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# Ultrastructure of the intestine of the bacteriophagous nematodes *Caenorhabditis elegans*, *Panagrolaimus superbus* and *Acrobeloides maximus* (Nematoda : Rhabditida)

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**Summary –** The intestine of the three free-living rhabditid nematodes *Caenorhabditis elegans*, *Panagrolaimus superbus* and *Acrobeloides maximus* was studied using transmission electron microscopy of sections made at three locations along the intestine. Between the three nematode species, ultrastructural differences in cellular components are minor. However, two cell types present in all three nematode species are positioned differently along the intestinal tract. Furthermore, two different types of secretions into the intestinal lumen were identified, one only at the anterior intestine, the second present along the entire length of the intestinal lumen. F-actin staining of fifteen whole-mount nematodes revealed the presence of a well developed, cross-shaped intestinal muscle, only present in Cephalobid species.

**Résumé – *Ultrastructure de l'intestin des nématodes bactériophages* Caenorhabditis elegans, Panagrolaimus superbus *et* Acrobeloides maximus *(Nematoda : Rhabditida)*** – L'intestin de trois nématodes libres – *Caenorhabditis elegans*, *Panagrolaimus superbus* et *Acrobeloides maximus* – a été étudié en microscropie électronique à transmission sur des sections réalisées en trois localisations différentes. Entre les trois espèces, les différences ultrastructurales concernant les éléments cellulaires sont faibles encore que les deux types de cellules présents chez les trois espèces soient positionnés différemment sur le trajet de l'intestin. De plus, deux types de substances sécrétées dans la lumière intestinale ont été identifiés, l'un uniquement dans l'intestin antérieur, l'autre tout le long de l'intestin. Des colorations *in toto* de quinze nématodes à l'aide de la F-actine, ont révélé la présence d'un muscle intestinal bien développé, en forme de croix, présent seulement chez les Céphalobides.

**Key-words :** Bacteriophagous nematodes, TEM, intestine.

The digestive system of nematodes is a straight tube starting at the mouth and ending at the anus. It is subdivided into a stomodeum (consisting of the buccal cavity and a muscular cuticle-lined pharynx and the pharyngeal-intestinal valve), the intestine and a proctodeum or rectum. The intestine and rectum are separated by a sphincter muscle. The general morphology of the intestine is uniform in nematodes and most detailed information comes from studies of marine (Deutsch, 1978; Muss, 1985; Van De Velde & Coomans, 1989), predatory (Arpin & Kilbertus, 1981; Sauer & Arpin, 1989), animal parasitic (Davidson, 1983; Wright *et al.*, 1985; Endo & Nickle, 1991) and plant-parasitic (reviewed by Geraert, 1992) nematodes. In contrast, little information is available on the ultrastructure of the intestine of terrestrial bacteriophagous nematodes (Epstein *et al.*, 1971; Popham & Webster, 1979).

Although the nematode *Caenorhabditis elegans* and its close relative *C. briggsae* are widely studied, research of the adult intestine has primarily been limited to physiological and developmental investigations.

In the present study we report on the ultrastucture of the adult intestine of three bacteriophagous nematode species.

## Material and methods

### Worms

Three free-living nematode species were used belonging to three families within the Rhabditida : *Caenorhabditis elegans* var. *Bristol* (Rhabditidae), *Panagrolaimus superbus* (Panagrolaimidae), *Acrobeloides maximus* (Cephalobidae).

### Monoxenic culture

All nematodes were sterilized for monoxenic culture using alkaline hypoclorite solution according to Sulston and Hodgkin (1988). Nematodes were cultured on *E.*

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*coli* and generally handled according to Brenner (1974). Stock cultures were kept at 20 °C.

### Transmission Electron Microscropy

*C. elegans, P. superbus* and *A. maximus* were used for T.E.M. Nematodes were washed off agar plates and kept in M9 buffer for 30 min to allow digestion of remaining bacteria. This was done because the presence of bacteria in the intestinal lumen often deforms the microvilli, making detailed observations more difficult. Young females were collected in distilled water and fixed in Karnovsky solution at room temperature (2 % paraformaldehyde, 1 % glutaraldehyde and 2.5 % acrolein in 0.2 M Na-cacodylate buffer pH 7.2; see Van De Velde & Coomans, 1989). After 30 min, the nematodes were transferred to the Karnovsky solution minus acrolein and the nematodes were cut in half and incubated in complete Karnovsky fixative overnight at 4 °C. The fixed, cut nematodes were rinsed in 0.2 M Na-cacodylate buffer, pH 7.2; for 8 h at room temperature and then postfixed for 48 h in 2 % $OsO_4$ in 0.2 M Na-cacodylate buffer, pH 7.2 at 4 °C. The nematodes were transferred to 50 % alcohol and rinsed several times until the black colour disappeared. They were subsequently transferred to 2 % uranyl-acetate in 50 % alcohol for 1 h. Dehydration was achieved using 70 %, 90 % and 100 % alcohol three times, 20 min each at room temperature. The nematodes were placed in 100 % alcohol/Spurr's resin (1:1) overnight, brought to absolute alcohol/Spurr's resin (1:2) for 8 h and transferred to 100 % Spurr's resin and left overnight at 4 °C. Polymerization was done at 70 °C for 12 h. Eighty nm-thick sections were made using a Reichert OMU-2 Ultramicrotome. Formvar-coated single slot copper grids were used. The nematodes were sectioned at three sites along the intestinal tract; immediately posterior to the pharynx, at midbody, and anterior to the anus. Sections were poststained with an LKB ultrastainer for 30 min in uranyl acetate at 40 °C and 5 min in leadstain at 20 °C. Electron microscopy was done using a Siemens Elmiskop 1A, operating at 80 kV.

### Muscle staining

Staining of the intestinal muscles was achieved by staining the F-actin microfilaments of the muscles. Staining was done according to Goh and Bogaert (1991). Worms were washed off from the agar plates in PBS (50 mM $Na_2HPO_4$, 140 mM NaCl, pH 7.2), fixed for 24 h at 4 °C in fresh 3 % dissolved paraformaldehyde in PBS, and rinsed in distilled water. Whole worms were permeabilized through a 20, 40, 60 and 70 % ethanol series, 10 min each on ice followed by rehydration through an ethanol series in the reverse order. Nematodes were then incubated overnight at 4 °C in 0.1 % Triton X-100-PBS (pH 7.2) containing 4 µg/ml phalloidin-FITC (fluorescein isothiocyanate) or phalloidin-TRITC (tetramethylrhodamine isothiocyanate) (Sigma). Treated nematodes were rinsed three times in PBS, pH 8.0 and subsequently taken through a 20, 50, 75 % glycerol-PBS series and mounted in 90 % glycerol-PBS, pH 8.0, with 1 mg/ml paraphenylene-diamine (Sigma) or 1 mg/ml pyrogallol (Sigma) when TRITC was used. Slides were sealed with clear nail polish and observed 30 min after being sealed.

To assess differences in staining intensity as a result of different permeability characteristics of the cuticle of the different nematodes, a second permeabilization procedure was also performed using the cuticle digestion as described by Link *et al.* (1992). Whole animals were washed off from agar plates, rinsed in PBS and fixed for 24 h at 4 °C in a mixture of 1 % glutaraldehyde and fresh 4 % paraformaldehyde in PBS. Nematodes were washed three times in PBS and subsequently incubated for 48 h at 37 °C in a solution of 5 % β-mercaptoethanol, 1 % Triton X-100, 125 mM Tris, pH 7.4, with gentle agitation. Animals were then treated with 2 mg/ml collagenase (Sigma type IV, 460 units/mg) in 100 mM Tris-HCl pH 7.5, 1 mM $CaCl_2$ by agitating vigourously at 37 °C for 1 h. Nematodes were then rinsed five times in PBS and incubated with phalloidin-FITC as described above. For visualization of nuclei 4,6-diamidino-2-phenylindole (DAPI, Sigma) was added to the phalloidin at a final concentration of 1 µg/ml. Specimens were observed with a Leitz microscope equipped for epifluorescence observations.

### Immunohistochemistry

The protocol used was kindly communicated to us by B. Podbilewicz (MRC-LMB). Freeze-cracking was carried out according to Priess and Hirsh (1986). Slides were coated with 0.1 % poly-L-lysine (MW > 320,000, Sigma) then a drop of nematodes (in distilled water) was put on the slide and covered with a coverslip. Excess water was removed and the slide dipped in liquid nitrogen for 2 min. The coverslip was pried off and the slide immersed in pre-cooled (– 20 °C) methanol for 5 min and then in acetone (– 20 °C) followed by rehydration through a 10 % acetone series. The slides were rinsed in PBS for 10 min, followed by PBS containing 0.5 % Tween 20 (PBS-Tween). Fixed worms on slides were incubated in PBS-Tween 20 supplemented with 2 % dried milk for 15 min to reduce background. All the antibodies were diluted in PBS-Tween-milk. Mab MH27 (generously provided by R. Waterston) was raised against *C. elegans* tissue and stains the cell boundaries (adherens junctions) of all hypodermic cells and all desmosomes in the pharynx and intestine (Priess & Hirsch, 1986; Waterston, 1988) in *C. elegans*. Nematodes on slides were incubated in monoclonal antibody MH27 (diluted 1:300) for 45 min at room temperature. The slides were rinsed three times for 5 min in PBS-Tween. The worms were next incubated in FITC-labelled goat anti-mouse IgG (Sigma) at a 1:50 dilution for 45 min. Nematodes on slides were then rinsed three

times for 5 min each in PBS-Tween and once in PBS, for 5 min, briefly immersed in distilled water, and mounted with a coverslip as described above. Specimens were observed with a Leitz microscope equipped for epifluorescence observations.

## Results

In all three species sectioned, the anteriormost intestine fills the entire nematode body cavity and the lumen exhibits a star-like transverse shape. Posteriorly, the lumen gradually becomes sausage-shaped (Fig. 1 A-D).

The lumen of the anterior intestine in the *Caenorhabditis elegans, Panagrolaimus superbus* and *Acrobeloides maximus* is widest immediately posterior to the pharynx (Fig. 1 B). This is most obvious in *C. elegans.*

Anteriorly the lumen is surrounded by four intestinal cells in *C. elegans* and *A. maximus, versus* only two cells in *P. superbus.* These anterior cells contain considerable amounts of rough endoplasmic reticulum (RER), Golgi apparatus and mitochondria, as well as a small number of lipid and yolk vacuoles. The cytoplasm is coarsely granulated in *C. elegans* and *P. superbus* while it appears more uniform in *A. maximus.* In *C. elegans* the RER seemed more ordered, while it was more abundant and exhibited a random distribution in *A. maximus.* The single most distinct difference between the nematode species is the presence of electron transparent vacuoles. Anterior cells of *C. elegans* contain considerable numbers of these vacuoles, with widely varying sizes, while *P. superbus* and *A. maximus* contain very few to none.

In cross-sections of anterior intestine of the three nematode species it was observed that the anterior cells are interdigitated with the posterior cells of the pharyngeo-intestinal valve. Nothing similar was observed more posteriorly (Fig. 1 B).

At midbody the intestine is being squeezed more *(C. elegans, P. superbus)* or less *(A. maximus)* between the epidermis and the reproductive system (Fig. 1 C). In *C. elegans* and *P. superbus* the volume of the intestinal cells is considerably less than immediately posterior to the pharynx. The cells have generally fewer organelles while the number of inclusions (yolk and lipid) increases considerably. Very few electron transparent vacuoles are evident. In *A. maximus* the cells contain RER and electron transparent vacuoles at midbody. With the exception of the ring of four cells immediately posterior to the pharynx, two cells surround the lumen of the intestine in *C. elegans* and *P. superbus* along the remainder of the intestinal tract. In *A. maximus,* however, the intestine is surrounded by a varying number of cells, the maximum observed was eight and the minimum was five.

The posterior intestine resembles the intestine at midlevel although the cells diminish in size and the number of vacuoles decreases (Fig. 1 D). Using antibody MH27, it was observed only in *A. maximus* that the number of cells surrounding the lumen immediately anterior to the anus increased compared to the number of cells surrounding the intestine at midlevel (data not shown). In *P. superbus* a dark, thick basal membrane surrounded the basal side of the intestinal epithelium, this layer was much thinner in the other two species.

The intestine of *C. elegans* is known to contain 20 cells containing a total of 32 nuclei in the adult. Using DAPI stained nematodes we counted 45-50 nuclei in the intestine of adult female of *P. superbus* and 95-100 in the intestine of adult *A. maximus* female. Since some intestinal cells are at least binucleate, the actual number of cells is probably lower.

The microvilli of *C. elegans* and *P. superbus* are identical in shape and structure (Fig. 2 A-2 B). In both species each villus is constricted at its base and maintains its cylindrical shape over its entire length. This is less so in *A. maximus* where villi tend to narrow at their tip (Fig. 2 C). In all three species the tips of the microvilli exhibit a dark cap, and differences are present in dimensions and numbers of the microvilli according to their position along the intestinal tract. In *C. elegans* the number of microvilli decreases from anterior to posterior; the length of the microvilli is 0.4 µm anteriorly, 0.8 µm at midlevel and 0.4 µm posteriorly. *P. superbus* exhibits an identical pattern as *C. elegans* with decreasing number of microvilli from anterior to posterior. The microvilli in *P. superbus* are somewhat longer : 0.5 µm anteriorly, 1.0 µm at midlevel and 0.6 µm posteriorly. In *A. maximus* the number of microvilli increases at midlevel and decreases again posteriorly. The length of the microvilli in *A. maximus* is : 1.2 µm anteriorly, 3.0 µm at midlevel and 1.0 µm posteriorly. In *A. maximus* the villus is slender and gives a less robust appearance (Fig. 2 C). On the lateral villus membrane small, fine extensions were visible emanating from the lateral villus surface (Fig. 2 C). Identical extensions have been reported for *C. elegans* (Vanfleteren, 1980) but our micrographs did not allow identification of these in *C. elegans* or *P. superbus.* The arrangement of the cytoskeleton could not be determined satisfactorily for comparison, although the internal skeleton seemed more strongly developed in *C. elegans* and *P. superbus* than in *A. maximus.* The glycocalyx in the three nematode species was visible as a grey, fuzzy coat between and sometimes above the microvilli.

### Terminal Web (TW)

The TW showed considerable differences in the three species studied. In *C. elegans,* it appeared as a continuous, thick (60-70 nm) electron dense band along the base of the microvilli in which circular apertures (diameter : 70 nm) could be observed at irregular in-

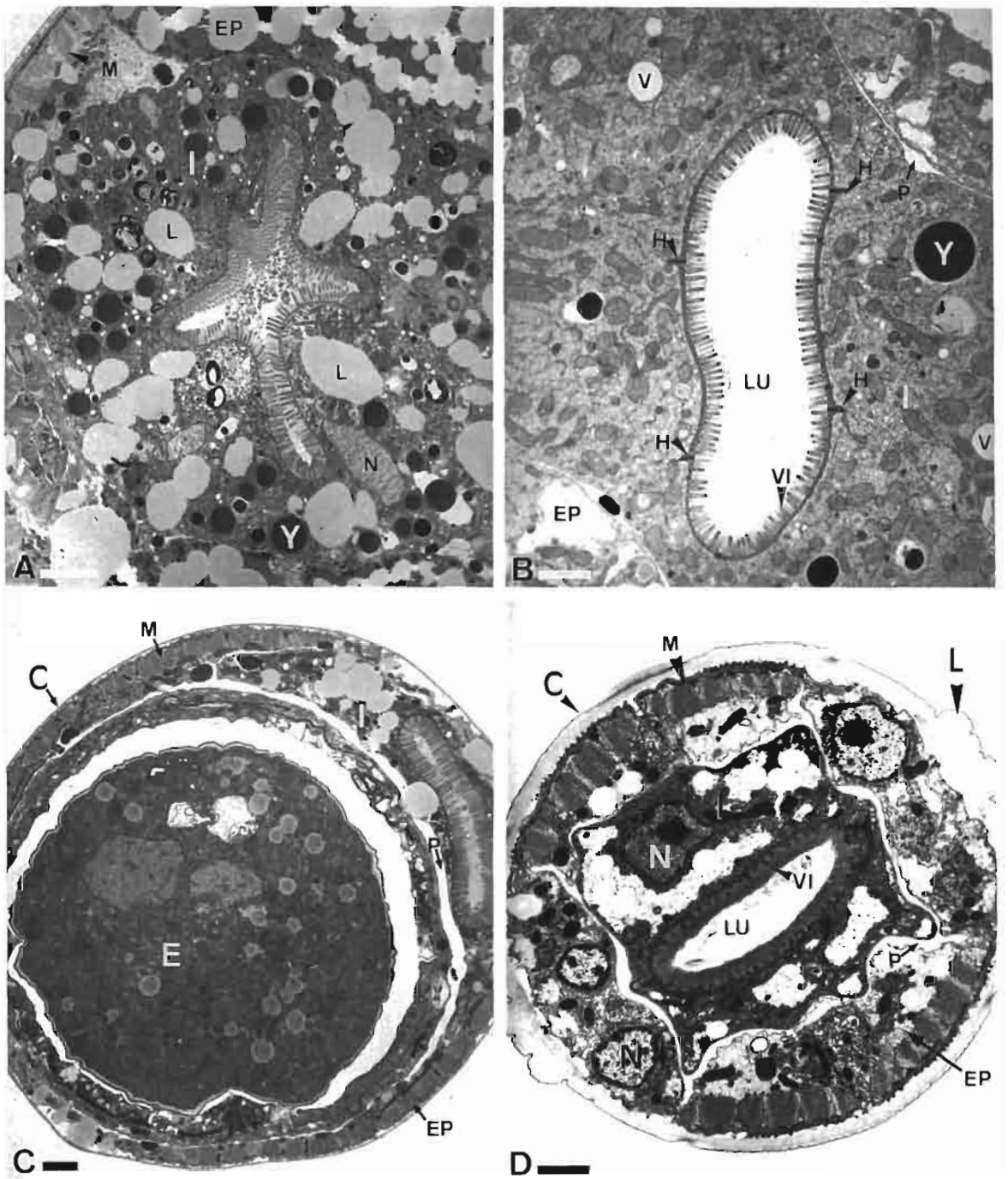


***Fig. 1.*** *Ultrastructure of nematode cross-sections at different levels along the intestinal tract. A :* Acrobeloides maximus *cross-section through anterior intestine exhibiting star shaped lumen; B :* Caenorhabditis elegans *cross section through the anterior ring of four cells immediately posterior to the pharynx; C :* C. elegans *cross-section at midlength showing intestine being squeezed between the reproductive system and the epidermis; D :* Panagrolaimus superbus *posterior intestine (C = cuticle; L = lateral fields; Ep = epidermis; P = pseudocoelomatic cavity; I = intestine; H = hemidesmoses; N = nucleus; M = muscle cells; LU = lumen; VI = microvilli; Y = yolk vacuole; L = lipid vacuole; V = electron-lucent vacuole; E = embryo.* Bar = 5 μm*).*

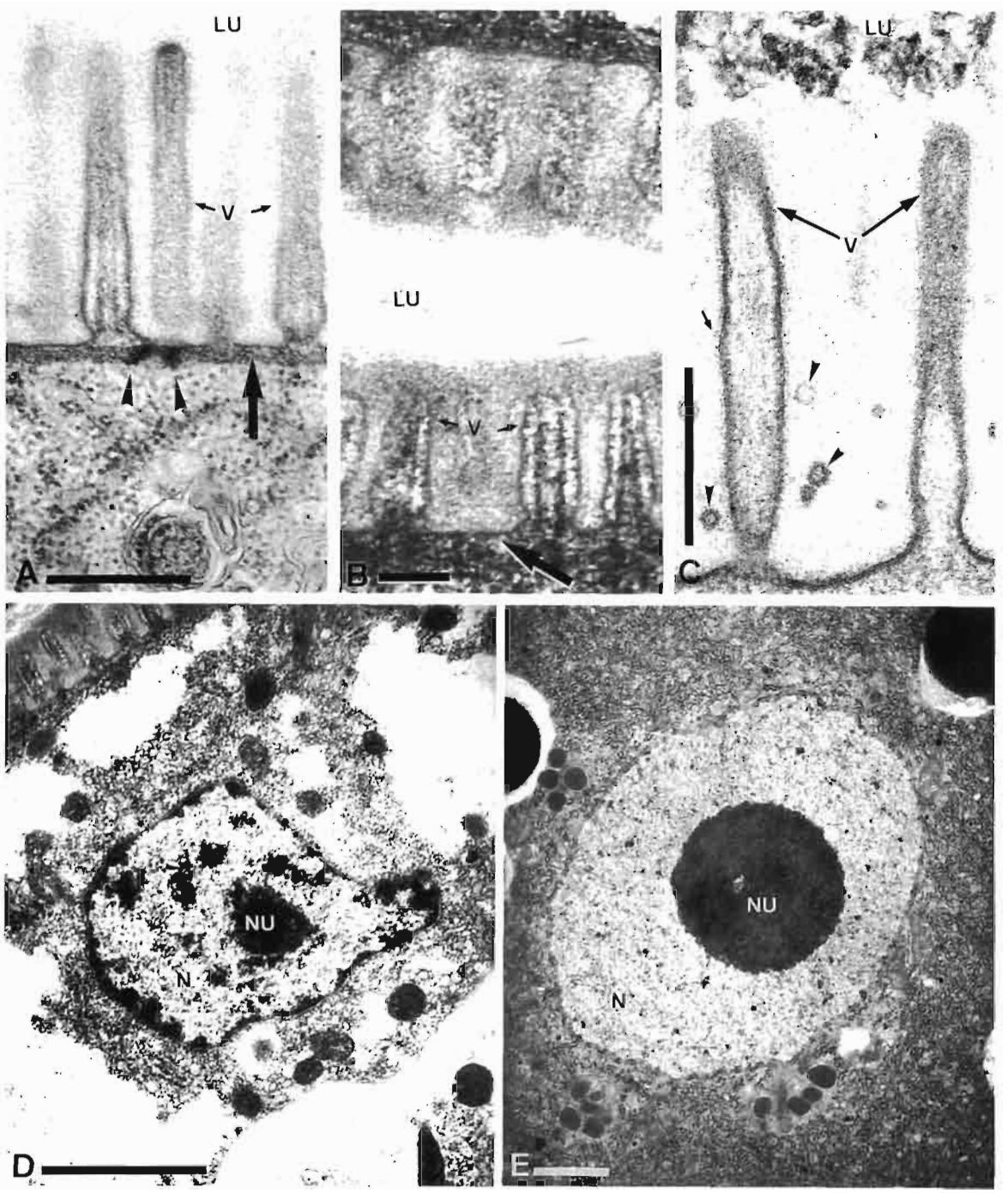


***Fig. 2.*** *Ultrastructure of intestinal microvilli shape in three rhabditid nematodes. A :* Caenorhabditis elegans *with well developed Terminal Web (arrow) and circular aperture present as two dark bands (arrowheads); B :* Panagrolaimus superbus *TW less developed (arrow), glycocalyx obvious as a grey layer between and on top of microvilli; C :* Acrobeloides maximus *absence of TW, presence of double walled vesicles (arrowheads) and remnants of double layered membranes above the microvilli tips. Lateral surface membrane possesses small fibrillar-like extensions (small arrow); D, E : Difference in nucleus and nucleolus morphology between* P. superbus *(D) showing amoeboid shape and* A. maximus *(E) more rounded shape (LU = lumen; V = microvilli; N = nucleus; Nu = nucleous.* Bar = 0.5 µm).

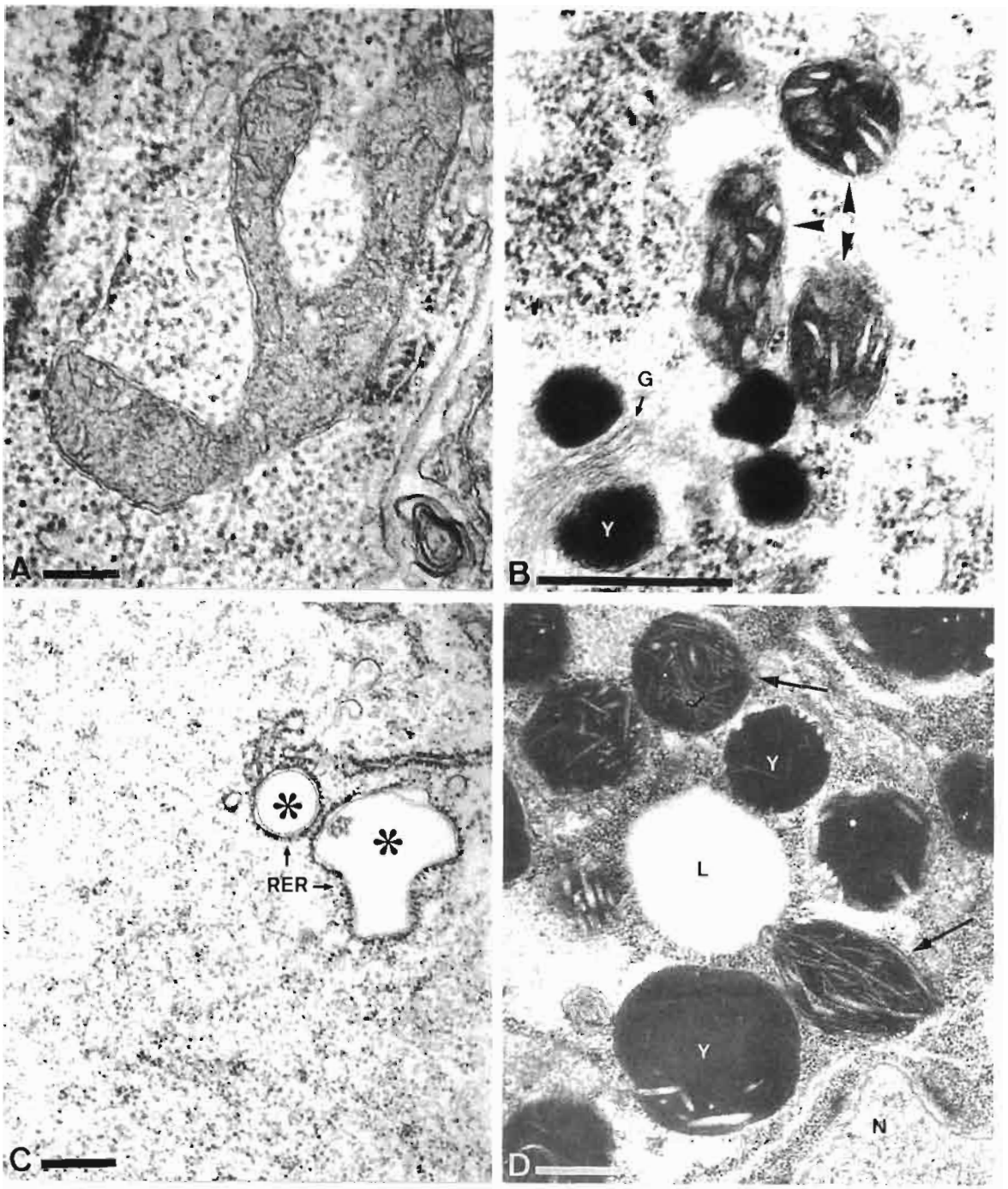


***Fig. 3.*** *Intestinal cell organelles. A :* Caenorhabditis elegans *branched mitochondria; B : Rounded mitochondria (arrowheads) present in* Panagrolaimus superbus; *C : Unidentified cytoplasmic floccular patch* (P. superbus) *bordered by RER some of which contain grossly dilated lumen (*); D : Typical cellular yolk (Y) and lipid (L) vacuoles in* Acrobeloides maximus. *Some of the yolk vacuoles contain randomly oriented tubular cavities indicate of yolk crystallization (arrows) (Y = yolk vacuole; L = lipid vacuole; G = Golgi apparatus; RER = Rough endoplasmic reticulum; N = Nucleus.* Bar = 0.5 μm).

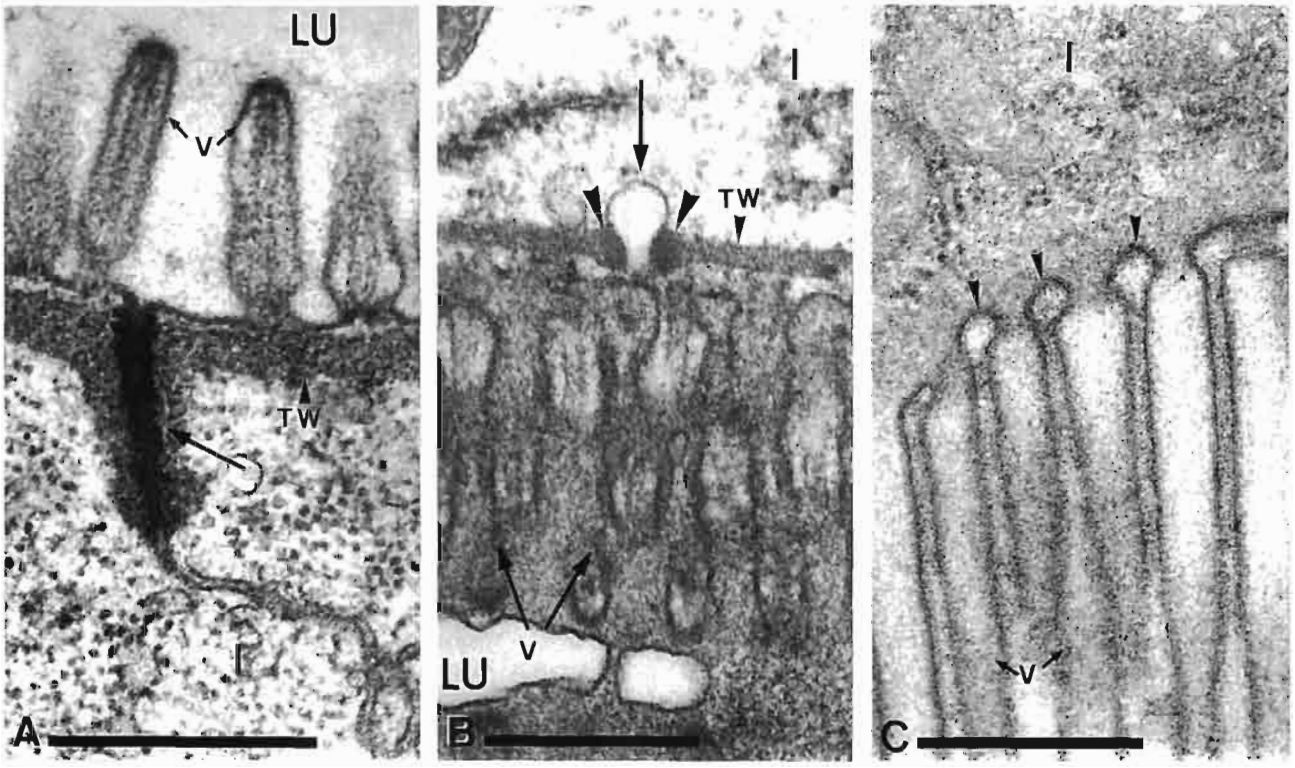


***Fig. 4.*** *A : Detail of belt desmosome (arrow) in* Caenorhabditis elegans; *B : A vesicle (arrow) can be seen passing through the circular apertures (large arrowheads) in the* C. elegans *Terminal Web, indicating possible function; C : Invaginations (arrows) at the crypt between intestinal microvilli of* Acrobeloides maximus *(I = intestinal cell; TW = Terminal web; V = villi; LU = lumen.* Bar = 0.5 μm).

tervals (Fig. 2 A). Some photographs showed vesicles emanating through these openings (Fig. 4 B). In *P. superbus,* the TW was visible as a more diffuse layer (Fig. 2 B), without circular structures. In *A. maximus,* no TW structure was observed (Fig. 2 C). These results were indirectly confirmed by F-actin staining using phalloidin. F-actin staining along the entire length of the brush border/TW area is visible in *C. elegans* as a sharp continuous line but is less intense and more diffuse in *P. superbus* and *A. maximus.* An identical result was obtained if the phalloidin staining was performed after collagenase digestion of the cuticle, thus reducing the possibility that differences among species are due to differences in permeability characteristics of the cuticle (Fig. 5 C).

Two types of secretions have been identified. The presence of fibrillar peritrophic membranes has been described (Borgonie *et al.,* unpubl.). A second type of secretion was observed in all three nematode species in the star-shaped region of the intestinal lumen, where huge numbers of small vesicles were seen between the microvilli and in the lumen (Fig. 2 C). These vesicles clearly possessed a double membrane. They were spherical close to the intestinal plasma membrane but became enlarged and ruptured once in the lumen.

The intestinal cell nuclei are distinctly more rounded and smaller in proportion to total cell content in *A. maximus* than in *C. elegans* and *P. superbus* which have big, oval to amoeboid nuclei (Fig. 2 D, E). The intestinal nuclei of all three species are larger than the nuclei in other tissues.

There were some differences in mitochondrial shape between the three species (Fig. 3 A, B). In *C. elegans,* the mitochondria were mostly sausage-shaped and sometimes branched (Fig. 3 A). In *P. superbus* and *A. maximus* all mitochondria were rounded in shape; branched and elongated forms were not observed (Fig. 3 B).

Sometimes in *C. elegans* and *P. superbus* the intestinal cell cytoplasm contained patches of floccular-like material not surrounded by any distinct layer that occasionally comprised a considerable part of the cell cytoplasm, especially in *P. superbus* (Fig. 3 C). Large amounts of RER were observed at the periphery of these patches, sometimes exhibiting a greatly enlarged lumen. On one occasion a similar, smaller patch was observed in the epidermis of *C. elegans.*

Lipid vacuoles were evident in the intestine in all three nematode species. In *A. maximus,* the intestinal cells and epidermis contained considerable numbers of such vacuoles. These vacuoles can reach considerable size and

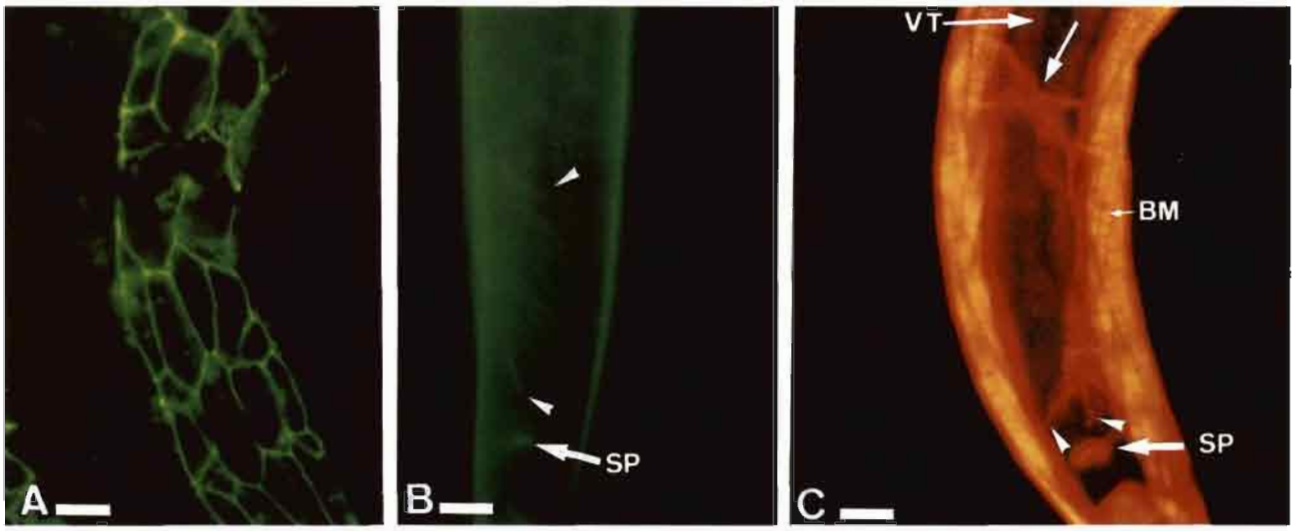


***Fig. 5.*** *A : Fluorescent visualization of MH27 antibody bound to intestinal desmosomes of squashed* Acrobeloides maximus *intestinal tissue; B : FITC-conjugated phalloidin staining of posterior intestinal muscles in* Caenorhabditis elegans *showing fine muscles around intestine (between arrowheads); C : TRITC-conjugated phalloidin staining of cross-shaped, strongly developed posterior intestinal muscle (arrow) and finer, weakly-stained muscles (arrowheads) close to the sphincter in* A. maximus. *Note the staining of the body wall muscles and faintly staining of F-actin in the intestinal microvilli/Terminal Web. Anterior top (SP = sphincter muscle; N = nucleus; BM = body wall muscles; VT = F-actin staining in the intestinal microvilli/Terminal Web.* Bar = 20 μm).

were clearly observed to be in contact with one another (Fig. 1 A). Such vacuoles in the epidermis were only occasionally observed in both *C. elegans* and *P. superbus*. Some of the yolk vacuoles present in the intestine exhibited tubular cavities surrounded by randomly oriented striations (fig. 3 D).

The intestinal cells in all three nematode species were linked to each other by belt desmosomes having identical morphology (fig. 4 A) and stained with MAb MH27 (Fig. 5 G). Desmosome staining with MH27 was considerably weaker in *P. superbus* than in the other two species.

Budding vesicles in *C. elegans* (Fig. 4 B) and deep invaginations at the base of the microvilli in *A. maximus* (Fig. 4 C) could be observed at the intestinal cell membrane. However, since no markers were used we could not determine whether it concerns secretion or endocytotic activity.

Phalloidin staining allowed comparison between the intestinal muscles positioned anterior to the intestinal-rectal sphincter among nematode species. *C. elegans* and *P. superbus* exhibited very similar positioning and structure of these muscles (Fig. 5 H). In *A. maximus,* however, a prominent cross-shaped muscle encircled the posterior intestine (Fig. 5 I). From these cross-shaped bands, several fainter muscle bands ran ventro- and dorsoposteriorly, joining the sphincter. Staining of intestinal muscles in fifteen nematode species showed that the cross-shaped muscles were only observed in nematode species of the family Cephalobidae.

## Discussion

The intestine in the three species studied is formed by a single layer of epithelial cells. At the ultrastructural level, the differences in the general intestinal morphology and cell organelles are minimal between the three species. Strong conservation of cellular structures is especially evident when considering the intestinal belt desmosomes : in all three species they exhibit identical morphology, and stain with monoclonal antibody MH27, indicating strong evolutionary conservation of these structures. No stratification of cell contents is observed, unlike in some other nematodes (Guttekova & Zmoray, 1980). Organelles are randomly scattered throughout the cytoplasm. The anterior intestinal cells occupy the entire body cavity and the lumen is wider than at midlevel and posteriorly.

All intestinal cells bear apical microvilli that vary in length and number along the intestinal tract. The structure of the microvilli is identical in *C. elegans* and *P. superbus,* but less complex in *A. maximus*. The microvilli are comparable with descriptions of microvilli in other organisms (Weiser *et al.,* 1988). Intestinal microvilli have been described in free-living nematodes (Popham & Webster, 1979), carnivorous nematodes (Arpin & Kilbertus, 1981), primitive plant-parasitic nematodes (Geraert, 1992), and in some animal parasitic nematodes (Wright *et al.,* 1985). The number of microvilli in the free-living nematodes (marine or terrestrial habitat) is dependent on the food source. Relatively few micro-

villi are present in *Chromadorina germanica* that feed upon the contents of prey pierced open by their buccal armature (Deutsch, 1978), compared to numerous microvilli present in *A. maximus* that feed on bacteria. In none of the three nematode species studied did we identify any " specialized " microvilli, nor did we observe that in the intestinal microvilli, the internal, central cisterna was continuous with the ER in the cytoplasm as shown for *Diplolaimella* sp. (Deutsch, 1978).

The presence of a well developed Terminal Web (TW), however, is apparently more variable. Although " absence " of a TW on our micrographs does not mean that there is none, it may indicate that it is not strongly developed, and therefore difficult to visualize using the fixation and staining techniques employed. The Terminal Web can be made more visible using different preparation techniques (Sandoz & Laine, 1985). A well developed TW occurs in all feeding types of nematodes, albeit rather rarely. Although the TW is considered to have an anchoring function for the microvilli (Weiser *et al.,* 1988), it remains unclear why it is so variably developed in different nematode species. A well developed TW could give the intestine of the nematode additional overall strength, especially when moving very actively, but this explanation cannot be generalized. Several nematode species we have in culture, which possess a well developed TW can hardly be considered very active (e.g. *Aphelenchoides rutgersi*), and some very active nematodes (e.g. *P. redivivus*) have a more weakly developed TW than *C. elegans*. TW structure as an antagonist for internal pressure raises the question why other species do not have such a well developed TW since all nematode species studied here possess the high internal pressure. *Caenorhabditis elegans* and *P. superbus* feed ferociously, and the food moves continuously along the intestinal tract. The possibility that a cortical network is necessary to maintain the microvilli upright during feeding is possible although it cannot be generalized to other nematodes. Some of the stylet bearing nematodes *(A. rutgersi)* have a well developed TW (Shepherd *et al.,* 1980), but it is difficult to imagine the same forces being exerted on the microvilli by the uptake of fluid through the stylet. Although the internal skeleton in the microvilli of *A. maximus* is more weakly developed and the microvilli are considerably longer than in the other species, structural strength could come from the sheer number and close packing of microvilli.

The exact structure of the circular apertures present in *C. elegans* TW is unknown. These structures are identical to those reported in the closely related species *C. briggsae* (Epstein *et al.,* 1971). The vesicles seen emanating through these apertures indicate that they may facilitate intracellular traffic from and to the intestinal plasma membrane through the dense network of TW fibrils. *Caenorhabditis elegans* and *C. briggsae* might not be the only nematodes possessing it. Himmelhoch and coworkers (1973), studying the ultrastructure of *Turbatrix aceti* mentioned the presence of openings in the TW, although no high magnification photographs of the structure are available for comparison with what is observed here.

It is remarkable that only the Cephalobidae studied have such a well developed set of cross-shaped, intestinal muscles. The possibility that it would accomodate for the larger size of the intestine (e.g. *A. maximus*) is unlikely since considerably smaller Cephalobidae (e.g. *A. nanus*) also have the same structure. The cross-shaped muscles could allow for the complete constriction of the intestine, thereby compartimentalizing the posterior intestine to prevent emptying of the entire intestine content during defecation (Thomas, 1990).

In *C. elegans* and *P. superbus* the intestine contains two multifunctional cell types. The first cell type is capable of secretion and intracellular digestion. It contains a very limited number of yolk and lipid vacuoles, various amounts of electron transparent vaucoles and huge amounts of RER, Golgi and mitochondria. These cells are positioned immediately posterior to the pharynx in *C. elegans* and *P. superbus*. The second type is still capable of secretion and digestion but is mainly a storage cell. These cells contain huge amounts of yolk and lipid vacuoles. Some of the yolk vacuoles exhibit identical randomly oriented tubular cavities indicative of the onset of yolk crystallization as reported by Yoshizaki (1990) in *Xenopus*. Fewer Golgi, RER and mitochondria are present than in the first cell type. Electron transparent vacuoles are rarely or never observed in these cells. They line the rest of the intestine in *C. elegans* and *P. superbus*. In *A. maximus* two cell types can also be identified but whith different characteristics. The first type is also found immediately posterior to the pharynx. It contains very few yolk vacuoles but huge amounts of lipid vacuoles, Golgi, RER and mitochondria. It differs from the anterior cells of *C. elegans* and *P. superbus* in that it lacks the electron transparent vacuoles. The second cell type is present along the remainder of the intestine and is identical to the cells of the other two nematode species except that these cells do contain numerous, small electron transparent vacuoles. A similar division of the intestine as observed for *C. elegans* and *P. superbus* is also reported for the marine nematode *Diplolaimella* sp. (Deutsh, 1978; Van De Velde & Coomans, 1989).

Two types of secretion products were observed. The first occurred mainly at the anterior intestine and consisted of small, spherical, double-walled vesicles emanating at the plasma membrane into the lumen, where they swelled and eventually burst. The observation that they appeared close to the intestinal plasma membrane excludes the possibility that they originated from the pharyngeal glands and were transported into the anterior intestine. No information could be obtained about the content of the vesicles. The fact that they were observed being secreted in huge amounts in the anterior intestine suggests, however, that they contained diges-

tive enzymes, since they would encounter the incoming food as soon as it reaches the intestine. This is an important consideration since nematodes like *C. elegans* are known to ingest huge amounts of bacteria but also defecate the entire content of the intestine every 40-45 seconds at 20-25 °C (Thomas, 1990) leaving little time for enzymes to act. Small secretory vesicles were also reported in the anterior intestine in *Diplolaimella* sp. but were thought to be involved in maintaining the " fibrillar matrix " present on top of the microvilli (Deutsch, 1978).

Deutsch (1978) reported only one cell type in *C. germanica* and found that these cells go through different phases of activity at different nematode life stages. Being first absorptive and digestive, they then become secretory and package digestive enzymes which are released. The cell ultimately dies and is sloughed into the lumen. An indication of such a cycle is the presence of a variety of membrane bound vacuoles representing different stages in food digestion. Only young females were used for the present study, and we did not find any indications of a possible cell cycle, although biochemical differences as a result of aging in intestinal cells have been described in *C. elegans* (Bolanowski *et al.*, 1983).

As reported for several marine nematodes (Nuss, 1985), whatever the number of cells surrounding the lumen, there were no differences in cell contents between cells in the same cross-section. This is in contrast with Gastrotrichs, believed to be closely related to the Nematoda, where different cell types are present at the dorsal side of the mid-intestinal region (Teuchert, 1977). Another difference is that in Gastrotrichs all the intestinal cells are interdigitated with one another. The only interdigitation observed in our nematodes was between the posterior cells of the pharyngeal-intestinal valve and the ring of four cells in the anterior intestine, and was also reported for the nematode *G. disjuncta* (Van De Velde & Coomans, 1989). Interdigitation is believed to confer more structural strength (Teuchert, 1977).

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