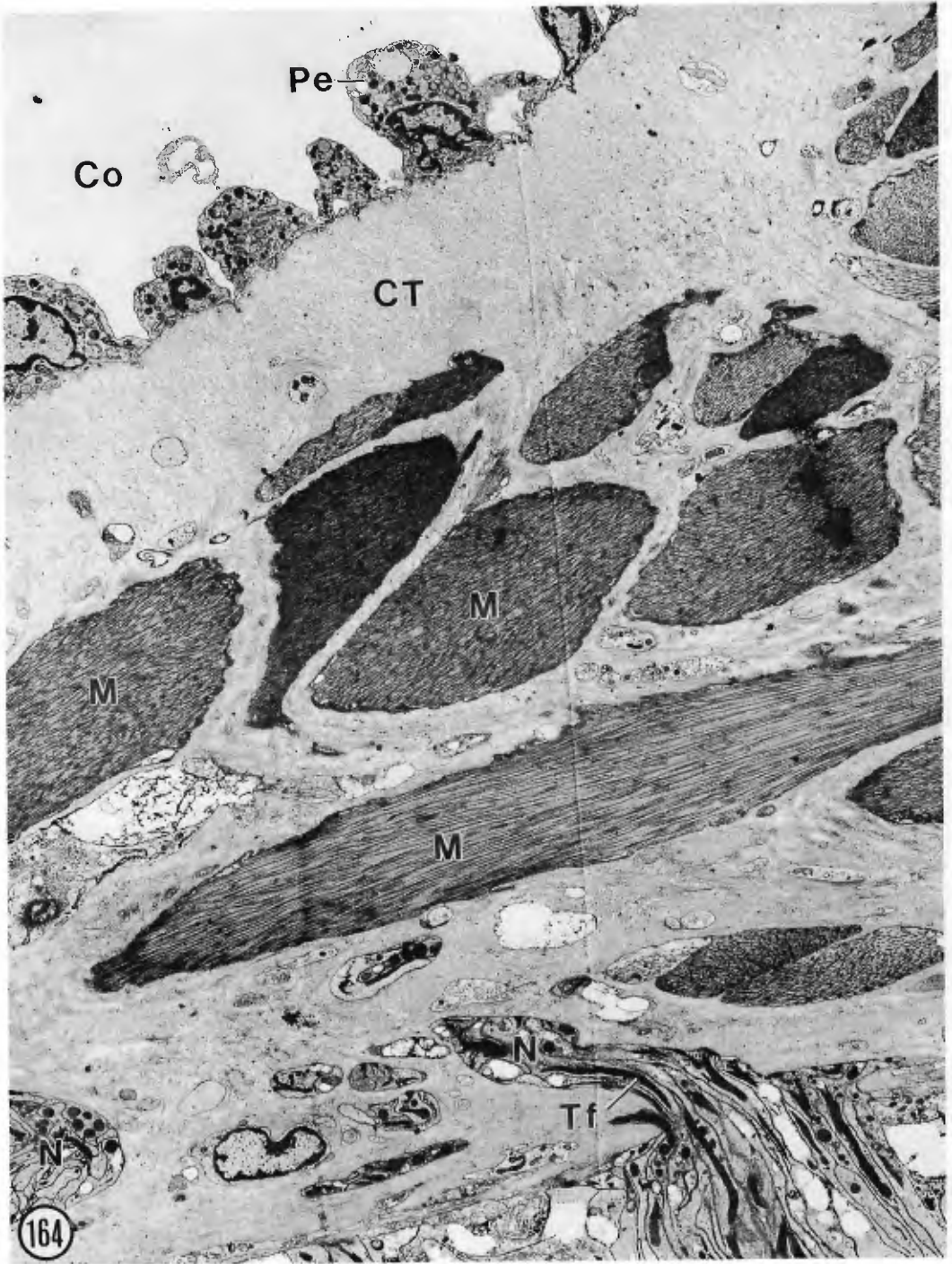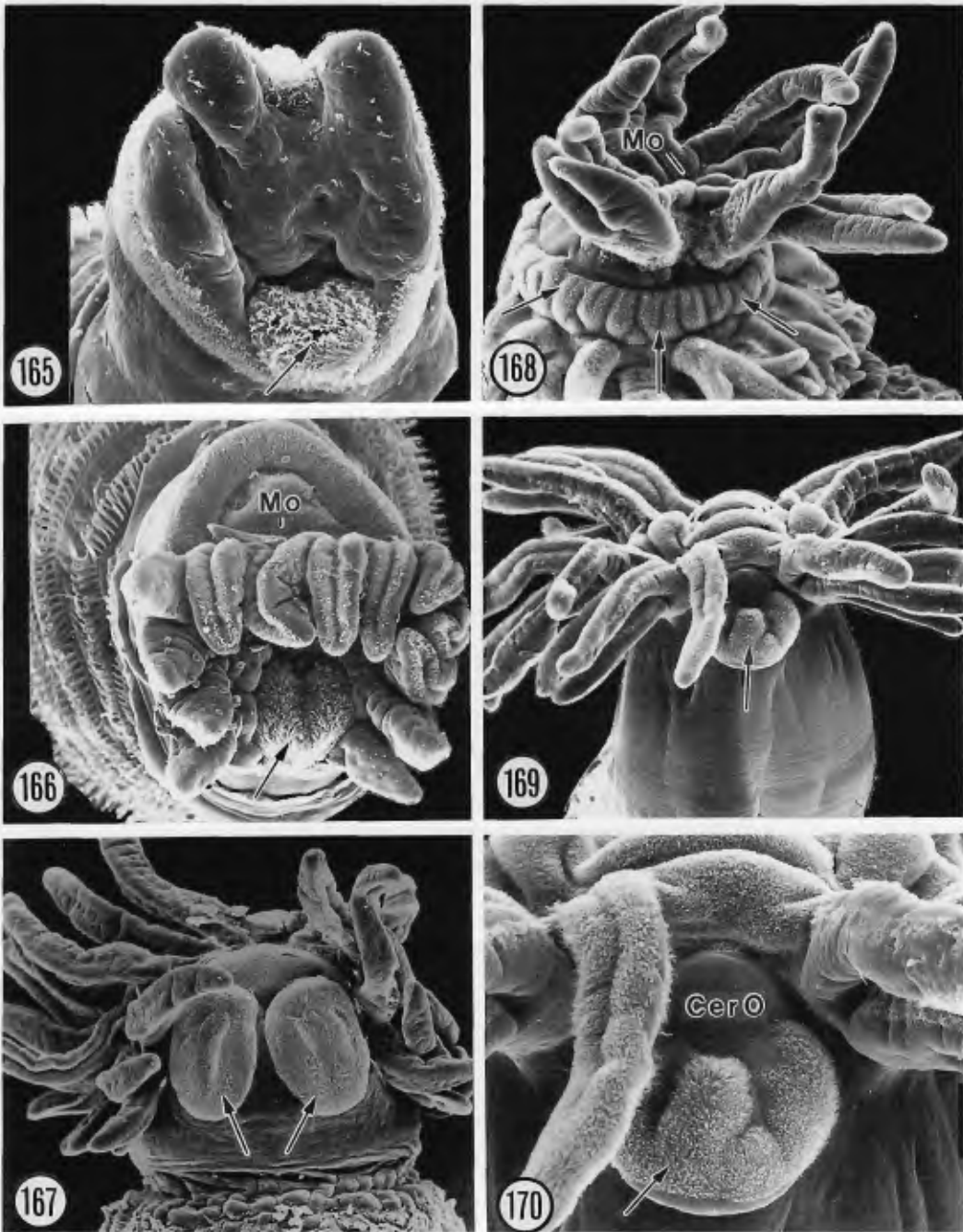
Fig. 164.   Brain capsule of *Phascolosoma agassizii*. Co, coelom; CT, connective tissue; M, muscle fibers; N, nerves traversing capsule; Pe, peritoneum; Tf, tonofilaments in glial cells of nerves. Parasagittal section. TEM. ×3,000. (From Hermans and Eakin, 1975.)

Figs. 165–170.   Heads of sipunculans to show nuchal organs and compare their form and position relative to tentacles in five species, representing five families. Organs are located dorsally on the head. Arrows point to nuchal organ of each species. SEM.
Fig. 165.   *Aspidosiphon brocki*. Nuchal organ is a ciliated pad within the dorsal arc of tentacles. ×261.
Fig. 166.   *Phascolosoma perlucens*. Apical view of head, showing mouth (Mo) and nuchal organ (arrow) in an arrangement similar to *Aspidosiphon brocki*. ×111.

Fig. 167.   *Nephasoma pellucida*. Nuchal organ is bilobed. ×96.
Fig. 168.   *Phascolion cryptus*. Nuchal organ is a ciliated, lobulated band encircling the dorsal side of the head between the upper primary and lower secondary tentacles. Primary tentacles surround the mouth (Mo). ×50.
Fig. 169.   *Themiste lageniformis*. The multilobed nuchal organ is outside and below the circle of tentacles. ×80.
Fig. 170.   Enlargement of Fig. 169, showing ciliation of nuchal organ and position of cerebral organ (CerO). ×200.

mediate in the sequence of photoreceptor complexity is *Phascolosoma agassizii*, subject of ultrastructural studies by Hermans and Eakin (1969, 1975). In this species ocelli lie at the distal ends of optic tubes that connect to the exterior on either side of the cerebral organ (Fig. 161). The cuticle continues into the tube, forming an interior lining. The ocellus is composed of two types of columnar cells: supportive and photoreceptive (Fig. 171). Cells lining the tube share characteristics of both cell types. The supportive cells, containing an abundance of melanin-like granules, comprise the pigment component of the ocellus. The cells are elongate with truncated apices from which long microvilli extend into the cuticular layer of the lumen. Bundles of tonofilaments pass longitudinally through the cell and into the apical microvilli. Expanded apices of neighboring photoreceptor cells may overlie the truncated apices of the pigmented supportive cells (Fig. 172). The widest part of the supportive cells is near the apex, the tapered base extending between the bases of the photoreceptor cells. The nucleus is more chromatin rich than that of the photoreceptor cell and located more apically. The pigment granules, about 0.7 μm in diameter, are membrane bound and in greatest numbers in the widest part of the cell, between the nucleus and apex. Although occasional basal bodies occur near the apical surface, no cilia are present. There is often a prominent Golgi apparatus.

Interspersed among the pigmented supportive cells, the photoreceptor cells are distinguished by their numerous microtubules, lack of pigment, and their expanded apices, which spread out into the lumen of the ocellus, bearing at least one cilium per cell, usually to one side, and an abundance of microvilli distributed irregularly over the surface (Figs. 172, 173). The latter are presumed to be the photoreceptive component. The shaft of the cilium is recessed into the cytoplasm, the tip projecting beyond the cuticle into the lumen. The ciliary structure is variable, ranging from eight doublets of microtubules, arranged within an axoneme, around one in the center to nine peripheral doublets around a central pair.

The photoreceptor cells are about 30 μm in length, continuing basally as axons that associate with the neurons and glial cells of the brain. The cells are widest, 6μm, at the level of the nucleus toward the basal part of the cell. The narrower portion, toward the apex and between the widest and most heavily pigmented part of the supportive cells, is 1–2 μm in diameter and termed the shaft. In the shaft region are numerous longitudinally oriented microtubules that project into the expanded apices of the cell.

Photoreceptor cells are joined with supportive cells near the margin of the lumen of the ocellus by zonulae adherentes and more distally by septate junctions. The cytoplasm of the perinuclear region is marked by many small mitochondria, Golgi complexes, and endoplasmic reticulum. Combining features of both the photoreceptor cells and the supportive cells, the cells lining the ocular tube possess cilia, sometimes several per cell, tonofilaments, and microtubules. The cuticular mass within the tube is fenestrated with channels and spaces, allowing the passage of seawater through the tube into the lumen of the ocellus.

Evidence for a light-sensitive function of this organ is found in its similarity in morphology and fine structure to those of annelids, molluscs, and arthropods whose function is more certain (Hermans and Eakin, 1969, 1975). The extensive microvilli of the presumed photoreceptive cells in *Phascolosoma agassizii* resembles that in other described rhabdomeric photoreceptors of protostomes. The cilia, although present, do not show the membranous elaborations typical of ciliary photoreceptors. Experimental evidence that sipunculans respond to light has been reported by Peebles and Fox (1933) for *Themiste zostericola*. In response to bright light, sipunculans withdrew the introvert; moreover, the dorsal side, the site of the ocelli, proved more sensitive than the ventral side.
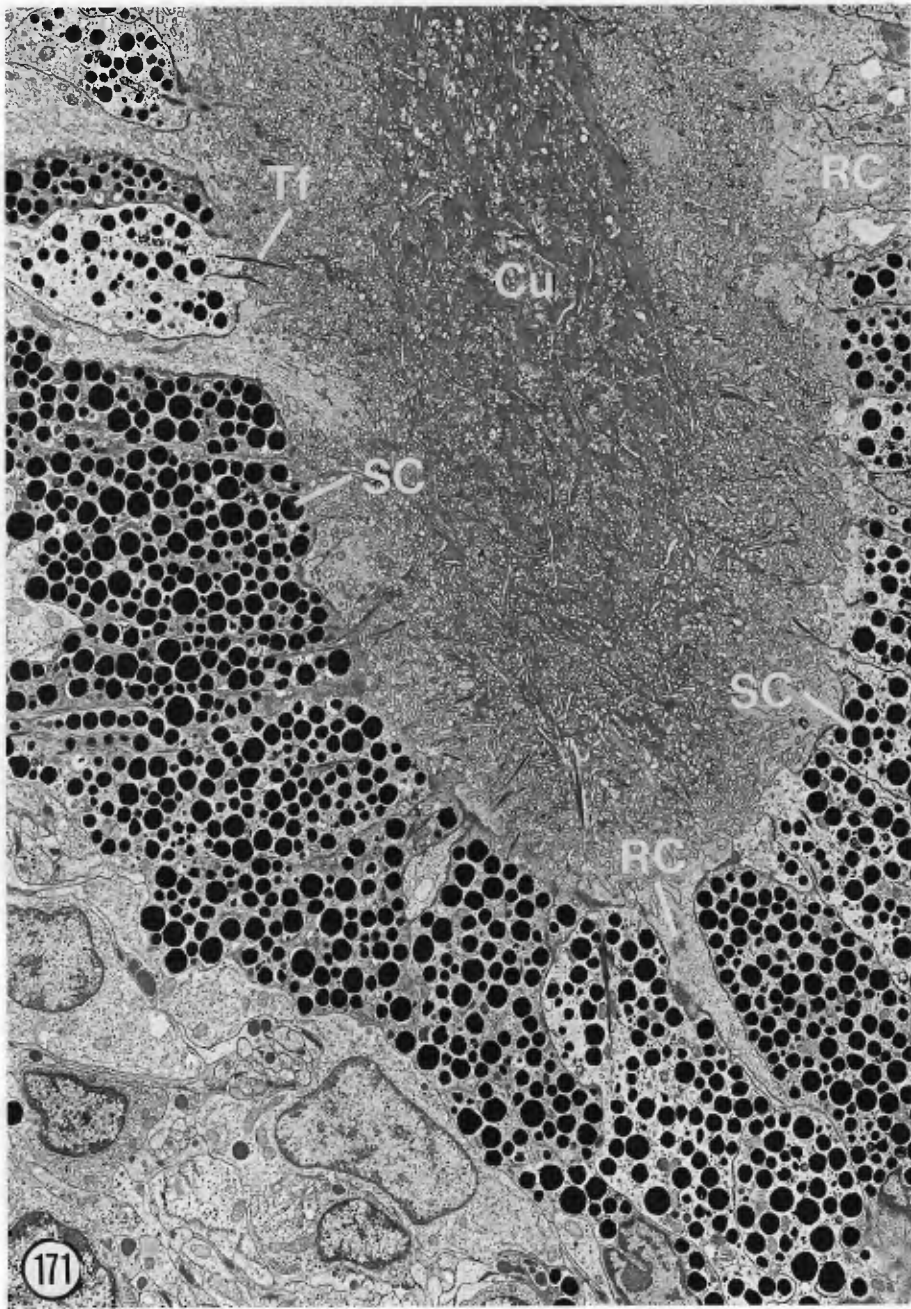
Fig. 171.   A sagittal section through the ocellus of *Phascolosoma agassizii*, showing photoreceptor cells (RC) and granule-packed supportive cells (SC). The ocellus is lined by a mass of cuticle (Cu), microvilli, and cilia. Supportive cells have elongate microvilli, containing bundles of tonofilaments (Tf). TEM. ×4,900. (From Hermans and Eakin, 1969.)

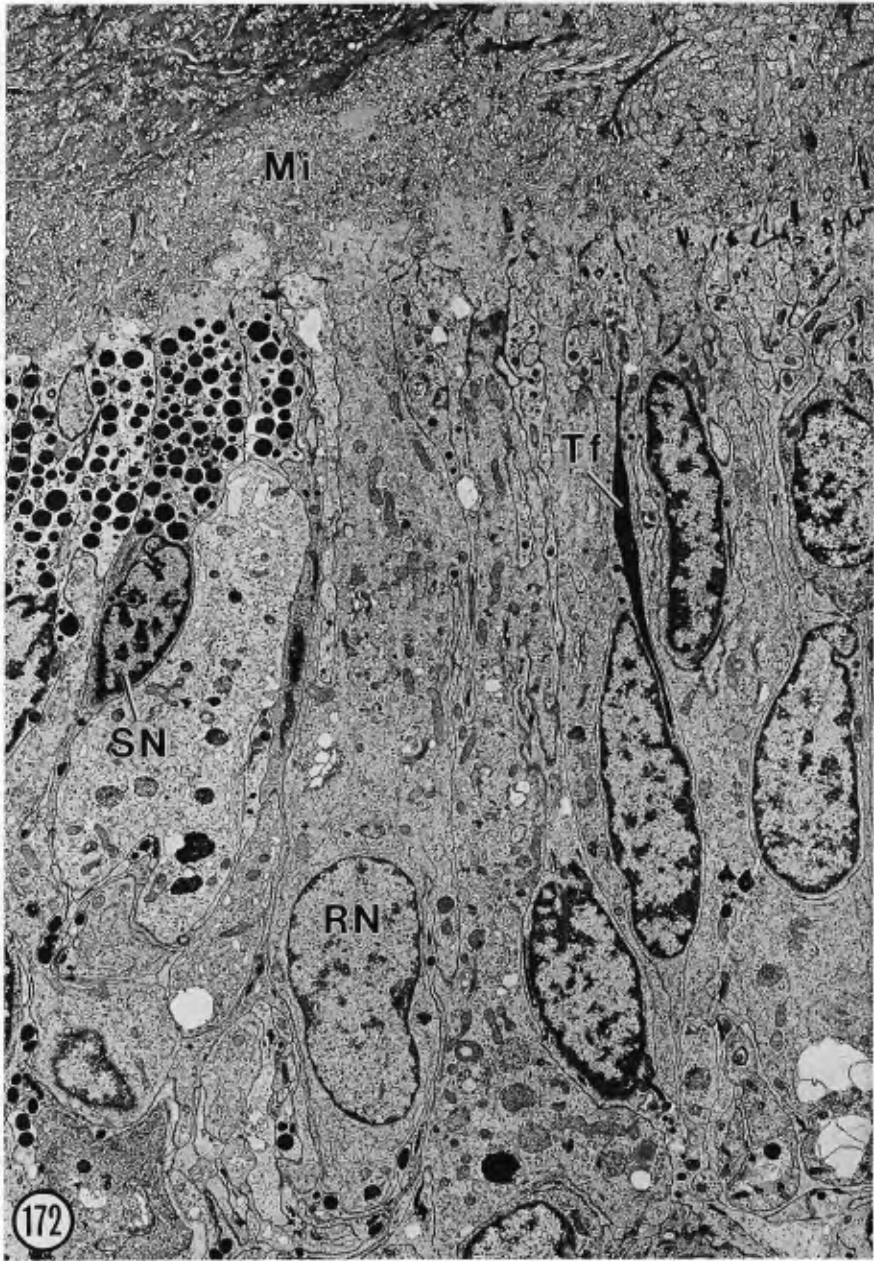Fig. 172. A longitudinal section of a photoreceptor cell at the margin of the ocellus of *Phascolosoma agassizii*. Mi, microvilli at apex of photoreceptor cell; RN, nucleus of photoreceptor cell; SN, nucleus of supportive cell; Tf, tonofilament. TEM. ×4,700. (From Hermans and Eakin, 1975.)
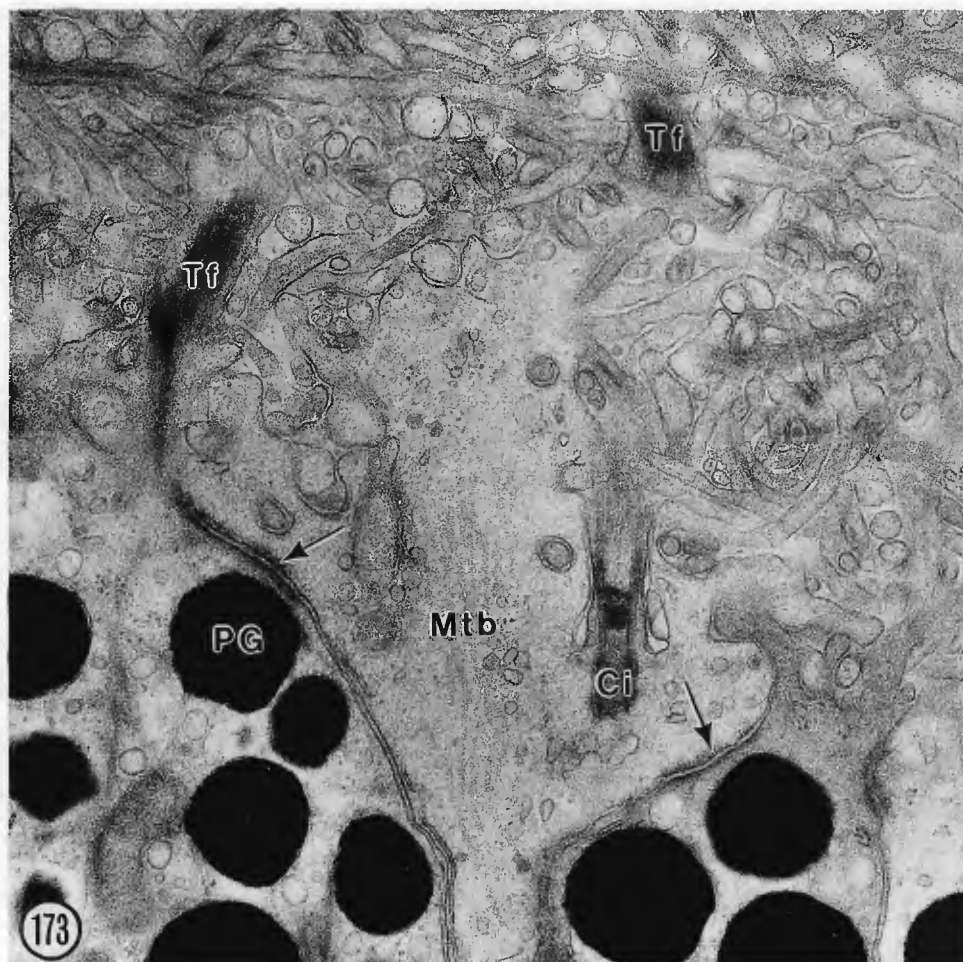
Fig. 173.   An oblique section through the apices of a photoreceptor cell (center) and two supportive cells in the ocellus of *Phascolosoma agassizii*, showing basal body of cilium (Ci) and microtubules (Mtb) of the receptor cell and tonofilaments (Tf) and pigment granules (PG) of the supportive cells. Arrows point to zonulae adherentes. TEM, ×27,000. (From Hermans and Eakin, 1969.)

REPRODUCTIVE SYSTEM

Sipunculans are dioecious with the exception of one known hermaphroditic species, *Nephasoma* (=*Golfingia*) *minuta* (Åkesson, 1958). Commonly, eggs and sperm are spawned into the seawater, where fertilization takes place. Males and females usually occur in equal numbers, but there is one reported parthenogenic species, *Themiste lageniformis*, in which only 4% of the population is males; in this species eggs are able to develop in the absence of sperm (Pilger, 1987). Other examples of unusual sex ratios are summarized by Rice (1975). Asexual reproduction by transverse fission or budding has been reported in two species (cf. Rice, 1975, for review).

Gonad

There is no external sexual dimorphism in sipunculans. Internally, the gonad, either male or female, is located at the base of the

ventral retractor muscles as a narrow, digitated band of tissue, extending from the lateral edge of one muscle, under the ventral nerve cord, to the lateral edge of the other muscle (Figs. 2, 11, 13, 14). It is attached to the muscle by a strand of peritoneal mesentery that continues from the peritoneal lining of the muscle to surround the gonad (Fig. 174). Early differentiation of gametes occurs within the gonad, after which they are released into the coelom as freely suspended cells to complete their development (Figs. 175–182). Spermatogonia differentiate into early spermatocytes before their release as loosely associated clumps; oocytes are released at the diplotene stage of the first meiotic prophase. In a cytological study of oogenesis in *Golfingia vulgaris*, Gonse (1956a) reported seven stages from proximal to distal regions of the ovary, ranging from the more proximal oogonia, resembling peritoneal cells, through various transitional stages marking the leptotene, zygotene, pachytene, and, finally at the distal border, the diplotene stage of the first meiotic prophase. The origin of germ cells has not been resolved. Several authors have noted similarities of early oogonia and/or spermatogonia to peritoneal cells (cf. Rice, 1975, for review; Klepal, 1993). Gerould (1906) reported "reproductive cells" at the base of ventral retractor muscles in larvae of *Golfingia vulgaris* and *Phascolopsis gouldii* at 2–3 weeks of age, but he did not determine their embryological derivation.
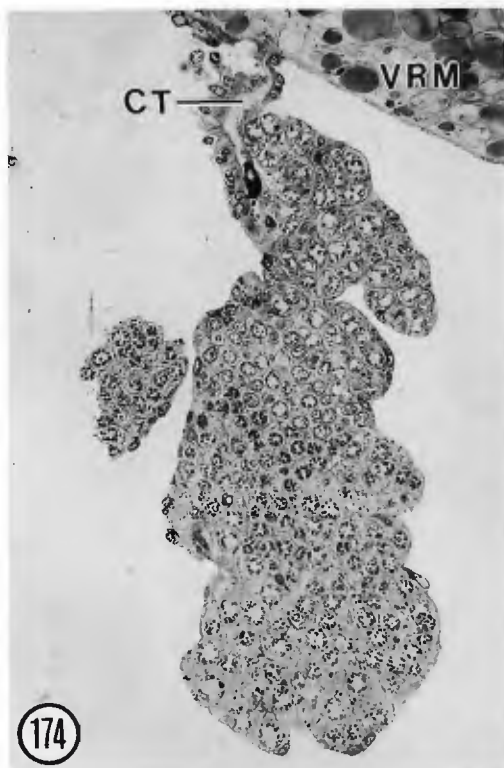

Fig. 174. Light micrograph of the ovary of *Phascolosoma agassizii*, showing a gradient of stages in the first meiotic prophase from proximal to distal ends. Note the mesenteric attachment to the ventral retractor muscle (VRM) with the central core of connective tissue (CT). Sagittal section (1 μm thick). ×342. (From Rice, 1974.)

Coelomic Oogenesis

Oocytes commonly break off from the ovary in small clumps of 10 to 20 cells, interspersed with peritoneal cells (Fig. 175). The clumps are soon dispersed into individual cells, each covered by a thin layer of small follicle cells, presumed to be derived from peritoneal cells (Figs. 176, 177). As the oocytes grow within the coelomic cavity, the follicle cells are lost (Fig. 178). Exceptions are found in species studied in the family Phascolosomatidae, in which oocytes detach from the ovary as single cells that lack follicle cells (Figs. 179–182). (Rice, 1974, 1975, 1983). During growth and development in the coelomic cavity, oocytes of species of Phascolosomatidae and Aspidosiphonidae are exceptional in that they change from spherical to ovoid in shape. In *Phascolosoma agassizii*, oocytes transform into flattened ellipsoids, manifesting a polarity and symmetry while developing as freely floating cells in the coelomic fluid (Figs. 179–182). Oocytes of other species studied maintain a spherical shape throughout the period of coelomic growth (Figs. 175–178).

In the most comprehensive study of coelomic oogenesis in sipunculans, Gonse
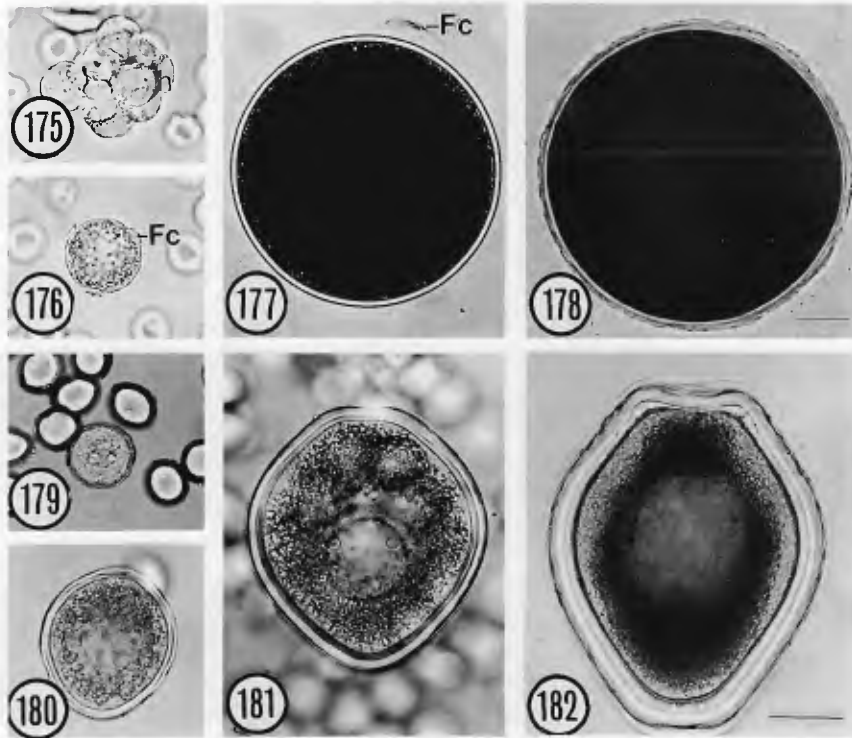
Figs. 175–182. Light micrographs of developing stages of living coelomic oocytes. Hemerythrocytes are seen in the background out of focus. Fc, follicle cells. Scale, 25 μm. (From Rice, 1974, 1975.)
Figs. 175–178. *Golfingia pugettensis.*
Figs. 179–182. *Phascolosoma agassizii.*

(1956a,b, 1957a,b) investigated cytological, cytochemical, and physiological properties of coelomic oocytes in *Golfingia vulgaris*. In cytological and cytochemical studies, based on light microscopic techniques, he devised criteria for developmental stages, reviewing the process of vitellogenesis and the sequence in appearance and position of carbohydrates, lipid bodies, and proteinaceous yolk granules. He further demonstrated two peaks in exogenous respiration during coelomic oogenesis, the earlier corresponding to the beginning of carbohydrate synthesis and both corresponding to periods of high concentrations of ribonucleic acid, coupled with metabolism of hexoses and pentoses (see Rice, 1975, for review).

The only ultrastructural studies of coelomic oogenesis in sipunculans are those of Sawada et al. (1968) and Sawada (1975), which concentrated on *Golfingia ikedai*, but included notations on *Phascolosoma scolops* and *Siphonosoma cumanense*. These observations are summarized in Figure 183. Prominent microvilli and the presence of numerous peripheral endoplasmic vesicles are presumed to provide the mechanism for nutrient uptake from the coelomic fluid. Two types of yolk granules, increasing in number with the growth, were noted: one formed in association with the Golgi apparatus and the other with mitochondria and endoplasmic reticulum. Formation of the egg envelope, absent in the earliest stage, was proposed to result from
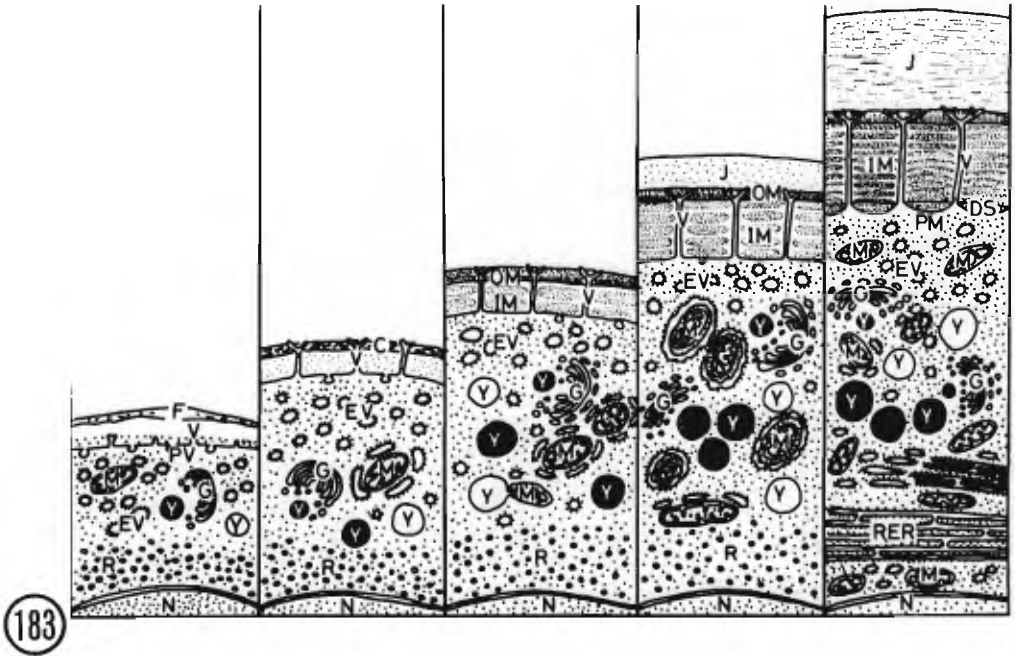
Fig. 183. Schematic representation of the growth of oocytes in *Golfingia ikedai*, based on electron microscopic observations. C, chorion or egg envelope; DS, diffused substances; EV, endoplasmic vesicles; F, follicle; G, Golgi body; IM, inner membrane; J, jelly layer; M, mitochondria; N, nucleus; OM, outer membrane; PM, plasma membrane; PV, pinocytotic vacuole; R, ribosome cluster; RER, rough endoplasmic reticulum; V, microvillus; Y, yolk granule. (From Sawada et al., 1968.)

precursor substances extruded from the cell surface and condensed in succession to form the numerous layers. The fully formed egg envelope of *Golfingia ikedai* consists of three primary layers, the middle of which is subdivided into 14 layers. Microvilli extend through pores in the egg envelope to the surface of the egg, often branching at the surface. This basic structure is characteristic of all sipunculan eggs. In *Phascolosoma agassizii*, as in most species of Phascolosomatidae and Aspidosiphonidae, there are only three layers, the middle layer lacking subdivisions (Fig. 184) (Rice, 1989).

Coelomic Spermatogenesis

Spermatocytes break off from the testis as loosely associated clumps of cells that undergo meiosis and differentiate into sperma-

tids while floating as cell clusters in the coelomic fluid (Figs. 185, 186). The spermatid clumps of some species (e.g., *Golfingia pugettensis, Themiste pyroides, Themiste alutacea*) break up into individual sperm, remaining as free cells in the coelom for prolonged periods, whereas in other species, such as many species of Phascolosomatidae, they do not separate until immediately before uptake into the nephridia and spawning. Mature spermatozoa of sipunculans are of the primitive type, as classified by Franzén (1956). They are essentially similar in form, consisting of a rounded head, a short midpiece with mitochondrial spheres, and a long filamentous tail (Fig. 187) (Rice, 1989).

Electron microscopic investigations of spermatogenesis have been carried out on three species of sipunculans: *Golfingia ikedai*
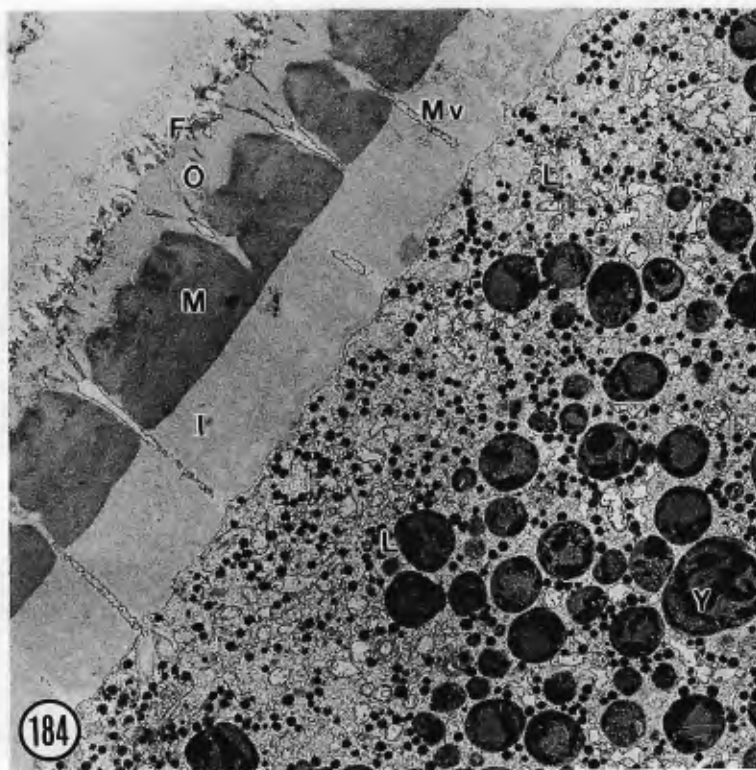
Fig. 184.   Developing coelomic oocyte of *Phascolosoma agassizii*, showing three layers of the egg envelope: outer (O), middle (M), and inner (I). Note microvilli (Mv) extending through the pores of the egg envelope, fuzz (F) on the outer surface of the envelope, small lipid bodies (L), and heterogeneously stained yolk granules (Y). TEM. Scale, approximately 1 μm. (From Rice, 1975.)

(Sawada, 1980), *Aspidosiphon muelleri* (Klepal, 1993), and *Phascolion cryptus* (Reunov and Rice, 1993). The process of coelomic spermatogenesis in *Golfingia ikedai* is summarized in Figure 188. In this species the flagellum begins to develop in the early spermatid after the second meiotic division and the condensation of the nuclear material. At this stage many Golgi bodies appear and give rise to preacrosomal vesicles. The smaller vesicles fuse to form a larger curved acrosomal vesicle with an aggregate of small dense particles, the subacrosomal substance, on the concave side. As the mature sperm takes shape, the acrosomal vesicle, formed in the tail region, moves to the apical region above

the nucleus, and the numerous small mitochondria fuse to four large structures at the base of the flagellum. A peculiar annular structure is noted around the base of the tail in *Golfingia ikedai*.

In *Aspidosiphon muelleri*, Klepal (1993) has observed beginning spermiogenesis in the zygotene stage of the primary spermatocyte within the gonad. Associated within one region of the cell are the centrioles, Golgi apparatus, stacks of rough endoplasmic reticulum membranes, proacrosomal vesicle and dense amorphous bodies, probably lipid (Fig. 192). Also observed at this stage are the characteristic synaptonemal complexes (Fig. 193). A close association of the early spermatogonia
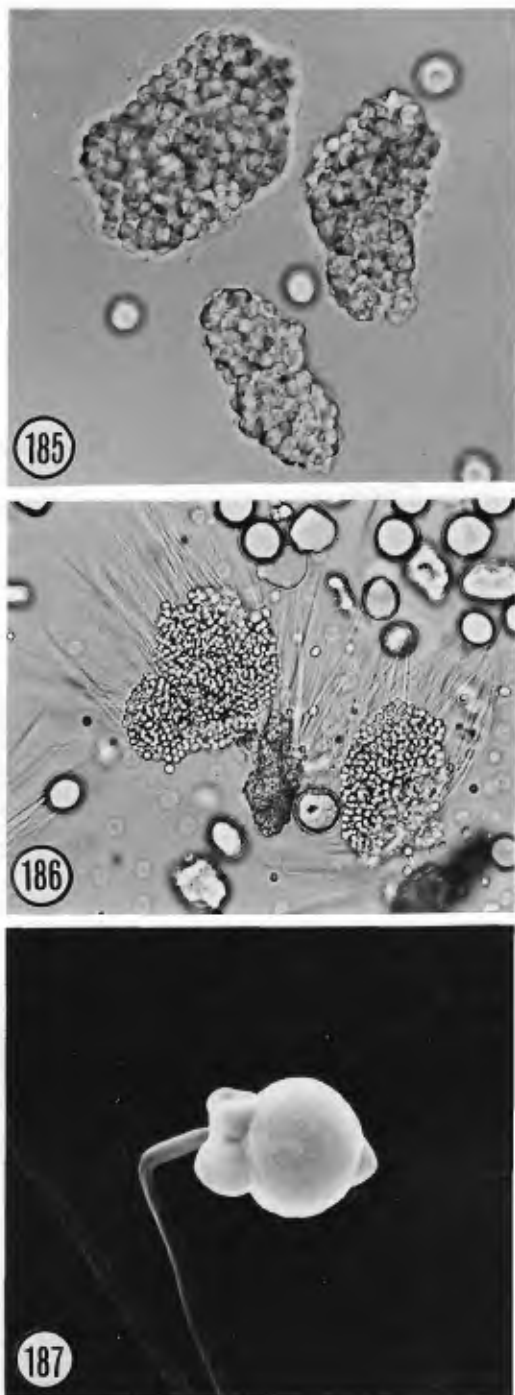
Fig. 185.   Light micrograph of coelomic sperm plates of *Phascolosoma turnerae*. ×373.
Fig. 186.   Light micrograph of coelomic spermatid clusters of *Apionsoma misakiana*. ×373.
Fig. 187.   A mature spermatozoan of *Apionsoma misakiana*. SEM. ×10,000.

and the peritoneum around the gonad suggests an interactive role and possible nutritive function (Figs. 189–191). Cytoplasmic processes of the peritoneal cells extend between the spermatogonia, and vesicles from these processes are also seen within the cytoplasm of the spermatogonia. At the time of detachment from the gonad, spermatocytes lose their contact with the peritoneal cells, but are often in association with granulocytes (Fig. 194).

Spermatogenesis in *Phascolion cryptus* differs in that both primary and secondary spermatocytes are connected by intercellular bridges, as are the later spermatids (Fig. 195) (Reunov and Rice, 1993). Cells within the clusters are tightly bound and develop synchronously. Primary spermatocytes are approximately 3–3.5 μm in diameter, whereas secondary spermatocytes are somewhat smaller and slightly ovoid, ranging from 2.5 to 3 μm. Spermatids develop fully formed flagella and, at this stage, the acrosomal vesicle moves to the apical region of the nucleus (Figs. 196–198). Mature sperm remain in clusters; their flagella are active and propel the clusters through the coelomic fluid. The flagellum, surrounded at its base by five mitochondria, has the standard set of axonemes (9 + 2) (Figs. 199, 200). The rather elongate, conical head is 3 μm in length. The mature sperm of *Aspidosiphon muelleri* differs in having a more rounded head of approximately 3 μm in diameter and a length (including midpiece) of 4 μm. The nucleus is characterized by an apical indentation that is filled by an extension of granular postacrosomal material (Fig. 201). On the basal side of the nucleus there are four to five mitochondria, at least one amorphous lipid-like body, and a pair of centrioles at right angles to one another. Along the length of the distal centriole is a pericentriolar complex, which serves as an anchoring fiber apparatus (Fig. 202). Extending from the centriole are nine primary processes, each of which gives rise to two secondary processes that insert on an annulus. It has been suggested that such a perinuclear complex, also found in other invertebrates, is
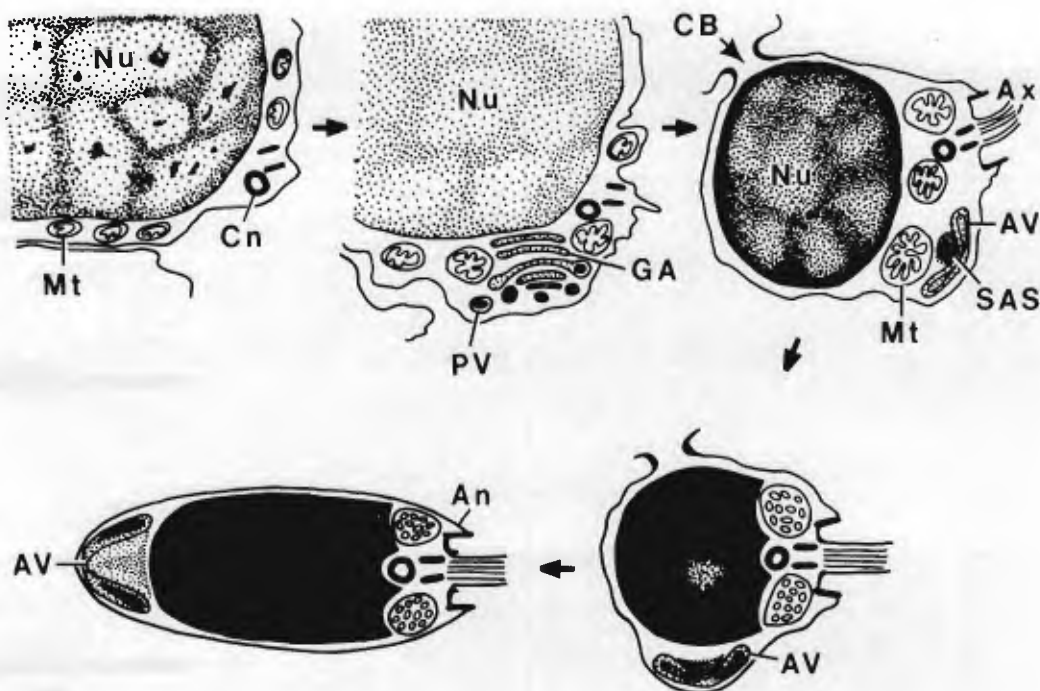
Fig. 188. Diagram of coelomic spermatogenesis in *Golfingia ikedai*. An, annulus; AV, acrosomal vesicle; Ax, axoneme of flagellum; CB, cytoplasmic bridge; Cn, centriole; GA, Golgi apparatus; Mt, mitochondria; Nu, nucleus; PV, preacrosomal vesicles; SAS, subacrosomal substance. (Redrawn from Sawada, 1980.)

associated with sperm having short, rounded heads (cf. Gardiner and Jones, 1985).

Maturation and Fertilization

Maturation of the oocyte commonly begins in the coelom with the breakdown of the germinal vesicle immediately before uptake into the nephridium prior to spawning. Exceptions have been observed in *Sipunculus nudus* on occasional spawnings of eggs with intact germinal vesicles (Rice, 1989). The egg is generally spawned at the first meiotic metaphase, and maturation is completed after sperm penetration (Figs. 199–204) with the extrusion of the first and second polar bodies (Figs. 205–211). Sperm penetration occurs by the formation of a hole in the thick egg envelope. Examined with light microscopy, the egg envelope and cortical cytoplasm in contact with the tip of the penetrating sperm showed an increased homogeneity. The sperm entry hole in the egg envelope persists for several days in the developing embryo (Rice, 1989).

As observed by Nomarski optics in living eggs of *Apionsoma misakiana*, sperm penetration is followed within 10 minutes by a withdrawal of the egg cytoplasm from the egg envelope at the vegetal pole and the migration of the metaphase spindle from a central position toward the animal pole (Fig. 209). Within 55 minutes, after release of the first and second polar bodies, the female pronucleus is formed, completing maturation of the egg (Figs. 210–212). The male pronucleus was first distinguished in the center of the egg
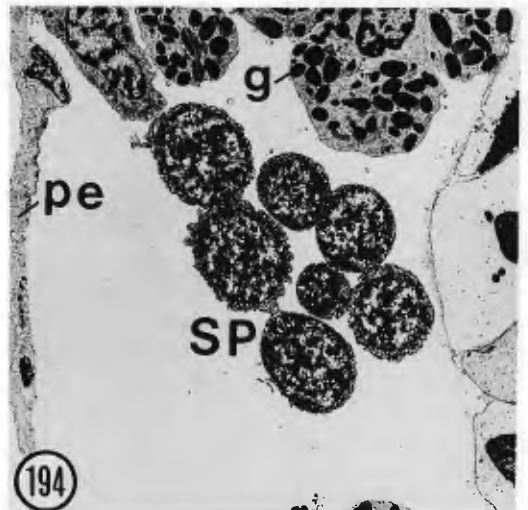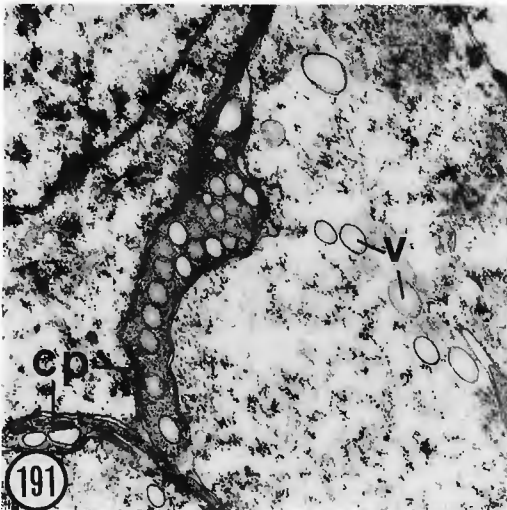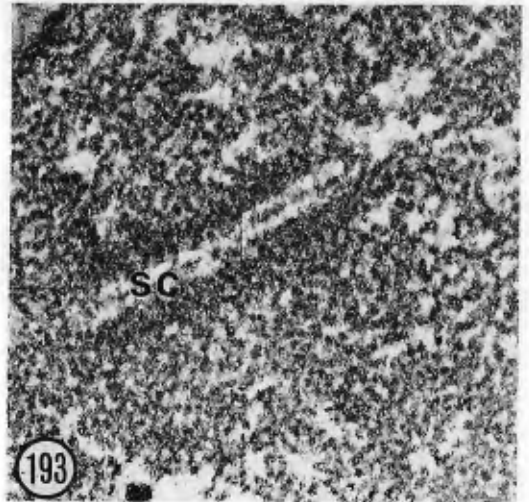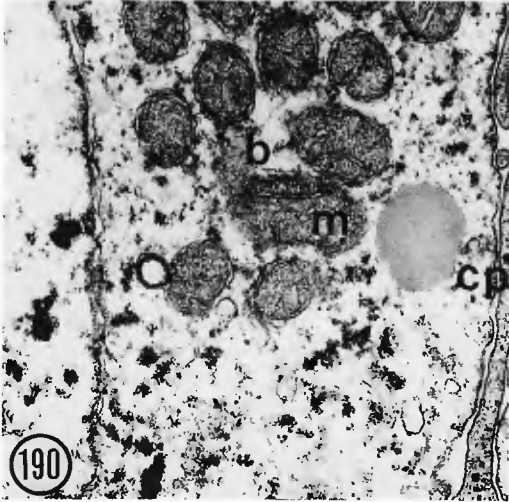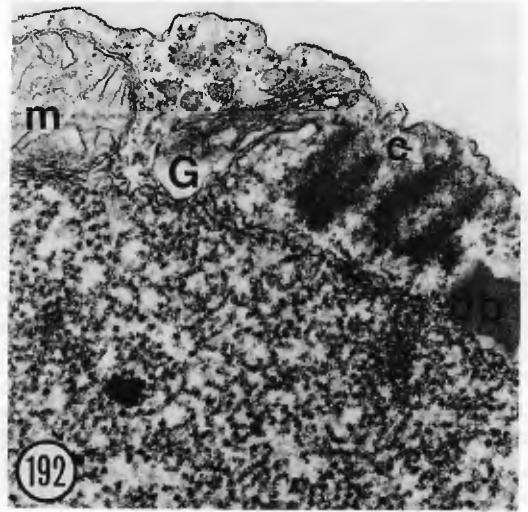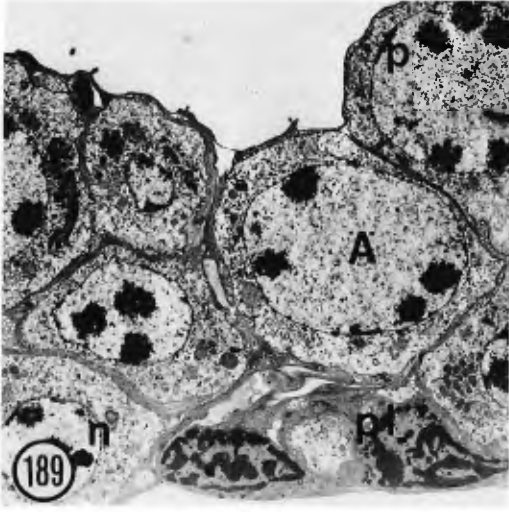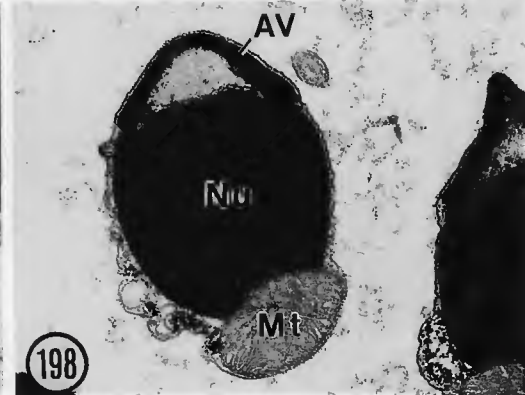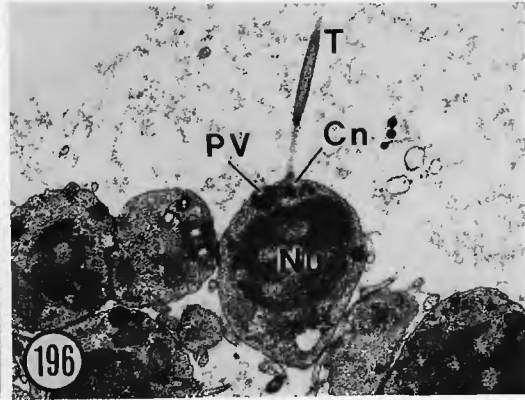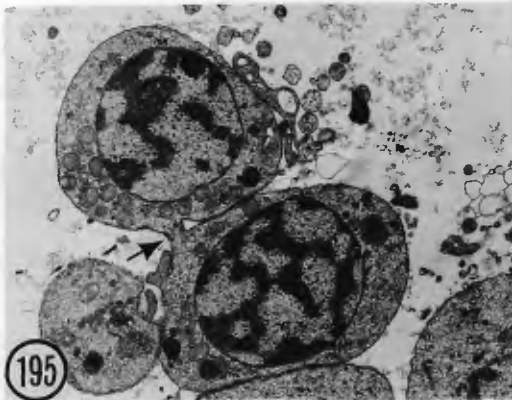
Figs. 195–198. Spermatogenesis in *Phascolion cryptus*. TEM. (From Reunov and Rice, 1993.)
Fig. 195. Secondary spermatocytes connected by intercellular bridge (arrow). ×10,560.
Fig. 196. Section through spermatid cluster. Cn, centrioles; Nu, nucleus; PV, proacrosomal vesicles; T, tail. ×11,645.

Fig. 197. Section through spermatid with flattened acrosomal vesicle (AV) in basal region. ×16,790.
Fig. 198. Section through spermatid with acrosomal vesicle (AV) in the apical region. Mt, mitochondrion; Nu, nucleus. ×17,760.

Figs. 189–194. Spermatogenesis in *Phascolosoma granulatum*. TEM. (From Klepal, 1993.)
Fig. 189. Section through spermatogonia A, in ovary, surrounded by peritoneal cells (pl). Nucleoli (n) and plaques of karyoplasm (p) are at the periphery of the nucleus. ×2,600.
Fig. 190. Section through spermatogonia A, showing mitochondria (m), sometimes connected by bars (b) and cytoplasmic processes (cp) of peritoneal cells. ×49,000.
Fig. 191. Section of spermatogonia B, separated by cellular processes (cp) of peritoneal cells. Vesicles (v) of peritoneal processes are in the cytoplasm of the spermatogonia, where they appear to fuse. ×14,860.
Fig. 192. Section through spermatocyte in zygotene stage. Note centrioles (c), dense amorphous body (db), Golgi apparatus (G), and mitochondrion (m). ×39,060.
Fig. 193. Section through spermatocyte in zygotene stage showing synaptonemal complex (sc). ×55,860.
Fig. 194. Section through loosely associated clump of spermatocytes (SP) at stage of release from peritoneum (pe) of gonad. Note nearby granulocytes (g). ×3,000.

35 minutes after sperm entry. The two pronuclei migrate toward one another, and, within 85 minutes postpenetration, they are completely fused to form the zygote nucleus (Figs. 213, 214). (Rice, 1989).

ACKNOWLEDGMENTS
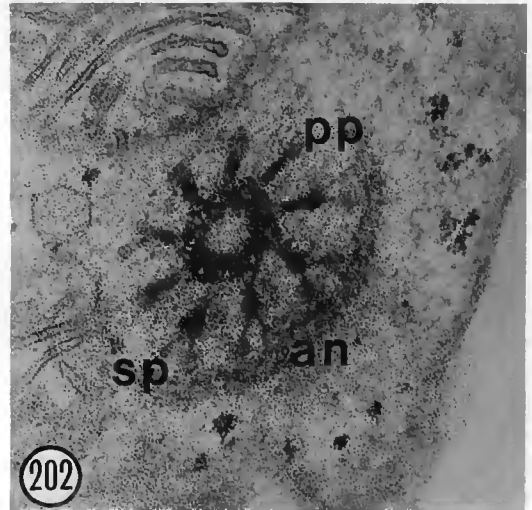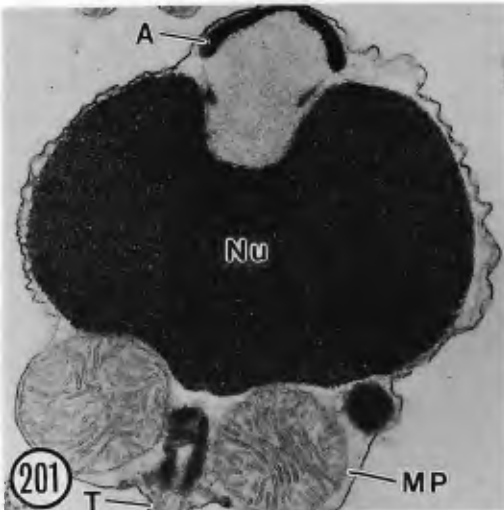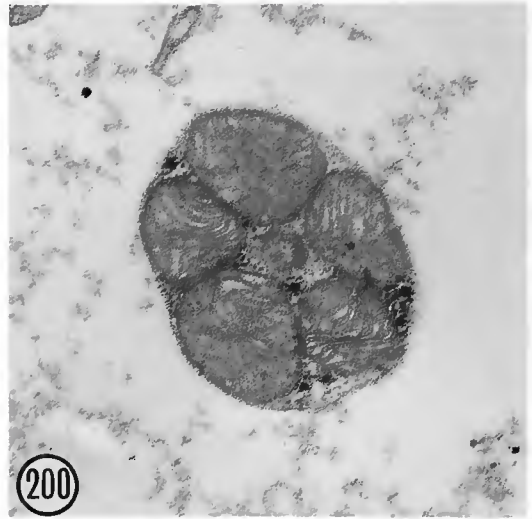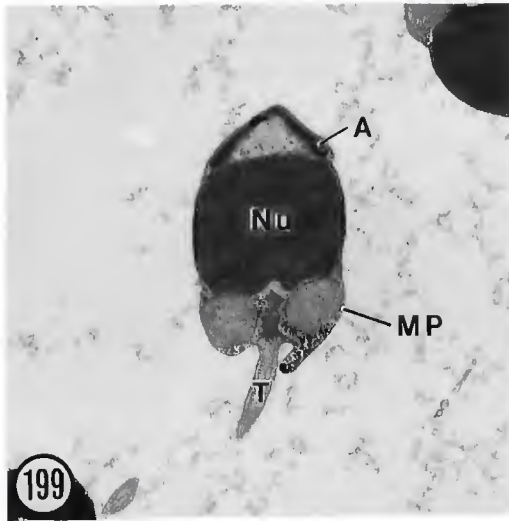
Figs. 199–202.   Mature spermatozoa. TEM.
Fig. 199.   Longitudinal section through head of mature sper-matozoan of *Phascolion cryptus*. A, acrosome; MP, midpiece; Nu, nucleus; T, tail. ×11,520. (From Reunov and Rice, 1993.)
Fig. 200.   Cross section through midpiece of mature spermato-zoan of *Phascolion cryptus* showing ring of five mitochondria around the central centriole. ×16,575. (From Reunov and Rice, 1993.)

Fig. 201.   Section of mature spermatozoan of *Phascolosoma granulatum*. A, acrosome; MP, midpiece; Nu, nucleus; T, tail. ×20,900. (From Klepal, 1993.)
Fig. 202.   Cross section of pericentriolar complex of mature spermatozoan of *Phascolosoma granulatum*. Two secondary processes (sp) arise from each primary process (pp) and "insert" on an annulus (an). ×61,055. (From Klepal, 1993.)
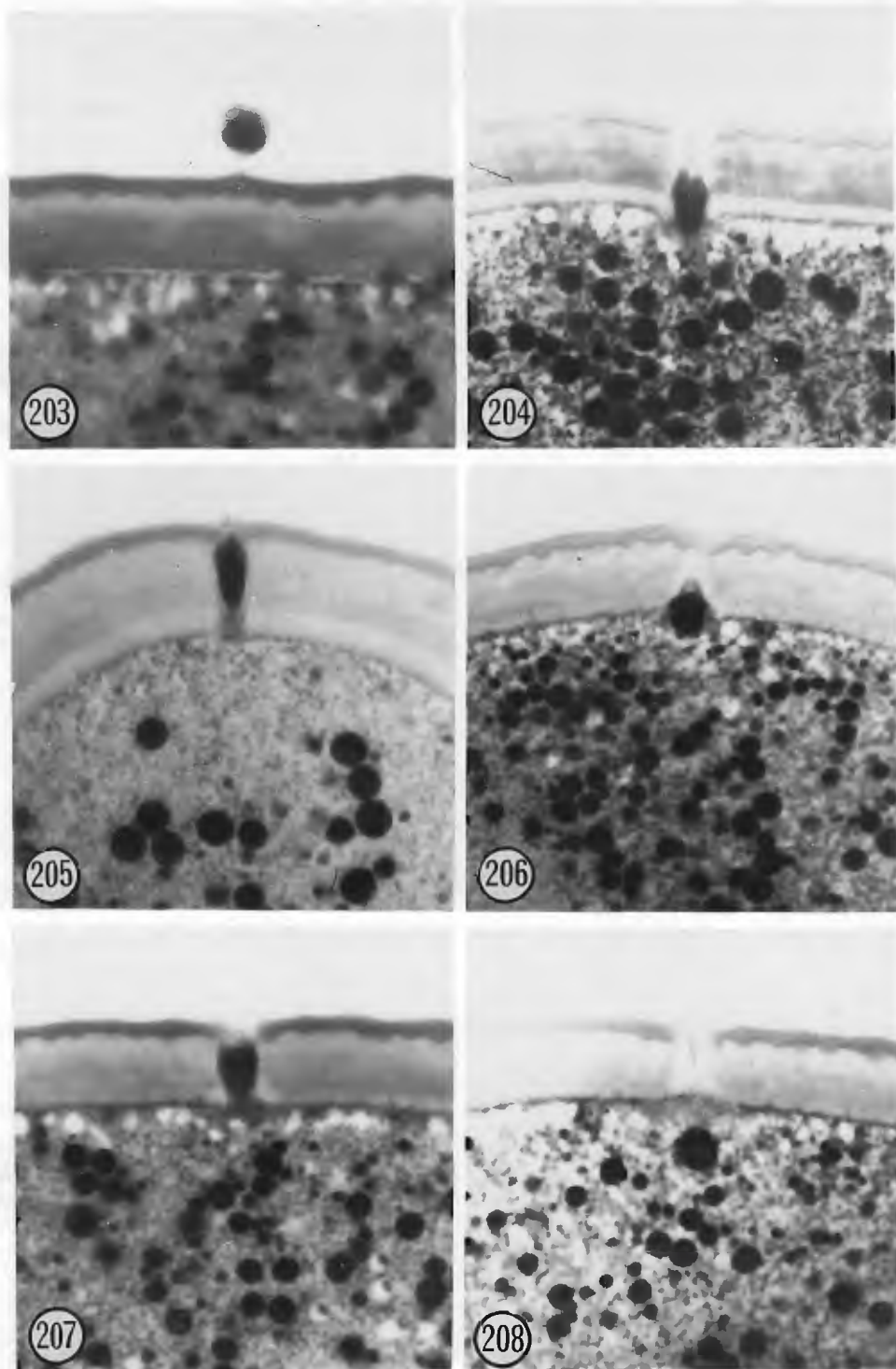
Figs. 203–208.   Light micrographs of sperm penetration in eggs of *Phascolosoma agassizii*. Sections (1 μm thick) of six eggs fixed within 15 minutes after combination with sperm. ×2,560.
Fig. 203.   Sperm is attached to outer border of egg envelope by acrosomal filament.
Figs. 204–207.   Sperm is in various stages of penetration. Note sperm entry holes in egg envelopes.
Fig. 208.   Sperm head is incorporated within egg cytoplasm.
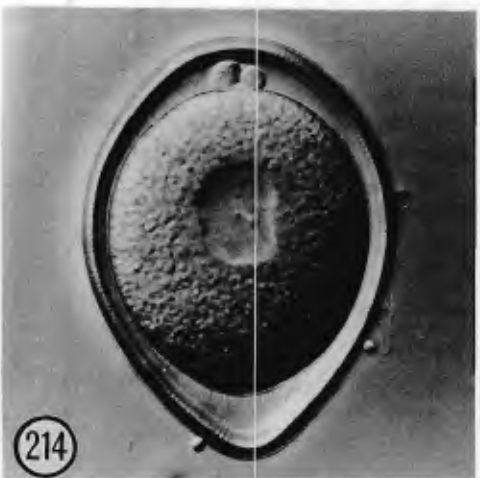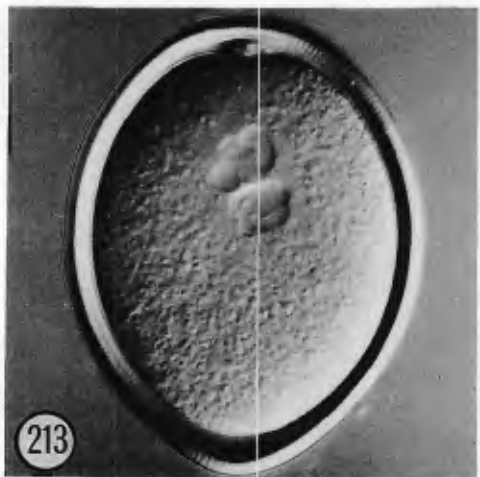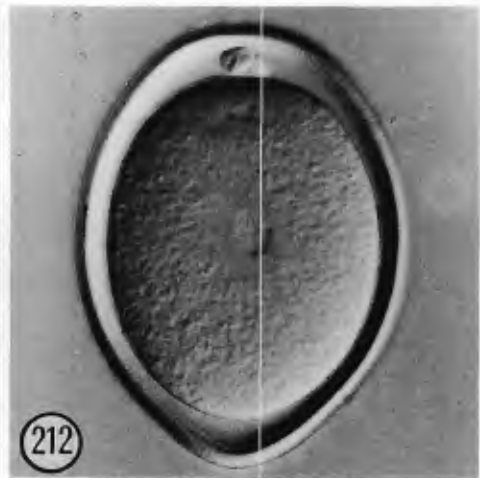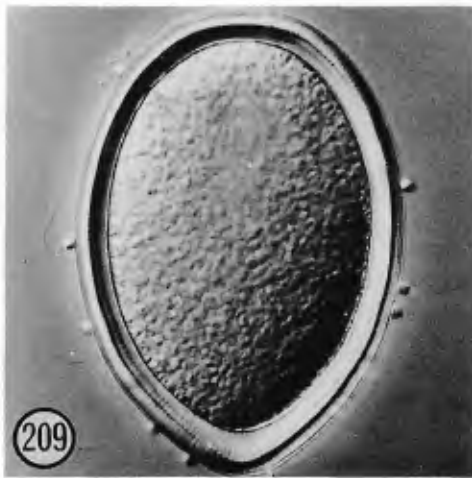
Figs. 209–214. Completion of maturation and fertilization in eggs of *Apionsoma misakiana*. Light micrographs of living eggs after combination with sperm. Nomarski optics. ×550. (From Rice, 1989.) Figures 209–213 are the same egg.

Fig. 209. Nine minutes. Cytoplasm has withdrawn from vegetal pole. First meiotic spindle has moved from center toward animal pole.

Fig. 210. Twenty-nine minutes. First polar body stage.

Fig. 211. Fifty-two minutes. Second polar body stage.

Fig. 212. Sixty minutes. Female pronucleus has formed at the animal pole and the male pronucleus in the center.

Fig. 213. Seventy-five minutes. Male and female pronuclei are in contact.

Fig. 214. Eighty-five minutes. Union of male and female pronuclei to form zygote.

micrographs and observations from their studies on sipunculan ultrastructure. Scientific illustrators gratefully acknowledged for their outstanding contributions are Bonnie Bower-Dennis for rendering all of the line drawings in the chapter and Carolyn Gast and Charissa Baker for their illustrations of the external and internal anatomies of sipunculans (see Figs. 1, 2, 4–21). Finally, the author thanks Ed Ruppert for reading the manuscript and for his enthusiastic support and expert advice throughout this endeavor. This is contribution No. 311 of the Smithsonian Marine Station at Link Port.

LITERATURE CITED

Abercrombie, R.K., and R.M. Bagby (1984) An explanation for the folding fibers in proboscis retractor muscles of *Phascolopsis* (=*Golfingia*) *gouldi*. II. Structural evidence from SEM of teased, glycerinated muscles and light microscopy of KOH-isolated fibers. Comp. Biochem. Physiol. *77A*:31–38.

Abercrombie, R.K., R.M. Bagby, and Y. Matsumoto (1984) An explanation for the folding fibers in proboscis retractor muscles of *Phascolopsis* (=*Golfingia*) *gouldi*. I. Correlation between length–tension data and structure in living muscle. Comp. Biochem. Physiol. *77A*:23–29.

Åkesson, B. (1958) A study of the nervous system of the Sipunculoidae, with some remarks on the development of the two species *Phascolion strombi* (Montagu) and *Golfingia minuta* (Keferstein). Undersökningar over Öresund *38*:1–149.

Andreae, J. (1882) Beitrage zur Anatomie und Histologie des *Sipunculus nudus* L.Z. Wiss. Zool. *36*:201–255.

Andrews, E.A. (1890) Notes on the anatomy of *Sipunculus gouldii* Pourtales. Stud. Biol. Lab. Johns Hopkins Univ. *4*:389–430.

Arvy, L., and M. Gabe (1952) Particularites histochimiques du tube digestif de *Phascolion strombi* (Montagu). Bull. Lab. Mar. Dinard. *36*:24–31.

Awati, P.R., and L.B. Pradhan (1935) The anatomy of *Dendrostoma signifer* Selenka and de Man. I. J. Univ. Bombay *3*:102–113.

Awati, P.R., and L.B. Pradhan (1936) The anatomy of *Dendrostoma signifer* Selenka and de Man. II. J. Univ. Bombay *4*:114–131.

Bang, B.G., and F.B. Bang (1965) Mucus hypersecretion in a normally isolated non-innervated cell (I). Cah. Biol. Mar. *6*:257–264.

Bang, B.G., and F.B. Bang (1972) Mucous hypersecretion induced in isolated mucociliated epithelial cells by a factor in heated serum. Am. J. Pathol. *68*:407–418.

Bang, B.G., and F.B. Bang (1975) Cell recognition by mucus secreted by urn cell of *Sipunculus nudus*. Nature *253*:634–635.

Bang, B.G., and F.B. Bang (1980) The urn cell complex of *Sipunculus nudus:* A model for study of mucus-stimulating substances. Biol. Bull. *159*:571–581.

Bang, F.B. (1966) Serologic response in a marine worm, *Sipunculus nudus*. J. Immunol. *96*:960–972.

Bang, F.B., and B.G. Bang (1962) Studies on sipunculid blood: Immunologic properties of coelomic fluid and morphology of "urn cells." Cah. Biol. Mar. *III*:363–374.

Blitz, R. (1965) The clearance of foreign material from the coelom of *Phascolosoma agassizii*. Ph.D. Thesis. Univ. Calif. (Berkeley).

Boiledieu, D., and P. Valembois (1977) Natural cytotoxic activ-

ity of sipunculid leukocytes on allogenic and xenogenic erythrocytes. Dev. Comp. Immunol. *1*:207–216.

Brown, C.L., C. Michel, and E.J. DeVillez (1982) Properties and distribution of a chymotryptic-like enzyme from the sipunculan, *Phascolopsis gouldii*. Can. J. Zool. *60*:361–367.

Bullock, T.H., and G.A. Horridge (1965) Structure and Function in the Nervous Systems of Invertebrates. San Francisco: W.H. Freeman and Company.

Cantacuzène, J. (1922) Réactions d'immunité chez *Sipunculus nudus* vacciné contre une bactérie. Crit. Rev. Séanc. Soc. Biol. *87*:264–267.

Cuénot, L. (1900) Le *Phascolosoma* common (*Phascolosoma vulgare* de Blainv.). Zool. Descriptive Invertébrés (Paris) *1*:386–422.

Cushing, J.E., and D.K. Boraker (1975) Some specific aspects of cell-surface recognition by sipunculid coelomocytes. In W.H. Hildemann and A.A. Benedict (eds.): Immunologic Phylogeny. New York: Plenum, pp. 35–44.

Cutler, E.B., and P.E. Gibbs (1985) A phylogenetic analysis of higher taxa in the phylum Sipuncula. Syst. Zool. *34*:162–173.

Dybas, L. (1975) Cell within a cell or a circulating cell pair. Nature *257*:790–791.

Dybas, L. (1976) A light and electron microscopic study of the ciliated urn of *Phascolosoma agassizii* (Sipunculida). Cell Tissue Res. *169*:67–75.

Dybas, L. (1981a) Sipunculans and echiuroids. In N.A. Ratcliff and A.F. Rowley (eds.): Invertebrate Blood Cells 1. New York: Academic Press, pp. 161–188.

Dybas, L.K. (1981b) Cellular defense reactions of *Phascolosoma agassizii*, a sipunculan worm: Phagocytosis by granulocytes. Biol. Bull. *161*:104–114.

Eguileor, D.M., and R. Valvassori (1977) Studies on the helical and paramyosinic muscles. VII. Fine structure of body wall muscles in *Sipunculus nudus*. J. Submicrosc. Cytol. *9*:363–372.

Elyakova, A. (1972) Distribution of cellulases and chitinases in marine invertebrates. Comp. Biochem. Physiol. *43B*:67–70.

Ernst, V.V. (1970) The structure and function of the proboscis retractor muscle of the sipunculid, *Golfingia gouldii*. Ph.D. Thesis. Univ. Louisville.

Franzén, A. (1956) On spermiogenesis, morphology of the spermatozoan, and biology of fertilization among invertebrates. Zool. Bidr. Uppsala *31*:355–482.

Gardiner, S.L., and M.L. Jones (1985) Ultrastructure of spermiogenesis in the vestimentiferan tube worm *Riftia pachyptila* (Pogonophora: Obtuata). Trans. Am. Microsc. Soc. *104*:19–44.

Gerould, J.H. (1906) The development of *Phascolosoma*. 2. Studies on the development of the Sipunculidae. Zool. Jb. Anat. *23*:77–162.

Gibbs, P.E., and E.B. Cutler (1987) A classification of the phylum Sipuncula. Bull. Br. Mus. Nat. Hist. (Zool.) *52*:43–58.

Goffinet, G., M.-F. Voss-Foucart, and S. Barzin (1978) Ultrastructure of the cuticle of the sipunculans *Golfingia vulgaris* and *Sipunculus nudus*. Trans. Am. Microsc. Soc. *97*:512–523.

Gonse, P.H. (1956a) L'ovogenèse chez *Phascolosoma vulgare*. i. Définition cytologique des stades de croissance des ovocytes. Acta. Zool. Stockh. *37*:193–224.

Gonse, P.H. (1956b) L'ovogenèse chez *Phascolosoma vulgare*. ii. Recherches biométriques sur les ovocytes. Acta. Zool. Stockh. *37*:225–233.

Gonse, P.H. (1957a) L'ovogenèse chez *Phascolosoma vulgare*. iii. Respiration exogene et endogene de l'ovocyte. Effet de l'eau de mer. Biochim. Biophys. Acta *24*:267–268.

Gonse, P.H. (1957b) L'ovogenèse chez *Phascolosoma vulgare* iv. Étude chromatographique des sucres du plasma. Action des differents substrats et de malonate sur la respiration de l'ovocyte. Biochim. Biophys. Acta *24*:520–531.

Hansen, M.D. (1978) Nahrung und Fressverhalten bei Sedi-

mentfressern dargestellt am Beispiel von Sipunculiden und Holothurien. Helgolander Wiss. Meeresunters. *31*:191–221.

Hermans, C.O., and R.M. Eakin (1969) Fine structure of the cerebral ocelli of a sipunculid, *Phascolosoma agassizii*. Z. Zellforsch. *100*:325–339.

Hermans, C.O., and R.M. Eakin (1975) Sipunculan ocelli: Fine structure in *Phascolosoma agassizii*. In M.E. Rice and M. Todorovic (eds.): Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura, I. Belgrade: Naucno Delo, pp. 229–237.

Hérubel, M.A. (1907) Recherches sur les sipunculides. Mem. Soc. Zool. Fr. *20*:107–418.

Hyman, L.H. (1959) Phylum Sipunculida. In The Invertebrates, Vol. 5. New York: McGraw-Hill Book Co., pp. 610–696.

Iwamoto, H., S. Suzuki, and H. Mizobe (1988) Regulatory mechanism of contraction in the proboscis retractor muscle of a sipunculid worm, *Phascolosoma scolops*. Cell Tissue Res. *253*:15–21.

Jeuniaux, C. (1969) Nutrition and digestion. In M. Florkin and B. Sheer (eds.): Chemical Zoology, Vol. 5. New York: Academic Press, pp. 69–91.

Klepal, W. (1993) Spermatogenesis and spermatozoa of *Aspidosiphon muelleri* Sipunculida—An ultrastructural study. J. Submicrosc. Cytol. In press.

Klippenstein, G.L. (1980) Structural aspects of hemerythrin and myohemerythrin. Am. Zool. *20*:39–51.

Krohn, A. (1851) Über die Larve des *Sipunculus nudus*. Arch. Anat. Physiol. *1851*:368–379.

Kurtz, D.M. Jr., (1992) Blood and O_2 Carriers in Blood and Tissues. In C.P. Mangum (ed.): Advances in Comparative Environmental Physiology, Vol. 13. New York: Springer Verlag.

Manavalaramanujam, R. (1978a) Studies on the cuticle and related structures in Sipuncula—1. Structure of the cuticle of *Sipunculus robustus*. J. Madras Univ., Sect. B *41*:34–45.

Manavalaramanujam, R. (1978b) Studies on the cuticle and related structures in Sipuncula—2. Histochemical studies on the cuticle of *Sipunculus robustus*. J. Madras Univ., Sect. B *41*:184–197.

Manavalaramanujam, R. (1979) Studies on the carbohydrate component of the cuticle of sipunculan worms. Indian Zool. *3*:53–57.

Manwell, C. (1960) Histological specificity of respiratory pigments—II. Oxygen transfer systems involving hemerythrins in sipunculid worms of different ecologies. Comp. Biochem. Physiol. *1*:277–285.

Matsumoto, Y., and B.C. Abbott (1968) Folding muscle fibers of the *Golfingia gouldii*. Comp. Biochem. Physiol. *26*:927–936.

Metalnikoff, S. (1900) *Sipunculus nudus*. Wissenschaft. Zool. Leipzig *6*:262–322.

Moritz, K., and V. Storch (1970) Über den aufbau des Integumentes der Priapuliden und der Sipunculiden *(Priapulus caudatus* Lamarck, *Phascolion strombi* [Montagu]). Zeitschr. Zellforsch. Mikrosk. Anat. *105*:55–64.

Moya, J., and T. Serrano (1984) Podocyte-like cells in the nephridial tube of sipunculans. Cuadernos Invest. Biology (Bilbao) *5*:33–37.

Nemhauser, I., R. Ornberg, and W.D. Cohen (1980) Marginal bands in blood cells of invertebrates. J. Ultrastruct. Res. *70*:308–317.

Nicosia, S.V. (1979) Lectin-induced mucus release in the urn cell complex of the marine invertebrate *Sipunculus nudus* (Linnaeus). Science *206*:698–700.

Ocharan, F.J. (1974) Sobre los nefridios de *Phascolosoma granulatum* (Sipuncula). Rev. Fac. Ciencias *1*:21–42.

Ochi, O. (1975) An electron microscopic study on the coelomic cells of some Japanese Sipuncula. In M.E. Rice and M. Todorovic (eds.): Proceedings of the International Symposium on the Biology of Sipuncula and Echiura, I. Belgrade: Naunco Delo Press, pp. 219–228.

Ochi, O., and S. Ohnishi (1971) Fine structures of the coelomic cells in the sipunculid, *Phascolosoma scolops*. I. General observations. Mem. Ehime Univ. *6*:229–242.

Oglesby, L.C. (1982) Salt and water balance in the sipunculan *Phascolopsis goudii:* Is any animal a "simple osmometer"? Comp. Biochim. Physiol. *71A*:363–368.

Ohuye, T. (1937) On the coelomic corpuscles in the body fluid of some invertebrates. 7. On the formed elements in body fluid of some marine invertebrates which possess the red blood corpuscles. Sci. Rep. Tohoku Univ. Biol. *12*:203–239.

Ohuye, T. (1942) On the blood corpuscles and hemopoiesis of *Dendrostoma minor*. Sci. Rep. Tohoku Univ. Biol., Ser 4, *17*:187–196.

Ohuye, T., O. Ochi, and I. Miyata (1961) On the morphogenesis and histochemistry of the "urn" found in the coelomic fluid of a sipunculid *Phascolosoma scolops*. Mem. Ehime Univ. Sect. II (Sci.), Ser. B (Biol.) *4*:329–334.

Paul, G. (1910) Über *Petalosoma minuta* Keferstein und verwandte Arten, nebst Bermerkungen zur Anatomie von *Onchnesoma steenstrupi*. Zool. Jb. Anat. *29*:1–50.

Peebles, F., and D.L. Fox (1933) The structure, functions and general reactions of the marine sipunculid worm, *Dendrostoma zostericola*. Bull. Scripps Inst. Oceanogr. Tech. Ser. *3*:201–224.

Pilger, J.F. (1982) Ultrastructure of the tentacles of *Themiste lageniformis* (Sipuncula). Zoomorphology *100*:143–156.

Pilger, J.F. (1987) Reproductive biology and development of *Themiste lageniformis*, a parthenogenic sipunculan. Bull. Mar. Sci. *41*:59–67.

Pilger, J.F., and M.E. Rice (1987) Ultrastructural evidence for the contractile vessel of sipunculans as a possible ultrafiltration site. Am. Zool. *27*:152A.

Pinson, D.Y. (1990) The metanephridial system of *Themiste alutacea* (Sipuncula). Thesis. Clemson, SC: Clemson University.

Prosser, C.L. (ed) (1973) Comparative Animal Physiology. Philadelphia: W.B. Saunders Co.

Reger, J.F. (1964) Identification of two types of muscle fibre in the proboscis retractor of *Sipunculus*. J. Cell Biol. *23*:123–124A.

Reissig, M., B.G. Bang, and F.B. Bang (1979) Mucus secretion in the urn complex of *Sipunculus nudus*, abstracted. J. Cell Biol. *83*:SP2512.

Reunov, A., and M.E. Rice (1993) An ultrastructural investigation of spermatogenesis in *Phascolion cryptus* (Sipuncula). Trans. Amer. Microscop. Soc. In press.

Rice, M.E. (1969) Possible boring structures of sipunculids. Am. Zool. *9*:803–812.

Rice, M.E. (1974) Gametogenesis in three species of Sipuncula: *Phascolosoma agaassizii, Golfingia pugettensis,* and *Themiste pyroides*. Cellule *70*:295–313.

Rice, M.E. (1975) Sipuncula. In A.C. Giese and J.S. Pearse (eds.): Reproduction of Marine Invertebrates. New York: Academic Press, pp. 67–127.

Rice, M.E. (1983) Sipuncula. In K.G. Adiyodi and R.G. Adiyodi (eds.): Reproductive Biology of Invertebrates, Vol. 1: Oogenesis, Oviposition and Oosorption. New York: John Wiley & Sons Ltd., pp. 283–296.

Rice, M.E. (1989) Comparative observations of gametes, fertilization, and maturation in sipunculans. In J.S. Ryland and P.A. Tyler (eds.): Reproduction, Genetics and Distributions of Marine Organisms. 23rd European Biology Symposium. Fredensborg, Denmark: Olsen and Olsen, pp. 167–182.

Ruppert, E.E., and M.E. Rice (1983) Structure, ultrastructure, and function of the terminal organ of a pelagosphera larva (Sipuncula). Zoomorphology *102*:143–163.

Ruppert, E.E., and P.R. Smith (1988) The functional organization of filtration nephridia. Biol. Rev. *63*:231–258.

Sawada, N. (1975) An electron microscopic study on the oogenesis of sipunculan worms. In M.E. Rice and M. Todorovic (eds.): Proceedings of the International Symposium on the

Biology of the Sipuncula and Echiura, 1. Belgrade: Naunco Delo Press, pp. 169–176.

Sawada, N. (1980) An electron microscopic study on spermatogenesis in *Golfingia ikedai*. Acta Zool. (Stockh.) *61*:127–132.

Sawada, N., Y. Noda, and O. Ochi (1968) An electron microscope study on the oogenesis of *Golfingia ikedai*. Mem. Ehime Univ (Sci.) Ser B (Biol.) 6:25–39.

Schmidt, O. (1865) Über den Bau un die systematische Stellung von *Aspidosiphon muelleri* Dies. (*Lesinia farcimen* Schmidt). Mitt. Naturw. Ver. Steirm. *3*:56–66.

Selenka, E., J.G. De Man, and C. Bulow (1883) Die Sipunculiden. Reisen im Archipel Phillippinen von Dr. C. Semper. Leipzig and Wiesbaden C.W. Kreidel's Verlag, pt. 2, *4*:1–133.

Selensky, W. (1908) Untersuchungen über die sogenannten Urnen der Sipunculiden. Z. Wiss. Zool. *90*:536–595.

Serrano, T. (1987) El nefridio de *Phascolosoma granulatum* (Leuckart, 1828) (Sipuncula, Phascolosomatidae): Histologia, histoquimica e histofisiologia. Ph.D. Thesis. Universidad del Pais Vasco Euskal Herriko Universitatea.

Serrano, T., E. Angulo, A. Mateo, and J. Moya (1989) The fine structure of the nephridial coelothelium of *Phascolosoma granulatum* (Leuckart, 1828) (Sipuncula, Phascolosomatidae). Z. Mikrosk. Anat. Forsch. (Leipzig) *103*:414–424.

Serrano, T., E. Angulo, and J. Moya (1990–91) Histochemistry and ultrastructure of the nephridial granular cells of *Phascolosoma granulatum* (Leuckart, 1928) (Sipuncula). Biol. Struct. Morphogen. *3*:107–114.

Serrano, T., E. Angulo, M.A. Sevillano, and J. Moya (1990) Three types of thick myofilaments in the nephridial muscle cells of the sipunculan *Phascolosoma granulatum*. Cell Struct. Funct. *15*:73–77.

Serrano, T., and J. Moya (1982) Histochemical reactions in the nephridial epithelium of the sipunculan *Phascolosoma granulatum* Leuckart. Cuad. Invest. Biol. *3*:1–6.

Shipley, A.E. (1890) On *Phymosoma varians*. Q. J. Microsc. Sci. *31*:1–27.

Shitamori, K. (1936) Histology of the integument of *Siphonosoma cumanense* (Keferstein). J. Sci. Hiroshima Univ. Ser. B 4:155–175.

Stang-Voss, C. (1970) Zur Ultrastruktur der Blutzellen wirbelloser Tiere. II. Über die Blutzellen von *Golfingia gouldi* (Sipunculidae). Z. Zellforsch. *106*:200–208.

Stehle, G. (1952) Differenzierungen des Verdauungstraktus von *Phascolosoma elongatum* Kef. (Vorlaufige mitteilung). Ann. Univ. Saraviensis *1*:309–314.

Stehle, G. (1953) Anatomie und Histologie von *Phascolosoma elongatum* Keferstein. Ann. Univ. Saraviensis 2:204–256.

Stephen, A.C., and S.J. Edmonds (1972) The phyla Sipuncula and Echiura. British Museum (Nat. Hist.),London.

Storch, V., and K. Moritz (1970) Über die Regeneration des Integumentes aus Zellen im Ventralnervenstrang von *Phascolion strombi* (Montagu) (Sipunculida). Z. Zellforsch. *110*:258–267.

Storch, V., and U. Welsch (1972) Zur Ultrastruktur der Metanephridien des landlebenden Sipunculiden *Phascolosoma* (*Physcosoma*) *lurco*. Kiel Meeresforsch. 2:227–231.

Terwilliger, N.B., R.C. Terwilliger, and E. Schabtach (1983) Two populations of hemerythrin-containing cells in the sipunculan *Themiste dyscritum*. Am. Zool. *23*:1025.

Terwilliger, N.B., R.C. Terwilliger, and E. Schabtach (1985) Intracellular respiratory proteins of Sipuncula, Echiura, and Annelida. In W.D. Cohen (ed.): Blood Cells of Marine Invertebrates, Experimental Systems in Cell Biology and Comparative Physiology. New York: Alan R. Liss, Inc., pp. 193–225.

Tetry, A. (1959) Classe des sipunculiens. Traite Zool. *5*:785–854, 1068–1082.

Towle, A. (1962) Physiological changes in *Phascolosoma agassizii* Keferstein during the course of an annual reproductive cycle. Ph.D. Thesis, Stanford University.

Triplett, E.L., J.E. Cushing, and G.L. Durall (1958) Observations on some immune reactions of the sipunculid worm *Dendrostomum zostericolum*. Am. Nat. *92*:287–293.

Valembois, P., and D. Boiledieu (1980) Fine structure and functions of haemerythrocytes and leucocytes of *Sipunculus nudus*. J. Morphol. *163*:69–77.

Valembois, P., P. Roch, and D. Boiledieu (1980) Natural and induced cytotoxicities in sipunculids and annelids. In M.J. Manning (ed.): Phylogeny of Immunological Memory. Amsterdam: Elsevier/North-Holland Biomedical Press, pp. 47–55.

Volkonsky, M. (1933) Digestion intracellulaire et accumulation des colorants acides (Etude cytologique des cellules sanguines des Sipunculidés). Bull. Biol. Fr. Belg. *105*:136–275.

Voss-Foucart, M.F., S. Barzin, Ch. Jeuniaux, and J.C. Bussers (1977) Étude comparée de la composition chimique des régions souples et durcies de la cuticule de quatre espèces de Sipunculiens. Cah. Biol. Mar. *18*:135–145.

Voss-Foucart, M.F., S. Barzin, and C. Toussaint (1978) Étude comparée de la composition en acides amines d'extraits peptidiques de la cuticle de Sipunculiens et d'Annélides Polychètes. Arch. Zool. Exp. Gen. *118*:457–470.

Walter, M.D. (1973) Freesverhalten und Darminhaltsuntersuchungen bei Sipunculiden. Helgolander Wiss. Meeresunters. 25:486–494.

Ward, H.B. (1891) On some points in the anatomy and histology of *Sipunculus nudus*. Bull. Mus. Comp. Zool. Harv. *21*:143–182.

Wilkins, R.G., and P.C. Wilkins (1987) The coordination chemistry of the binuclear iron site in hemerythrin. Coord. Chem. Rev. 79:195–214.