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GUT STRUCTURE AND DIGESTIVE PHYSIOLOGY OF THE FREE-LIVING MARINE NEMATODES, *CHROMADORINA GERMANICA* (BÜTSCHLI, 1874) AND *DIPLOLAIMELLA* SP.

by

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ABSTRACT

Histochemical, cytochemical, and ultrastructural methods were used to study 1) the digestive tracts of two species of free-living marine nematodes, Chromadorina germanica and Diplolaimella sp. and 2) their ingestion and digestion of different species of algae and bacteria. In each species the digestive tract consists of a buccal cavity, esophagus, esophageal-intestinal valve, intestine, and rectum. Each digestive tract is described and there are a number of differences between the two species. Diplolaimella sp. has a small, unarmored buccal cavity. The esophagus is described. There are three esophageal gland cells and a small esophageal muscular bulb. The esophageal-intestinal valve consists of a second muscular bulb with several gland-like cells protruding into the bulb and posterior to it. The intestinal cells are of two types: the anterior four cells are secretory and the remaining cells are absorptive. There are unusual microvilli lining the luminal borders of these cells that contain a cisterna along the central axis that is continuous with the cisterna of the endoplasmic reticulum. This basic structure is shown to change with the nutritional state of the nematode. An unusual, structurally complex fibrillar matrix lines the luminal cell borders. Its structure is

described. An amoeba is found residing in the intestinal lumen wherever food is present. The process of digestion is described. Diplolaimella sp. ingests whole cells or large cell fragments. Digestion begins chemically in the intestinal lumen and is completed intracellularly in the pigment granules which are demonstrated to be lysosomes.

Chromadorina germanica has a larger buccal cavity with two sets of three teeth. Its esophagus is similar to that of Diplolaimella sp. but its gland cells and bulb are larger. The esophageal-intestinal valve consists of several protein-synthesizing cells. The intestine consists of secretory cells that are similar throughout its length. These cells have only a few short microvilli. They have a variety of cell inclusions, some of which are involved in digestion. The process of digestion is described. C. germanica pierces its food and ingests the cell contents from cells greater than 2.2 μm in diameter. Digestion proceeds in the intestinal lumen with completion in lysosomes. There is evidence that much of digestion occurs intracellularly. The intestinal cells go through a cycle of intracellular secretion and lysosomal digestion and then extracellular secretion, of the holocrine type, before dying.

Evidence is presented to show that Diplolaimella sp. ingests whole bacteria and small chlorophytes and large cell fragments of the larger chlorophytes but may not ingest diatoms. C. germanica is able to ingest the cell contents of diatoms and large chlorophytes but may not

ingest small chlorophytes or bacteria. As a result of the various differences in digestive physiology and gut ultrastructure between the two species, each species is adapted to a different diet. There is a discussion of how these differences may enable the two species to coexist.

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INTRODUCTION

A basic question of ecology is "Why are there so many species" (Hutchinson 1959)? This question is especially appropriate for the Nematoda, the free-living species of which occur in virtually every known benthic habitat. There have been several approaches to understanding why large numbers of nematode species live in any given benthic habitat. In studies of field populations, variables such as temperature, oxygen concentration, and salinity have been related to the abundance and distribution of nematode species (e.g., review by McIntyre 1969; Pamatmat 1968; Tietjen 1969, 1971; Coull 1970; Meyers et al. 1970; Warwick and Buchanan 1970; Warwick 1971; Ward 1973; Wieser and Schiemer 1977). Other approaches have used a laboratory setting to determine nematode requirements for or tolerances to environmental variables (e.g., Tietjen et al. 1970; Gerlach and Schrage 1971, 1972; Tietjen and Lee 1972; Hopper et al. 1973; Ott and Schiemer 1973; Schiemer and Duncan 1974).

An underlying concept of these approaches is that each species is defined by a series of environmental and biological parameters which together make up its niche and that no two species have the exact same niche requirements. The many parameters of the benthic environment vary spatially and temporally, creating many separate microenvironments

each suitable for different species. Food is among those factors, important in determining a nematode's niche, which do vary spatially and temporally. In shallow benthic environments a large array of potential foods for nematodes exists (e.g., Lee et al. 1973, Fenchel 1969). Many of these potential foods, especially algae, bacteria, and protozoa, exhibit great heterogeneity with respect to their spatial and temporal distributions (Lackey 1961). The heterogeneous distributions of different food species may account for much of the variety and large numbers of nematode species found in the benthos if nematodes are feeding on these different foods.

Nematodes are known to eat diatoms and algae (Chitwood and Timm 1954, Webb 1956, Perkins 1958, Wieser 1960, Tietjen and Lee 1973), bacteria (Webb 1956, Perkins 1958, Chitwood and Murphy 1964, Hopper and Meyers 1966a, Tietjen 1967, Tietjen and Lee 1972, Tietjen et al. 1970), fungi (Meyers et al. 1973), yeast (Hopper and Meyers 1966a, b), protozoa and micrometazoa (McIntyre 1969), and detritus (Tietjen 1967, Meyers et al. 1970). It has been suggested that nematode species which are found in the same benthic area live in different microenvironments and, presumably, on different diets (Tietjen and Lee 1972, 1973, 1977). It is possible, then, that nematodes have narrow diets. Each nematode species' diet would consist of only one or several species of the foods available to it.

There is little understanding of why narrow diets

are a necessary strategy for marine nematodes. Such resource partitioning is the stratgy of food specialists--they consume only the food type conferring maximum fitness (Rapport and Turner 1977). Food quality does appear to be important in conferring maximum fitness for some micro and meiofaunal species, such as copepods (Lee et al. 1976, Sellner 1976) and ciliates (Rubin and Lee 1976). Wieser theorized about the likelihood of nematodes being feeding specialists (having narrow diets) living in similar microenvironments but existing on different foods in the benthos (Wieser 1953, 1960). He grouped nematodes into feeding types according to the size of the buccal cavity and the extent and type of buccal armature. These groups are: selective deposit feeders, having a very small buccal cavity; nonselective deposit feeders, having a larger buccal cavity; epistrate feeders, having a small buccal cavity with teeth; and predators, having a large buccal cavity with large teeth. He reasoned that differences in nematode diets could be due to different mouthparts and therefore to differing abilities to ingest various food species. If nematodes can be grouped as feeding types, then selective feeding should be an observable phenomenon. Tracer feeding techniques have been used to demonstrate that selective feeding may occur in several nematodes (Tietjen et al. 1970, Tietjen and Lee 1973, 1977). Finally, if nematodes are feeding specialists, having narrow diets, then how and why does this strategy ensure their success?

It is generally assumed that nematode digestion, aside from the process of ingestion, conforms to a similar pattern throughout the taxon--free-living soil, freshwater, and marine as well as parasitic forms alike (reviewed by Lee 1965, Bird 1971, Lee and Atkinson 1976). The digestive tract is a straight tube differentiated into buccal cavity, esophagus, esophageal-intestinal valve, intestine, and rectum. There are three esophageal gland cells that empty their contents into the esophageal lumen. The esophagus is usually a pumping organ which forces food into the intestine. The intestine consists of a single layer of cells. These have microvilli and pigment granules whose function is unknown. Digestion is extracellular in nearly all species, although an intracellular phase does occur in some species (e.g., Colam 1971a, c). Seemingly slight differences in the digestive physiology of marine nematode species may reflect adaptations to different diets. The purpose of this study was to compare the digestive physiologies of two species of marine nematodes, including the microanatomy of their digestive tracts, to see ultimately how they might coexist in the benthos and to examine the extent and manner of their resource partitioning.

MATERIALS AND METHODS

Nematode Culturing

Two nematode species, originally isolated from a salt marsh at Towd Point, Southampton, Long Island New York, were cultured for this study: *Diplolaimella* sp. and *Chromadorina germanica* Bütschli 1874. Nematodes were maintained in plastic petri dishes with an overlay of 10 ml of 0.45 µm HA millipore-filtered sea water (mfsw) with a salinity of 26°/oo. A mixture of an antimycotic/antibiotic (penicillin 100 U/ml, fungizone 0.25 mcg/ml, streptomycin 100 mcg/ml) was added to the medium. The cultures were maintained at 20°-24°C under a light regime of 18 hr light/6 hr dark. Approximately 0.1 ml of suspended food organisms, at a concentration of 10^8 cells/ml, were added to each dish. These foods were obtained from stock cultures of algae grown on "S" medium (Lee et al. 1970). The seawater overlay was changed once a week. Nematode cultures were transferred about once a month. In addition to the added foods, other species of algae and bacteria grew in the culture dishes and thus cultures were considered to be agnotobiotic.

Electron Microscopy

An initial series of fixation schemes were tested on whole animals to develop the fixation procedure for routine ultrastructural studies. For these experiments, the

following stock fixatives were used: 70% glutaraldehyde, 4% osmium tetroxide, 20% formaldehyde, and 100% acrolein (all obtained from Polysciences, Inc.). All fixation schemes tested are summarized in Table 1. Fixation with formaldehyde alone was not tried, despite its use by Lippens (1974) on C. germanica, because the preservation of fine structure was not satisfactory.

The procedures finally adopted were as follows. Whole Diplolaimella sp. were fixed for 2 hr at 4°C in 3% glutaraldehyde in sea water (Table 1). Specimens were rinsed briefly in mfsw and post-fixed in 2% osmium tetroxide in sea water (Table 1) for 2 hr at 4°C. Whole C. germanica were fixed for 1 hr at 4°C in 3% glutaraldehyde in sea water but immediately after the fixative was added, the cuticle was cut open by either partially cutting through the nematode or by cutting off the anterior end of the nematode. Specimens were then processed as above.

After post-fixation, specimens of both species were dehydrated with acetone using the sequence 30% acetone for 10 min at 0°C, 70% acetone overnight or up to two days at -11°C, 90% acetone for 15 min at 4°C, three changes of 100% acetone 15 min each at 20°C. Specimens were placed in a mixture of 1:1 acetone to Spurr's embedding medium (1969), placed under vacuum for 45 min, and left uncapped in the hood for an additional 45 min. Finally, specimens were embedded in fresh plastic and polymerized at 70°C for 24 hr.

Primary fixation was carried out in small glass wells

which were in turn placed in petri dishes to prevent evaporation. Fixative was removed carefully by Pasteur pipette and several drops of 1% agar in mfsw at 47°C were added to the well. This was immediately mixed with a metal spatula to suspend the nematodes in the agar matrix. When the agar had hardened, it was transferred to a glass vial filled with the appropriate rinse solution. Processing continued with nematodes suspended in this agar for all steps up to and including embedding. To embed, small chunks of agar containing specimens were cut out with a razor and forceps under a dissecting scope and placed in flat embedding trays.

Light gold to silver to gray sections were prepared with an LKB ultramicrotome using a diamond knife. Sections were mounted on uncoated 200-mesh or 300-mesh copper grids (Polysciences, Inc.), stained with saturated aqueous uranyl acetate followed by 3% lead stain (Sato 1967), and viewed with a Philips 300 Electron Microscope at 80 kv. Kodak Electron Image plates were used for taking pictures.

Feeding Experiments

For this series of experiments, nematodes were fed a variety of different potential food species to test nematode ability to ingest and digest each food. About 30-50 adult nematodes were gently pipetted into sterile petri dishes taking care to transfer very little of the medium into the new dish. Individual microfloral species were harvested from stock cultures by centrifugation, washed and resuspended in 0.2 ml mfsw. The food cells chosen for

these experiments included six algal and two bacterial species and they were fed to nematodes as single foods. For controls, nematodes were pipetted into dishes without food. Dishes were examined and counted daily for live nematodes for up to 5 days. Some nematodes were removed after 1-2 days and processed for the study of gut contents. Food cells were processed along with the nematodes.

Histochemistry and Cytochemistry

Specimens were fixed in 3% formaldehyde in sea water for 2 hr. Specimens were washed briefly in mfsw and incubated for nonspecific esterase activity using the indoxyl acetate method of Holt (1958); lipase activity using Tween 80 (Gomori according to Pearse 1961); and arylamidase activity (Burstone and Folk 1956). Controls included specimens incubated without the substrate and after heat inactivation at 100°C for 5 min. Whole mounts were examined with the light microscope.

For visualization at the electron microscope level, nematodes were fixed in 1% glutaraldehyde in sea water for 15 min, washed with mfsw 1 min, incubated for acid phosphatase (Barka and Anderson 1962) at room temperature for 15 min, rinsed in mfsw, and then fixed again in 3% glutaraldehyde in sea water for 30 min. Routine procedures were followed for post-fixation, dehydration, and embedding.

A variety of procedures were used to test for the presence of glycosaminoglycans in a fibrillar matrix found to be lining the luminal borders of the intestinal cells of

Diplolaimella sp. Specimens were fixed according to a method adapted from Shepard and Mitchell (1976) in 3% glutaraldehyde in sea water (Table 1) and 0.1% Toluidine blue O, rinsed and either post-fixed routinely or not post-fixed. Specimens were also stained according to a method modified from Dystra and Aldrich (1976): nematodes were fixed in 3% glutaraldehyde in sea water and 1% Alcian blue. Then specimens were cut transversally through the intestine and post-fixed in 2% osmium tetroxide in sea water (Table 1) and 0.05% Ruthenium red in sea water or 0.05% Ruthenium red in 0.1 M cacodylate buffer with 0.75 M sodium chloride. Specimens were also fixed routinely, cut transversally through the intestine, and post-fixed in 2% osmium tetroxide and 0105% Ruthenium red in sea water, as modified from Luft (1971).

For visualization of surface proteins in the fibrillar matrix of Diplolaimella sp., specimens were fixed in 3% glutaraldehyde in sea water and 8% tannic acid in 0.015 M calcium chloride and routinely rinsed and post-fixed (Fedorko and Levine 1976). For histochemical procedures applied to free-floating sections, nematodes were fixed routinely and dehydrated and embedded without post-fixation. Test for glycoproteins were made on sections collected on uncoated grids and immersed for 5 min in 0.5% phosphotungstic acid in 0.1 N HCl, pH about 1 (Quintarelli et al. 1973).

RESULTS

Ultrastructure of the Digestive Tract: *Diplolaimella* sp.

Diplolaimella sp. has a typical nematode digestive tract, consisting of buccal cavity, muscular esophagus, esophageal-intestinal valve, intestine, and rectum.

Buccal Cavity

The buccal cavity consists of an anterior and posterior lumen separated by a short narrow channel (Fig. 1). The anterior buccal cavity is a circular opening approximately 1 µm in diameter surrounded by 12 rounded cuticular knobs. Both anterior and posterior buccal cavities are lined by a nonsculptured moderately electron-dense cuticle continuous with the elaborately sculptured external cuticle covering the body wall. The walls of the anterior buccal cavity consist of two cell types. Anteriorly, hypodermal cells occur beneath the cuticle of the buccal cavity. Posterior to the hypodermal cells are muscle bands attached at one end to the cuticular lining of each lumen of the buccal cavity. The other end radiates out to attach to the external wall of the esophagus so that these muscles run transversally. Hemidesmosomes attach the muscle filaments to the cuticle.

Esophagus

The posterior buccal cavity leads into the

triradiate esophagus which is cylindrical in shape anteriorly, but forms a bulb-like swelling at its posterior end. The esophagus is differentiated into three longitudinal sectors that are separated by the rays of the esophageal lumen. Each sector consists of: 1) two radial muscle bands, 2) an esophageal gland cell, and 3) at least one enteric nerve. There are also "marginal cells" between each sector.

Each radial muscle consists of filaments which run radially between the lumen wall and the outer periphery of the esophagus. The filaments form hemidesmosomes at each end so that the outer hemidesmosomes occur on the external wall of the esophageal lumen. The noncontractile portion of the muscle cells consists of mitochondria, sarcoplasmic reticulum, and glycogen particles. The mitochondria are generally positioned between the muscle filaments at the external wall of the esophagus. The muscles of this cylindrical portion of the esophagus are able to contract and thereby temporarily widen the lumen. This presumably causes a temporary vacuum, an action, which in conjunction with the more powerful action of the bulb muscles, helps move food into and down the esophagus.

One esophageal gland cell runs anteriorly from the basal portion of the pre-bulbar part of the esophagus in each of the three esophageal sectors. The gland cells have secretory granules throughout most of their length, from the anterior end of the gland. Towards the posterior third of

each gland is a nucleus. Posterior to the nucleus are the rough endoplasmic reticulum and Golgi apparatus of each cell. The dorsal esophageal gland opens into the posterior buccal cavity through a short cuticle-lined duct (Fig. 2). This gland is filled with membrane-bounded very electron-dense spherical granules. Short cuticular-lined ducts lead from the two subventral glands into the esophageal lumen about midway along its length (Fig. 3). These two glands contain identical-looking secretory granules which appear to be filled with an electron-transparent material, although some smaller denser granules also occur. Judging from the locations of the two types of granules relative to the secretory duct, the final secretory product appears to be the electron-transparent material. The secretory products of the three gland cells are probably squeezed out of the cell, through their respective ducts, when the muscle filaments surrounding the cell contract during ingestion.

The posterior bulb region of the esophagus is wider in diameter than the cylindrical region of the esophagus. The gland cells do not extend into this region. The muscle filaments are longer here than in the cylindrical region and they are well-supplied with mitochondria, especially at the external wall of the esophagus. The main force used to pump or suck in food is applied by the bulb muscles. These muscle filaments measure 3 μm in length whereas those of the cylinder are 2.6 μm in length.

The apices of the esophageal rays in the marginal regions are bordered by marginal cells (Fig. 2). Each marginal cell encloses the ray on both sides and extends from alongside each ray to the outer wall of the esophagus. Two to three sets of noncontractile filamentous bands run radially from the apex of the ray to the outer wall of the marginal cell. The bands are attached at either end by hemidesmosomes. These bands are probably noncontractile stabilizing structures that help to maintain the triradiate shape of the esophageal lumen. The marginal cells have extensive rough endoplasmic reticulum with distended cisternae, numerous mitochondria, and some Golgi complexes. The general appearance is one of active protein synthesis, although the exact nature of the membrane-limited granules occasionally found in the marginal cells is still unknown.

Esophageal-Intestinal Valve

Between the esophagus and the beginning of the intestine is a cuticular-lined esophageal-intestinal valve consisting of several cells (Fig. 5). The valve is separated from the esophagus by a small band of encircling muscle filaments. The valve also has sets of muscle filaments which run radially from the cuticle lining the valve lumen to the external wall of the valve. Just posterior to the muscle cells are several secretory cells or gland-like cells which form the rest of the valve. These secretory cells protrude anteriorly into the valve muscle bands for part of their length. They contain membrane-bounded inclusions

filled with fibrillar and electron-transparent materials (Fig. 4) or electron-dense materials (Fig. 5). The muscles of the valve may, because of the position of these secretory cells squeeze secretions out of the cells into the valve lumen when the muscles contract. No ducts were found, but the posterior valve lumen usually contained several dense granules that could have originated from these secretory cells. The extreme posterior end of the secretory cells also have flattened projections spanning the valve lumen and separating it from the intestinal lumen. These projections or flaps consist of cuticle on the valve lumen side and secretory cell cytoplasm on the intestinal lumen side. Fibrillar material lines the secretory cell cytoplasm where it borders the intestinal lumen. Together with the muscles of the valve, the flaps may prevent regurgitation of food. The basal lamina surrounding the valve exteriorly is continuous with that surrounding the esophagus and the intestine.

Intestine

The intestine is formed by a single layer of epithelial cells. In any cross-section only two cells circumscribe the intestinal lumen. These cells have very little stratification and exhibit cell organelles scattered at random throughout the cell.

The first two anterior sets of cells are typical secretory cells (Fig. 6). They each have a well-developed and extensive rough endoplasmic reticulum with distended

cisternae filled with a medium electron-dense product. There are several Golgi complexes. The nucleus of each cell is central and contains finely granular material with denser material near its periphery. The outer nuclear membrane is studded with ribosomes. A few electron-dense cell inclusions are scattered in the distal regions of the cells. The microvilli are approximately 0.12 μm in diameter and 0.5 μm in length. They are closely packed at the cell surface bordering the intestinal lumen. Microfilaments are oriented along the longitudinal axes of the microvilli (Fig. 7). In some specimens microfilaments were not found in the microvilli--a reflection of the "not-feeding" nutritional state of the animal. Instead, each microvillus (Fig. 11) had a cisterna along its central axis that was 0.02-0.04 μm in diameter and continuous with the endoplasmic reticulum. The cisterna ended blindly near the tip of the microvillus. A thin basal lamina bounds the intestinal cells at their external borders.

The remaining intestinal cells are all similar in structure and are not specialized along the length of the intestine (Fig. 9). Each cell has a well-developed rough endoplasmic reticulum and large numbers of mitochondria which are distributed throughout the cell. Occasionally, a multivesicular body occurs in these cells. Much of the cell volume is taken up by cell inclusions which have been described in the literature as "pigment granules." These are single membrane-bounded spheres containing very electron-

dense materials. Some of these granules are positive for acid phosphatase, showing that some are actually lysosomes (Fig. 8). Less electron-dense granules are found peripheral to the lysosomes (Fig. 39). They are not acid-phosphatase positive but may represent an earlier stage in the production of the lysosomes. Glycogen particles are also found in this region of the cell.

The luminal surfaces of these intestinal cells are modified to form the very unusual microvilli which were sometimes found in the anterior four intestinal cells. The microvilli are regularly spaced but not as closely packed as in the anterior cells. In well-fed specimens, these cells lose their usual appearance of a clearly defined cisterna along the central axis of each microvillus. Instead, the microvilli appear swollen and more electron-transparent (Fig. 25). Because these intestinal cells lack any secretory apparatus, they are most probably absorptive cells.

The entire intestinal lumen is coated along the luminal surfaces of the cells with a well-organized fibrillar matrix (e.g., Fig. 9). In transverse section it sometimes appears to be formed into four ridges that protrude into the lumen and four valleys or rays. Within the fibrillar matrix three zones of varying electron density could be distinguished. There is a narrow dense zone at the lumen-matrix interface which occasionally resembles a membrane but is not a typical unit membrane. There is a less dense homogeneously fibrillar matrix just under this zone.

The third zone, closest to the intestinal cell membrane, occupies the largest volume of the fibrillar matrix. This zone of the matrix is fairly uniformly diffuse but within it are electron-dense fibrillar globules, parallel sheets of fibrils, and membrane-bounded vesicles (Figs. 7, 9, 25). The vesicles appear to contain the same fibrillar material which is found outside them. These vesicles are most numerous at the very beginning of the intestine and sometimes occur in the matrix lining the rest of the intestinal cells. Their origin may be the anterior secretory cells of the intestine. The vesicles were found between the microvilli at their bases, throughout the inner matrix zone, and especially collected just beneath the middle zone. They may play a role in the maintenance and renewal of the fibrillar matrix.

The chemical composition of the matrix is unknown. Tests for glycosaminoglycans were negative. These molecules may be present in too low a concentration for visualization by the methods used. However, at a pH of about 1, there was staining of the narrow zone of the lumen-matrix interface with phosphotungstic acid applied to thin sections. The external cuticle also stained under these conditions. It is possible, then, that the lumen-matrix interface zone has proteins similar to those found in the cuticle.

There are specialized microvilli located immediately adjacent to the intercellular junction between the two circumluminal cells and in the area of the cell membrane

equidistant between the two cell junctions. These four sets of longitudinally aligned microvilli are filled with microfilaments running along the central axis of each. The tips of these microvilli have a very electron-dense cap just under the plasma membrane of the microvillus (Fig. 10). Trailing from the tip of each specialized microvillus is dense fibrillar material which is continuous with the middle zone of the fibrillar matrix. The microfilaments in these microvilli may anchor and stabilize the middle zone of the matrix. These microvilli may function to maintain the physical integrity of the matrix.

The terminal intestinal cells have extensive cell junctions and few short irregularly spaced microvilli. The latter have electron-dense cytoplasmic caps just under the cell membrane of the tip of the microvillus. The rectum is cuticular-lined and has muscle processes surrounding it. The lumen is very narrow.

Ultrastructure of the Digestive Tract: *Chromadorina germanica*

Buccal Cavity

C. germanica has a larger mouth than *Diplolaimella* sp. (Fig. 13). The mouth measures approximately 2.9 µm in diameter. An unusual set of small pads, analogous to lips, surround the buccal cavity (Fig. 12). The pads are two-layered structures. The outer layer of each pad is electron dense and coated with an amorphous substance on its outer leaf. The outer layer is immediately apposed by a second,

more electron-dense layer. Thin filaments are attached to the pads at this layer in a hemidesmosomal configuration. The filaments then run for a short distance obliquely just under the external cuticle and end in the very dense cuticle around the body wall. Just lateral to the pads, the external cuticle is sculptured with knob-like projections. The pads, together with these knobs, may help to hold and position food particles for subsequent ingestion.

The entire buccal cavity is lined with a dense unsculptured cuticle. The cuticle is modified to form three electron-dense sharp pointed teeth, or odontia, protruding from the base of the buccal cavity and surrounding the entrance to the esophagus (Fig. 13). Muscle fibers run posteriorly from their attachment points at the cuticle lining the buccal cavity to the esophageal musculature. These longitudinally running muscle fibers may help to temporarily enlarge the buccal cavity and expose the teeth. This contrasts with Diplolaimella sp. which has neither pads nor odontia and where all muscles around the buccal cavity run transversally.

Esophagus

The buccal cavity opens into the esophagus just posterior to the three teeth. The esophagus is lined with cuticular material continuous with that of the buccal cavity. Another set of three teeth, or onchia, which are pointed elaborations of cuticle, are embedded in the anterior end of the esophagus (Fig. 13).

While the esophagus is generally similar to that of Diplolaimella sp., it differs from the latter in several respects. The dorsal esophageal gland cell empties into the anterior portion of the esophageal lumen somewhat posterior to that of Diplolaimella sp. The electron-dense inclusions produced by this gland are similar to those in the dorsal esophageal gland cell of Diplolaimella sp. In contrast to Diplolaimella sp., however, the products of the subventral esophageal gland cells are loaded with granules enclosing finely fibrillar material or denser material (Fig. 14). These cells empty their contents into the prebulbar portion of the esophageal lumen somewhat posterior to the dorsal esophageal gland cell opening. Because the glandular cells in C. germanica are more massive, the muscle filaments of the esophagus are stretched longer around the circumference of the gland cells. This suggests that when the muscles contract during ingestion, the gland cells may be squeezed more and release more digestive enzymes into the lumen than is the case with Diplolaimella sp. Finally, the bulb of the esophagus of C. germanica is larger and wider than that of Diplolaimella sp. In contrast with the latter, each muscle filament is approximately 6 µm or twice the length in the bulb region. Thus, C. germanica can exert more pressure on potential food.

Esophageal-Intestinal Valve

The esophagus and intestine are separated by several cells forming a valve-like structure (Fig. 15). These

valvular cells probably engage in active protein synthesis, since each has an extensive rough endoplasmic reticulum with flocculent intracisternal material, Golgi apparatus, and some secretory vesicles. The lumen of the valve is lined with cuticle which is continuous with that of the esophagus. In some micrographs the floor of the lumen at the posterior end of the valve was dorsally displaced; the walls of the lumen were also laterally displaced. This specialized region could serve as a flap to prevent regurgitation of food.

Intestine

In contrast to Diplolaimella sp., six cells encircled the intestinal lumen of C. germanica in any cross-section. Only one cell type was distinguishable with no regional specialization along the length of the intestine (Figs. 17, 19, 20).

All intestinal cells have few short irregularly spaced microvilli. The cells show transmembrane activity, possibly endocytosis, as indicated by small invaginations of the cell membrane that were coated on their outer leaflets with fibrillar or fuzzy material that varies in thickness and extends from the outer leaflet of the microvillar membrane into the lumen (Fig. 42).

All intestinal cells appeared to have a potential for protein synthesis; each has typical protein-synthesizing features: extensive rough endoplasmic reticulum with distended cisternae, scattered Golgi complexes, and a prominent

nucleus containing finely diffuse chromosomal material bounded by a nuclear membrane studded with ribosomes. This high potential degree of synthetic activity contrasts with the majority of the intestinal cells of Diplolaimella sp. which are absorptive.

A variety of membrane-bounded cell inclusions were randomly distributed in the intestinal cells throughout the length of the intestine. It is impossible to tell, however, from ultrastructure alone, if these inclusions represent different stages in the processing of digestive material. It is possible, however, to reconstruct a probable chronological development of these inclusions.

The largest sized inclusion body, stage I (my terminology), contains small vesicular-shaped profiles and some membrane-like materials (Fig. 17). Stage II is characterized by whorls of lamellae (Fig. 20). Transition stages between I and II are common (Fig. 19). Stage III is characterized by several large dense spherical granules enclosed by a single membrane (Fig. 19). The last stage, stage IV, results from the possible coalescing of these granules to form inclusions which are sometimes uniformly very dense and sometimes grainy. At times these inclusions are not fully infiltrated by the plastic embedding material. Stage IV appears similar to the lysosomal inclusions (pigment granules) found in Diplolaimella sp. These inclusions are sometimes observed in the process of being extruded into the gut lumen, thereby representing the final stage in the

development of these inclusions. Another possibility is that these inclusions represent separate stages in two different processes. The stage I inclusion is similar to an autophagosome and may be digesting worn-out cell parts. Stage II may be the residual body of this process. Or, stage II may include the breakdown of cell membranes and may therefore function in cell membrane conservation. The stages III and IV would still represent steps in the lysosomal digestion of food molecules.

Several other types of cytoplasmic inclusions occasionally have been observed in the intestinal cells: multivesicular bodies; vacuolar inclusion bodies each characterized by an electron-transparent matrix, a peripheral halo of unstructured moderately electron-dense material, and a small eccentrically placed spheroid of amorphous material in the inside of the halo (Fig. 20). The vacuolar inclusion body is quite small and is often associated with a Golgi complex. Because this type of inclusion and some, but not all, of the stage IV inclusions are acid-phosphatase positive they can be considered lysosomes (Fig. 21). Depending on the nutritional state of the organism, the intestinal cells also contain lipid inclusions and glycogen particles. Glycogen particles, when they are present, usually surround the very dense inclusions of stage IV, a pattern similar to that observed in Diplolaimella sp.

The intercellular junctions of the intestinal cells are complex (Fig. 17). They are characterized by extensive

infoldings near the luninal borders of two adjacent cells, thereby effectively increasing the cell-to-cell surface area. The intercellular space is filled with amorphous electron-transparent material. For a short distance, about midway between the lumen and the basal lamina, the intercellular junction resembles a septate junction because it is filled with regularly spaced dense material (Fig. 16). The interdigitation characteristic of the junctions also occurs along the luminal borders of the intestinal cells (Fig. 22). The basal lamina is smooth-surfaced and is coated with a flocculent material on its outer leaf. This or similar material also fills the area surrounding the digestive tract.

There were occasional dying intestinal cells being sloughed off into the lumen throughout the length of the intestine (Fig. 22). The cytoplasm of each dying cell exhibits a gradient of density in contrast to that of typical intestinal cells in C. germanica, with the least dense region of the cell protruding furthest into the intestinal lumen. This region consists of some microtubules and membrane-like material scattered throughout an electron-transparent matrix. The region of the cell closer to the cell's basal lamina contains a cytoplasm of nearly normal density with intact organelles or, in the case of cells in advanced stages of dying, with swollen mitochondria, a few distended cell inclusions, and short strips of rough endoplasmic reticulum. Significantly there are no secretory products

in these cells. This phenomenon may represent a type of holocrine secretion whereby the cell dies immediately after secreting its packaged enzymes into the lumen.

Ingestion and Digestion of Foods

Digestion in *Diplolaimella* sp.

Reconstruction of the major events of digestion was deduced by following the natural food as it was broken down and by studying the digestive tracts of specimens in different nutritional states.

Food was generally ingested as whole intact cells by the pumping action of the esophagus. The dorsal esophageal gland secretions coat the food when it enters the buccal cavity and then the subventral esophageal gland secretions coat the food cells as they move down into the esophageal lumen. There was no histochemical evidence that these secretions were enzymatic in nature, although these gland cells have been shown to secrete hydrolytic enzymes in other species (e.g., Lee 1965). Tests for esterase, lipase and arylamidase activities were negative in this region. These glands may be secreting components of one or more digestive enzyme which only become activated when mixed together in the lumen or on the food. The food may be further coated by the secretions from the cells of the esophageal-intestinal valve, although these cells were also negative for hydrolytic enzymes.

Morphological evidence for the initial chemical breakdown of the food was found in the intestinal lumen.

Both intact and partially broken down food cells were distributed throughout the lumen (Figs. 25, 27, 28, 38, 39). Esterases are involved in the initial stage of digestion, as indicated by a positive reaction for nonspecific esterase activity along the entire intestinal lumen (Fig. 37). The esterases and other enzymes involved in luminal digestion may possibly be secreted by the esophageal gland cells, the valve cells, and the anterior secretory cells of the intestine. Luminal digestion may be the rate-limiting step in the digestive process because well-fed nematodes subsequently deprived of food for four days still had intestines full of undigested food cells. This might indicate low concentrations of enzymes in the lumen.

At the same time that food enters the intestinal lumen, the intestinal cells, possibly the anterior secretory cells, secrete material for the fibrillar matrix. This matrix becomes sparse in starving animals (Figs. 23 and 24) in contrast with well-fed specimens which have a very dense matrix with homogeneously finely fibrillar material (Fig. 25). Well-fed specimens also had vesicles sprinkled throughout the anterior fibrillar matrix and along the length of the intestinal lumen, suggesting that the production of the matrix is coincidental with the ingestion of food.

Once the food has been broken into molecular size in the intestinal lumen, it presumably passes through the fibrillar matrix. This is suggested by the complete absence of detectable food particles in the matrix.

The presence of large numbers of acid phosphatase-positive lysosomes in the intestinal cells indicates that there is an intracellular phase of digestion. This phase probably begins in the less electron-dense cell inclusions and ends in the more electron-dense lysosomes. There are greater numbers of the former type of cell inclusion in well-fed specimens compared to specimens on diets of low-density food: the latter have from 0-4 of these inclusions and the former have about 15-40 inclusions in transverse sections. Further evidence that these inclusions contain food molecules comes from the fact that specimens raised on bacterial diets had intracellular inclusions filled with homogeneous material (Fig. 9) and those raised on algal diets had inclusions filled with fine grainy material (Figs. 29 and 39). Similar grainy material was found in most of the stage IV inclusions of C. germanica and may be characteristic of algal feeding.

Well-fed specimens had large numbers of glycogen particles surrounding both types of cell inclusions, although the lysosomes are usually closer to the luminal border of the cell and thus closer to the edge of the glycogen-containing area of the cell. This suggests that glycogen may be an end product of intracellular digestion. Undigested food debris collects in the posterior portion of the lumen where it is periodically expelled through the rectum.

A protozoan with pseudopods, an amoeba, was found

in the intestinal lumen of Diplolaimella sp. (Figs. 34 and 35). Its role in digestion is unknown. Amoebae always occurred in the lumen wherever there was food and were absent from luminal areas devoid of food cells or food debris. In well-fed adults they occurred in numbers estimated at 50-100 individuals per worm. The amoebae appeared to be unaffected by the enzymes present in the lumen that breakdown the food cells: no partially digested remains were found in the lumen. This is consistent with the observation that arylamidase, a protease found in carnivore digestive tracts, was absent from the intestinal lumen. Thus, it may not be digested because of the low concentrations of proteases in the lumen or because, as a symbiont, it is resistant to attack by these enzymes. The amoeba was usually "resting" on the outer zone of the fibrillar matrix. Groups of amoebae were usually observed to be arranged in single file along the length of the intestinal lumen. Their morphology indicated that they were similar to other intestinal amoebae and probably actively absorb and digest food.

Digestion in Chromadorina germanica

In contrast to Diplolaimella sp., C. germanica has a set of three teeth to puncture food cells and ingest their contents. A second set of teeth probably macerate the food further and may prevent particles beyond a certain size from entering the esophagus. Thus, digestion begins with the mechanical breakdown of food in the buccal cavity.

Cell envelope fragments were never observed in the gut, so that only cellular contents and not cell envelopes must be sucked down through the esophagus. No enzymatic reactions were detected in the esophageal gland cells, suggesting that the action of these cells in secreting enzyme precursors is similar in both species. The food particles continue to the intestinal lumen. Here, they are further broken down by esterases (Fig. 36) and possibly other enzymes. Then food molecules are absorbed by the intestinal cells, presumably by endocytosis. Digestion then proceeds in the various cell inclusions, probably beginning in the stage I or stage III inclusions. The final stage of digestion occurs in the stage IV inclusion. The large amounts of secretory products found in the intestinal cells are primarily used in the lysosomal digestion of food. Digested food is stored as glycogen and, rarely, as lipid (Fig. 30).

Results of Feeding on Different Foods

Various types of algae and bacteria were fed to the two species of nematodes (Table 2). The bacterial cells were smallest, being about 2.2 µm in length. The chlorophytes ranged approximately from 2.2 to 4.9 µm in diameter or length. The diatoms were the largest at more than 10 µm long.

The normal morphology of the food organisms in culture was studied first in order to interpret changes which took place during digestion. The bacterial cell

envelopes are thin (Fig. 31). The cell contents consist of ribosomes, chromosomal material and ground cytoplasm. The smallest chlorophyte, Nanochloris sp., has a very thin inconspicuous cell wall and cell membrane (Fig. 25). The cell includes a prominent chloroplast and a starch body. Storage granules containing starch-like material adjoining the chloroplast also occur in the other algal species. The chlorophytes Chlorococcum sp. (Fig. 41), Dunaliella salina, and Dunaliella sp. (Fig. 39), had several starch-like storage granules each. The cell envelope varied. Dunaliella sp. has a very thin cell wall and cell membrane whereas that of D. salina is somewhat thicker. Chlorococcum sp. has a multilayered cell wall consisting of fibrillar material that surrounds the cell membrane (Fig. 41). The diatoms have dense, thick, sculptured siliceous cell walls and store polysaccharides, such as chrysolaminaran, and lipids (Fig. 33).

C. germanica survived on diets of the diatoms and three of the chlorophytes: Dunaliella sp., D. salina, and Chlorococcum sp.; but was unable to survive on Nanochloris sp. and the bacterial species tested (Table 3). Diplolaimella sp. was able to survive on the bacteria and chlorophytes but eventually died on diets of the diatoms. Since the cultures of both worms were contaiminated with bacteria, starvation was a lingering process for Diplolaimella sp. It was probably able to use this alternative food source, as bacteria were never entirely eliminated by the antibiotic

present in the liquid overlay in the culture dishes.

These results were borne out by examination of the guts of specimens fed various diets. The species of bacteria, D5-2 and A5-6, were found in the intestinal lumen of Diplolaimella sp. as both intact and partially digested cells (Fig. 28). No matter what else was offered to Diplolaimella sp., bacteria were always observed in the intestinal lumen of this animal (Figs. 25, 38, 39, 41). On the other hand, bacteria were rarely identified in the intestinal lumen of C. germanica (Fig. 32) and never as partially digested food. Intestinal cells of specimens exposed to bacteria for periods longer than 2 hr were in various stages of dying (Fig. 26).

Undigested and partially digested Nanochloris sp. cells were easily identified in the intestinal lumen of Diplolaimella sp. (Figs. 25 and 27). Intact or partially digested algal cells were never observed in the gut of C. germanica. As in the case of bacterial feeding, the intestinal cells of specimens offered a diet of Nanochloris sp. appeared to be undergoing cell death. Remnants of Chlorococcum sp., particularly the distinctive starch grains, were found in the intestinal lumina of both nematode species (Figs 40 and 42). Broken cell fragments of Dunaliella salina occurred in the lumen of Diplolaimella sp. (Fig. 29) whereas both intact cells (Fig. 39) and cell fragments (Fig. 38) of Dunaliella sp. were found in the lumen of this nematode. Remains of neither Dunaliella

species were found in C. germanica although the intestinal cells appeared normal and were not all dying.

Cell fragments of Amphora acutioscula (Fig. 33), a diatom, identifiable as small spherical lipid-like droplets and starch granules, were found in the lumen of C. germanica (Fig. 30). The lipid droplets were similar to those in the diatom's chloroplast. No cellular debris was found in the lumen of Diplolaimella sp. on this diet. Those specimens that did survive for a time on this diet had bacteria in the lumen. No recognizable fragments of Cylindrotheca closterium were found in the lumen of either nematode species. The intestinal cells of C. germanica appeared normal--an indication that the food was being digested.

DISCUSSION

The nematode species studied here have different methods of ingestion and digestion which are intimately related to their respective diets (Table 4). Diplolaimella sp., due to its lack of buccal armature, ingests food solely by the sucking action of the esophagus. Its diet is limited to whole cells somewhat less than 3.9 µm in diameter, which includes bacteria and the smaller chlorophytes, and cell fragments from cells greater than 3.9 µm in diameter, which includes the thin-walled chlorophytes. This nematode is not anatomically equipped to exert enough pressure on the thicker, more rigid diatom wall to remove diatom cellular contents. The cell size limitation is actually not a direct result of the buccal capsule size, which is only about 1 µm in diameter. More likely, the cell size is a function of the diameter of the temporarily widened buccal capsule and esophagus during ingestion. The Diplolaimella sp. digestive tract, with the major portion of digestion occurring in the lumen, is adapted for processing whole bacterial and chlorophyte cells and chlorophyte cell fragments. It is interesting to note, in this regard, that Monhystera denticulata, a species closely related to Diplolaimella sp., produces β-glucuronidase in the intestine (Jennings and Deutsch 1975). Chromadorina germanica does

not produce this enzyme. This enzyme is probably utilized in hydrolyzing the cell envelope of ingested food cells because it is able to attack the glycan or polysaccharide portion of peptidoglycan (Ghuysen et al. 1966), a major constitutent of the bacterial cell envelope, and the uronic acid residues composing the mucilages forming many algal cell walls (O'Colla 1962).

In contrast, the buccal capsule armature of C. germanica enables it to ingest algal cell contents. It seems unable to ingest food cells less than 2.2 µm in diameter probably because the pads and knobs forming the mouth are unable to hold and position these small food cells for piercing by the teeth. In contrast, Tietjen and Lee (1977) found, using tracer feeding methods, that a small Nanochloris sp. was ingested but not assimilated by C. germanica. The size of this species was not measured. Cells less than 2.9 µm, the oral diameter, may either bounce off the teeth during attempts at ingestion or be filtered out by the esophageal teeth, the onchia, during ingestion. C. germanica also has a larger esophageal bulb which enables it to exert more suction on the diatom cell contents. Its digestive tract, therefore, is adapted for processing small algal food particles denuded of their cell envelopes.

The function of the nematode esophageal-intestinal valve has generally thought to be, as its name implies, valvular (e.g., Chitwood and Chitwood 1950, Bird 1971). In Diplolaimella sp., the circular muscle fibers between the

esophagus and valve may function as a valve, but the position of the radiating muscle fibers of the valve itself suggest that this part of the valve, like the esophagus, pumps food through its lumen into the intestine. The protein synthesizing cells of the valve suggest a protein secretory role for these cells and they may be secreting digestive enzymes involved in the digestive process in the intestinal lumen. This secretory function was also pointed out be de Man (1880) when he described a closely related species, Monhystera vulgaris, and observed a "cone-like group of gland cells" between the esophagus and the beginning of the intestine. In C. germanica, the valve is less complex and lacks any muscle fibers. Its ultrastructure also suggests that it may be secreting digestive enzymes, as well as acting as a valve structure.

The fibrillar matrix of Diplolaimella sp. has not been found in any other nematodes, including free-living and parasitic forms. This matrix may be acting as a molecular sieve, allowing only individual food molecules to pass into its regions. Although the present study did not test this idea, the concept of a molecular sieve is supported by evidence showing that the interface zone of the matrix contains cuticular proteins or cuticular protein-like molecules. If this zone is like a cuticle in chemical composition, then it would certainly limit the size of substances passing through it. The fibrils of the inner matrix zone may function in a type of contact digestion (Ugolev

1965) in which food molecules may become adsorbed onto the fibrils where enzymatic activity may then further hydrolyze them. This may be the reason for this matrix zone increasing in density with increased ingestion of food. The matrix may also be similar in function to the peritrophic membrane found in the midgut of certain insects (e.g., Smith 1968) and crustaceans (e.g., Sheader and Evans 1975) where it is believed to protect the intestinal cells from abrasion by hard particulate food. The peritrophic membrane encloses the food being digested and breaks up in the rectum to pass out, as fragments, with the feces (Jennings 1972). In Diplolaimella sp., the fibrillar matrix was never observed to enclose the food, food was never seen embedded in it, nor did it occur with the undigested food debris. Nevertheless, the fibrillar matrix may function, in part, to protect the microvilli from abrasion by the relatively large food particles, in addition to its probable role of molecular sieve and substrate site for contact digestion.

Microvilli similar to those found in Diplolaimella sp. have been described in other nematodes (e.g., Feldman 1972, Sheffield 1964, Colam 1971b). The general structure is that of a microvillus with a central core of electron-transparent material bounded by a number of minute filaments forming a tube. In Diplolaimella sp. the electron-transparent material in the microvillus is bounded by a continuation of the endoplasmic reticulum. This cisterna

may be involved in the transport of food molecules from the microvillus to the lysosome, particularly since the microvillus swells in response to high levels of food being digested in the lumen.

Most nematodes have both lipid and glycogen storage inclusions in their intestinal cells (e.g., Bird 1971). The lack of lipid in Diplolaimella sp. and its low amount, relative to glycogen, in C. germanica may be an adaptation to short-term storage of food (Calow and Jennings 1974).

The amoeba found in the Diplolaimella sp. intestine is probably ingesting some of the food of this nematode. It is possible that the amoeba is secreting enzymes that may be used by the nematode in luminal digestion or the amoeba is excreting partially digested food that the nematode then utilizes. Any studies measuring food utilization by Diplolaimella sp., such as those for other free-living nematodes (Duncan et al. 1974, Marchant and Nicholas 1974, Nicholas et al. 1974), would have to consider that not all the food ingested by the worm is available for digestion.

Although one type of cell is present in the intestine of C. germanica, this cell probably goes through different phases of activity at different stages of its life. In a probable scheme, these cells may first be absorptive and digest food within their lysosomes. They therefore secrete and package digestive enzymes destined to be released into inclusions containing food molecules. Older cells then become secretory and package digestive

enzymes which are eventually released into the lumen. Then the cell dies. A similar cycle of growth of the intestinal cells described here does occur in a marine nematode, Pontonema vulgaris, (Jennings and Colam 1970) where the cells are first absorptive and then, when they have aged, undergo a period of merocrine secretion.

A major difference between digestion in these two nematodes is in the emphasis on either luminal or lysosomal digestion. C. germanica has relatively fewer luminal stages to its digestion of food, as the major part of digestion occurs intracellularly by virtue of the elaborate set of cell inclusions and lysosomes described here. Diplolaimella sp. relies much more on luminal digestion with only the final stages of digestion occurring intracellularly. In general, nematode digestion was believed to be entirely luminal due to the absence of obvious phagocytosis or pinocytosis occurring in the intestinal cells. In the case of Diplolaimella sp., food molecules may be small enough, as a result of greater extracellular digestion to be transported individually across the cell membrane to undergo lysosomal digestion.

The advantage of lysosomal digestion is that the cells need to secrete relatively few enzyme molecules to produce an optimum concentration of enzyme (Jennings 1972). But extensive lysosomal digestion also means that each cell must be able to synthesize a full complement of enzymes for the sequential breakdown of food molecules. In luminal

digestion, the intestinal cells can become more specialized, with different cells secreting different enzymes or one cell secreting different enzymes at different times. Luminal digestion therefore permits cells to become more specialized so that their functioning is more efficient.

There may be an absolute limit on the number of different digestive enzymes one cell is capable of synthesizing. Thus, C. germanica, with its emphasis on lysosomal digestion probably secretes fewer species of enzymes. To optimize its digestion, C. germanica must have a fairly narrow diet which would present the intestinal cells with fewer different molecules to hydrolyze, as each food cell has its own unique set of macromolecules. This nematode feeds only on algal cells and actually only ingests the cell contents, thereby avoiding the necessity of secreting enzymes to hydrolyze the algal cell envelope. The algal cell contents present a variety of different macromolecules to be digested. In a greatly simplified scheme, membranes would be hydrolyzed by different endo and exopeptidases and esterases; nuclei and nucleic acids would be hydrolyzed by deoxyribonuclease and ribonuclease; and storage inclusions would be hydrolyzed by lipases, esterases, and a host of carbohydrases. The quality and quantity of the different enzymes needed for digestion will be different for each species of food.

Diplolaimella sp., with its emphasis on luminal digestion, has a somewhat different strategy. Its few

secretory cells may actually be capable of synthesizing a greater number of different enzymes. Its diet, compared to C. germanica, is broader because it feeds both on algae and bacteria. To digest its food it must produce enzymes to hydrolyze the cell envelopes of both chlorophytes and bacteria as well as their cell contents. For example, the gram-positive bacterial cell envelope is hydrolyzed by endo-N-acetylmuramidases or lysozymes, endo-N-acetylglucosaminidases, endopeptidases, and acetylmuramyl-L-alanine amidases (Ghuysen et al. 1968). For gram-negative bacteria, both a lysozyme and an endopeptidase, such as trypsin, are necessary for cell lysis (Braun et al. 1974). The cell wall of the Chlorophyceae would be hydrolyzed by carbohydrases that attack cellulose, xylan, mannon, or other polysaccharides (Mackie and Preston 1974).

In general, a restriction in the variety of species forming the diet of these two nematodes may enable them to increase the efficiency of both the lysosomal and luminal phases of digestion by minimizing the number of different enzymes the digestive tract cells must synthesize. This strategy may be particularly important to these nematode species, which are apparently at the lower size limit for metazoans (Swedmark 1964) and therefore have relatively few cells assigned to function in digestion.

The strategy of a narrow diet appears to be adopted by these two species. The phenomenon of selective feeding is shown here to be a function of the size of the food and

also, for Diplolaimella sp., the type of cell envelope. As a result, these two species have the potential to overlap in their utilization of food resources when chlorophytes of a size utilizable by both are available but do not compete for diatoms and bacteria. Other studies also show that C. germanica prefers diatoms and Diplolaimella sp. prefers bacteria (Tietjen and Lee 1977). They are able to coexist because of their different food preferences. The strategy of selective feeding, then, may be a method for optimizing digestion--extracting the maximum usable molecules from the ingested food--and also may serve to reduce interspecific competition. One difference between marine nematodes in any benthic environment is probably in the quality and quantity of their digestive enzymes, also shown for the snail species Hydrobia ulvae, H. ventrosa, and H. neglecta (Hylleberg 1976), as well as in the general strategy of the digestive process.

The two nematode species studied here had fairly different diets and were not very closely related taxonomically. In most benthic envrionments, however, there are many very closely related species living, seemingly, in the same microenvironments. These species, such as the chromadorids, may be much more similar with respect to their morphology but might differ in the quality and quantity of the digestive enzymes they secrete. Such closley related species may especially be using resource partitioning or selective feeding to optimize their digestion. Each species

may be adapted to ingesting and digesting somewhat different spectra of food. Resource partitioning may be an important way for similar species to coexist in a community (Schoener 1974, Ivester and Coull 1975, 1977). A large number of nematode species in a benthic area could be avoiding interspecific competition while exploiting, as a taxon, a large diverse food source. Perhaps, if success is defined as the presence of a large number of species in an area, then the Nematoda have been very successful by using the strategy of narrow partitioning of food resources to optimize each species' food utilization.

TABLE 1

FIXATION SCHEMES TESTED FOR DETERMINING ROUTINE ELECTRON MICROSCOPY OF TWO NEMATODE SPECIES

Fixative[a]	Time, hr
Diplolaimella sp.	
6% glutaraldehyde in sea water[b]	0.5
6% glutaraldehyde in sea water[b]	1
3% glutaraldehyde in sea water[c]	2
3% glutaraldehyde in sea water[c]	3
5% glutaraldehyde + 0.2 M phosphate buffer pH 7.4 + 0.27 M sodium chloride	2
5% glutaraldehyde + 0.2 M phosphate buffer pH 7.4 + 0.27 M sodium chloride	3
2.5% glutaraldehyde + 0.2 M phosphate buffer pH 7.4 + 0.27 M sodium chloride	3
2.5% glutaraldehyde + 0.2 M phosphate buffer pH 7.4 + 0.27 M sodium chloride	24
Chromadorina germanica	
6% glutaraldehyde in sea water[b]	0.5
6% glutaraldehyde in sea water[b]	1
3% glutaraldehyde in sea water[c]	2
3% glutaraldehyde in sea water[c]	3

[a]All procedures carried out at 4°C unless otherwise noted.

[b]10 ml 70% glutaraldehyde + 107 ml millipore filtered sea water (mfsw).

[c]10 ml 70% glutaraldehyde + 107 ml mfsw, diluted 1:1 with mfsw.

TABLE 1--Continued

Fixative[a]	Time, hr
Chromadorina germanica	
3% glutaraldehyde in sea water[c] with cutting through cuticle	1
3% glutaraldehyde in sea water[c] with cutting through cuticle	2
3% glutaraldehyde in sea water[c] + 1% dimethyl sulfoxide (DMSO)	2
3% glutaraldehyde in sea water[c] + 5% DMSO	2
3% glutaraldehyde in sea water[c] + 10% DMSO	2
2.5% glutaraldehyde + 0.2 M phosphate buffer pH 7.4 + 0.27 M sodium chloride	3
5% glutaraldehyde + 0.2 M phosphate buffer pH 7.4 + 0.27 M sodium chloride	2
4% paraformaldehyde + 6% glutaraldehyde in sea water[b]	2
4% paraformaldehyde + 6% glutaraldehyde in sea water[b]	3
4% paraformaldehyde + 6% glutaraldehyde in sea water[b] at 60°C	2
1% acrolein in sea water[d]	1
1% acrolein in sea water[d]	1.5
10% acrolein in sea water[e]	0.5

[a]All procedures carried out at 4°C unless otherwise noted.

[b]10 ml 70% glutaraldehyde + 107 ml millipore filtered sea water (mfsw).

[c]10 ml 70% glutaraldehyde + 107 ml mfsw, diluted 1:1 with mfsw.

[d]1 ml 100% acrolein + 99 ml mfsw.

[e]10 ml 100% acrolein + 90 ml mfsw.

TABLE 2

SPECIES OF FOOD FED TO NEMATODES

Code Number	Species Name	Approximate Dimensions	
		length, µm	width, µm
Bacteria			
D5-2	gram-negative rod	2.2	0.6
A5-6	gram-negative rod	2.1	0.7
Chlorophytes			
41	*Nanochloris* sp.	2.2	1.8
55	*Dunaliella* sp.	3.9	2
13	*Dunaliella salina*	4.9	3.4
38	*Chlorococcum* sp.	4.9	4.6
Diatoms			
RF-8	*Amphora acutioscula*	10.4	5.5
8	*Cylindrotheca closterium*	10.6	2.2

TABLE 3

NUMBER OF LIVE NEMATODES IN CULTURE DISHES WITH DIFFERENT FOOD SPECIES OVER TIME

	Diplolaimella sp.				*Chromadorina germanica*			
Food	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
A5-6	20	15	13	13	20	12	2	2
D5-2	20	15	14	13	20	10	4	1
RF-8	20	12	5	4	20	11	11	10
8	20	11	6	5	20	12	11	10
38	20	15	12	12	20	12	10	8
55	20	13	13	12	20	14	14	12
41	20	14	13	11	16	5	7	2
13	20	16	15	14	20	12	10	10
no food	20	10	4	5	20	10	6	1

TABLE 4

COMPARISON OF THE ULTRASTRUCTURE OF THE DIGESTIVE TRACTS OF TWO NEMATODE SPECIES

	Diplolaimella sp.	*Chromadorina germanica*
Buccal Cavity	unarmored 1-2 µm diameter opening transverse muscles dorsal esophageal gland empties here	3 teeth, pads, knobs about 3 µm diameter opening longitudinal muscles
Esophagus	narrow esophageal cylinder smaller gland cells smaller muscular bulb	wide esophageal cylinder/3 teeth larger gland cells larger muscular bulb
Esophageal-Intestinal Valve	anterior circular muscle band muscular bulb several gland-like cells	several protein-synthesizing cells
Intestine	2 types of cells: 4 anterior secretory-like + remaining absorptive structurally complex fibrillar matrix microvilli usually with a cisterna along central axis 2 types of cell inclusions, lysosomes	1 type of cell: secretory-like irregular fibrillar coat small few microvilli large variety of cell inclusions, lysosomes dying cells part of holocrine secretory process

Fig. 1. An obliquely longitudinal section through the anterior end of Diplolaimella sp. showing the anterior and posterior buccal cavities lined with cuticle and transverse muscles; bc = buccal cavity, c = cuticle, ck = cuticular knobs, h = hypodermis, m = muscle. X 20,500. Bar is 0.5 µm.

Fig. 2. Transverse section through the posterior buccal cavity of Diplolaimella sp. showing the dorsal esophageal gland emptying into it. Secretory product appears in the cell and in the lumen. Marginal cells are also visible; c = cuticle, d = duct, D = dorsal, gc = gland cell, lu = lumen, m = muscle, mc = marginal cell, mf = microfilaments. X 14,600. Bar is 1 µm.

Fig. 3. Transverse section through the esophagus of Diplolaimella sp. where the two subventral glands empty into the lumen. Their secretory products are electron-transparent, in contrast to the products of the dorsal gland, also shown; c = cuticle, d = duct, el = esophageal lumen, gc = gland cell, m = muscle, s = secretory product, SV = subventral. X 25,000. Bar is 0.5 µm.


c
bc
bc
lu
2

Fig. 4. A detailed view of the esophageal-intestinal valve showing its packaged secretory products which appear as fibrillar and electron-transparent materials; lu = lumen, m = muscle, s = secretory product. X 18,750. Bar is 0.5 μm.

Fig. 5. Semi-longitudinal section of Diplolaimella sp. showing part of the muscle band that encircles the valve lumen, the valve's muscular bulb, and its gland-like cells. The anterior intestine is also visible. The secretory products are very electron dense. The arrow points to secretory-like material in the valve lumen. The flaps separating the valve lumen from the intestinal lumen are also shown; b = bacterial cell, bl = basal lamina, c = cuticle, eiv = esophageal-intestinal valve, fl = flaps, gc = gland-like cell, i = intestine, lu = lumen, m = muscle, n = nucleus, s = secretory product. X 15,750. Bar is 1 μm.

Fig. 6. Transverse section through the beginning of the intestine of Diplolaimella sp. showing one of the four secretory cells; bl = basal lamina, c = cuticle, fm = fibrillar matrix, G = Golgi complex, lu = lumen, mv = microvilli, n = nucleus, rer = rough endoplasmic reticulum. X 12,200. Bar is 1 μm.

Fig. 7. Transverse section through a secretory cell showing the microvilli characteristic of these cells in well-fed specimens and the high concentration of vesicles in the fibrillar matrix; fm = fibrillar matrix,

lu = lumen, mv = microvilli, n = nucleus, rer = rough endoplasmic reticulum, v = vesicle. X 50,000. Bar is 0.2 µm.

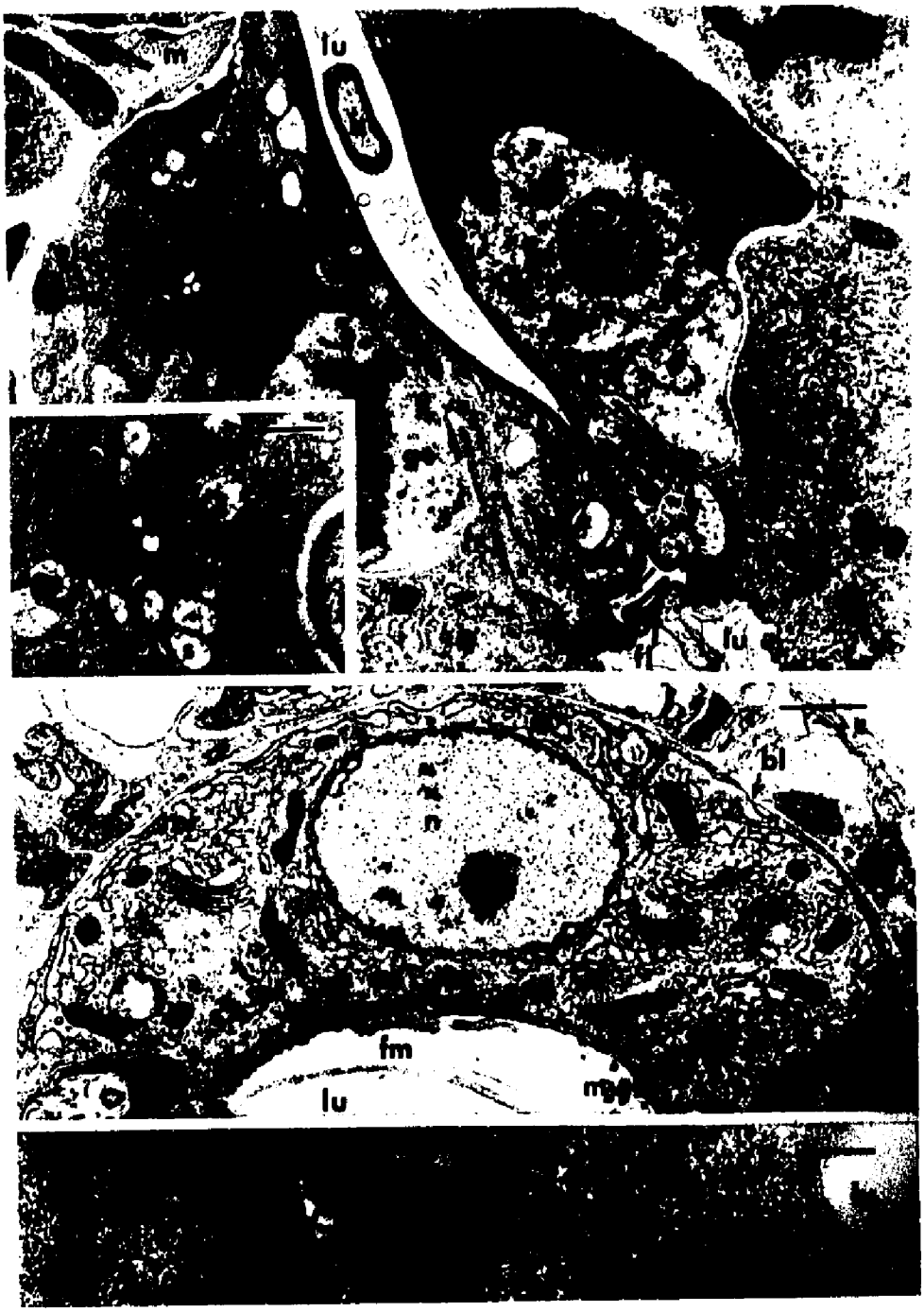
m
lu
bl
lu
bl
n
fm
lu
mg

Fig. 8. An oblique section through the intestine of Diplolaimella sp. showing lead deposits in the "pigment granules" from the acid phosphatase reaction, not counterstained; ly = lysosome, mv = microvilli. X 20,800. Bar is 0.5 µm.

Fig. 9. Typical transverse section through two intestinal cells of an adult female Diplolaimella sp. Unusual microvilli are at the four "corners" of the "square" outlined by the fibrillar matrix; cj = cell junction, fd = food debris, fm = fibrillar matrix, gl = glycogen, lu = lumen, ly = lysosome, mv = microvilli. X 9,300. Bar is 1 µm.

Fig. 10. Detail of the unusual microvilli associated with the fibrillar matrix of Diplolaimella sp.; fm = fibrillar matrix, lu = lumen, mv = microvilli. X 50,000. Bar is 0.2 µm.

Fig. 11. Detail of the microvilli of the intestinal cells of Diplolaimella sp. showing the cisterna of the microvillus (*) continuous with the cisterna of the endoplasmic reticulum (+). X 118,300. Bar is 0.1 µm.

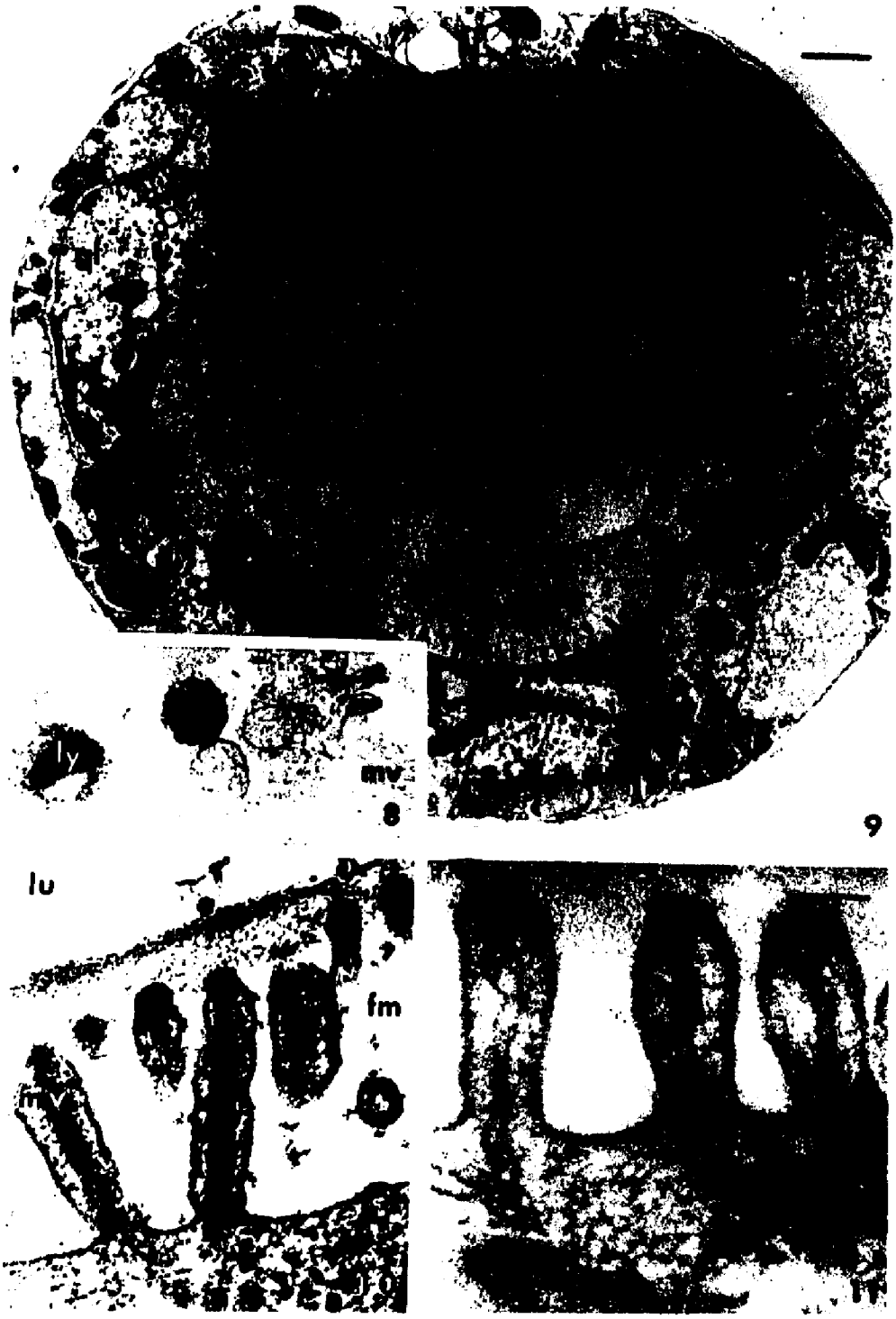
ly
mv
8
9
lu
fm
mv

Fig. 12. Detail of a pad surrounding the buccal cavity of Chromadorina germanica showing the stabilizing filaments; c = cuticle, f = filaments. X 27,000. Bar is 0.3 µm.

Fig. 13. Longitudinal section through the anterior end of C. germanica showing the pads forming the mouth, the two sets of teeth (odontia and onchia), and longitudinal muscles; bc = buccal cavity, c = cuticle, ck = cuticular knob, el = esophageal lumen, m = muscle, od = odontia, on = onchia, p = pad. X 14,900. Bar is 1 µm.

Fig. 14. Transverse section through the esophagus of C. germanica showing the secretory products of the two subventral esophageal gland cells; c = cuticle, lu = lumen, m = muscle, mc = marginal cell, n = nucleus, s = secretory product, SV = subventral. X 25,000. Bar is 0.5 µm.

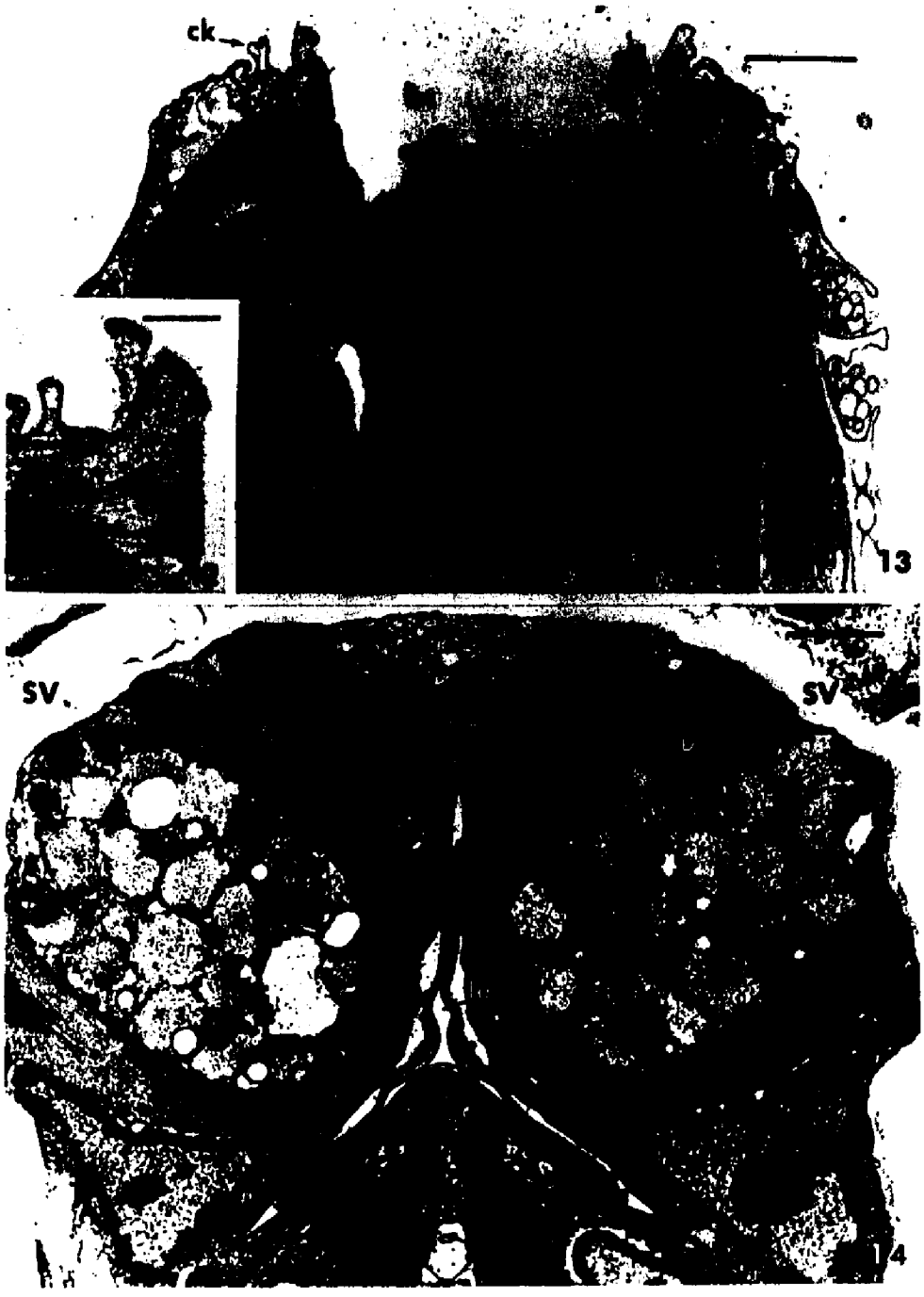
ck
13
SV
SV
14

Fig. 15. Longitudinal section of C. germanica showing the esophagus, esophageal-intestinal valve, and anterior intestine; c = cuticle, e = esophagus, eiv = esophageal-intestinal valve, i = intestine, lu = lumen. X 19,700. Bar is 0.5 µm.

Fig. 16. Detail of the septate junction along the interfacing membrane of two adjacent cells of the intestine of C. germanica. X 72,700. Bar is 0.1 µm.

Fig. 17. Transverse section through the intestine of C. germanica showing the typical features of these cells, including some of the different cell inclusions and the intercellular junctions. The arrow points to a septate junction; cj = cell junction, G = Golgi complex, lu = lumen, mv = microvilli, SI = stage I inclusion, SIV = stage IV inclusion. X 22,800. Bar is 0.5 µm.

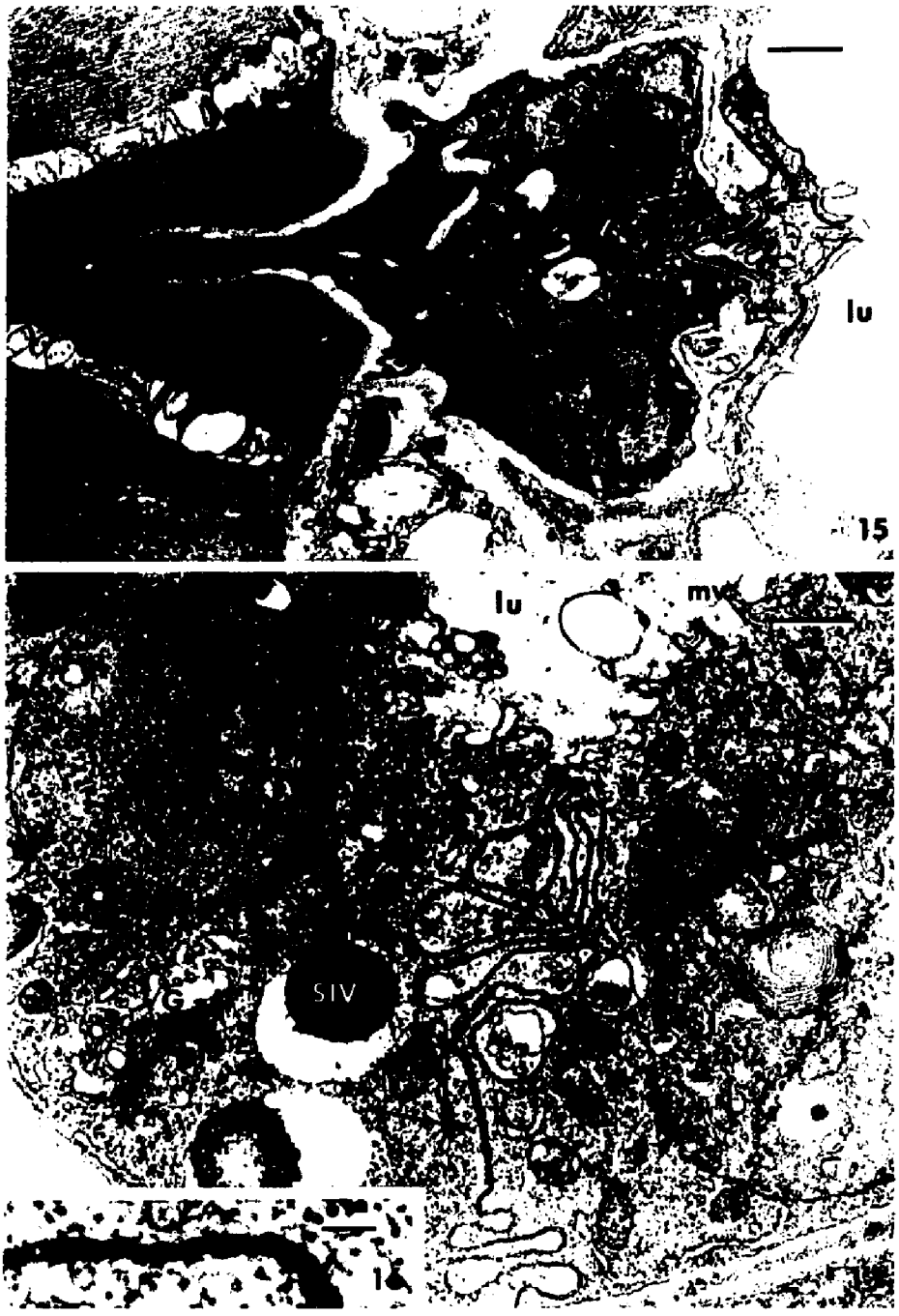
lu
15
lu
my
SIV
14

Fig. 18. Detail of an intestinal cell of C. germanica showing possible endocytosis. Note the outer leaflet of the plasma membrane coated with fuzzy material; lu = lumen. X 105,000. Bar is 0.1 µm.

Fig. 19. Transverse section through the intestine of C. germanica showing some of the different cell inclusions; lu = lumen, mv = microvilli, n = nucleus, rer = rough endoplasmic reticulum, SI-II = stage I-II inclusion, SIII = stage III inclusion. X 25,000. Bar is 0.5 µm.

Fig. 20. Transverse section through the intestine of C. germanica showing some of the different cell inclusions, including the electron-transparent inclusion; cj = cell junction, in = inclusion, lu = lumen, SII = stage II inclusion. X 13,800. Bar is 1 µm.

Fig. 21. Transverse section of the intestine of C. germanica showing lead deposits, in a variety of cell inclusions, from the acid phosphatase reaction, not counterstained; lu = lumen, SII = stage II inclusion. X 12,500. Bar is 1 µm.

Fig. 22. Longitudinal section through the intestine of C. germanica showing a dying cell being shed into the lumen. Note the electron-transparent cell matrix in the part of the cell furthest from the cell junctions. Near normal density, but with no cell organelles, occurs in the cell region immediately adjacent to the cell junctions; dc = dying cell, lu = lumen. X 17,000. Bar is 1 µm.


lu
SII
lu
18
SII
lu
21
lu
22

Fig. 23. Tranverse section through the intestine of a starved specimen of Diplolaimella sp. showing an empty lumen and a thin fibrillar matrix; c = cuticle, fm = fibrillar matrix, lu = lumen, ly = lysosome. X11,400. Bar is 1 µm.

Fig. 24. Detail of another area of the intestine of a starved specimen of Diplolaimella sp.; fm = fibrillar matrix, mv = microvilli. X 28,300. Bar is 0.5 µm.

Fig. 25. Transverse section through the intestine of a well-fed specimen of Diplolaimella sp. showing the alga Nanochloris sp. and bacteria in the lumen. Note the rather dense fibrillar matrix, the vesicles in the matrix, and the swollen microvilli--all characteristic of a well-fed specimen; a = algal cell, b = bacterial cell, fm = fibrillar matrix, gl = glycogen, lu = lumen, ly = lysosome, mv = microvilli, v = vesicle. X 18,300. Bar is 1 µm.

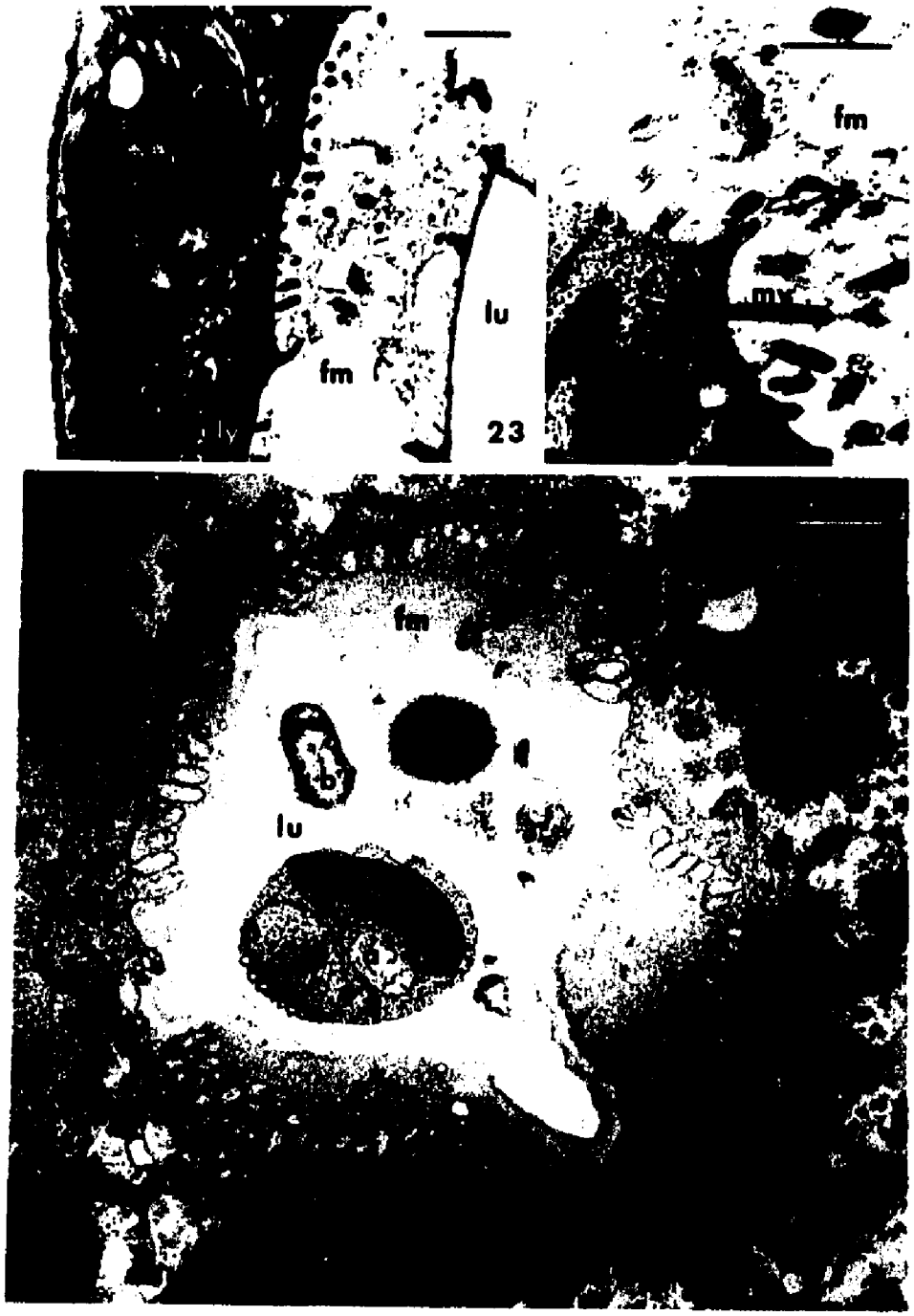
lu
fm
23
fm
my
fm
lu

Fig. 26. Longitudinal section of the intestine of C. germanica after being exposed to bacterial food for 24 hr showing dying intestinal cells with distorted cell organelles and no organized cell matrix; dc = dying cell, lu = lumen. X 18,000. Bar is 0.5 µm.

Fig. 27. Transverse section of the intestine of Diplolaimella sp. fed Nanochloris sp. for 4 days showing partially broken down cells in the lumen; ad = algal cell debris, lu = lumen. X 11,500. Bar is 1 µm.

Fig. 28. Semi-longitudinal section through the intestine of Diplolaimella sp. fed on bacterium D5-2 for 24 hr showing this food intact and partially broken down in the lumen; b = bacterial cell, bd = bacterial cell debris, fm = fibrillar matrix, lu = lumen. X 12,300. Bar is 1 µm.

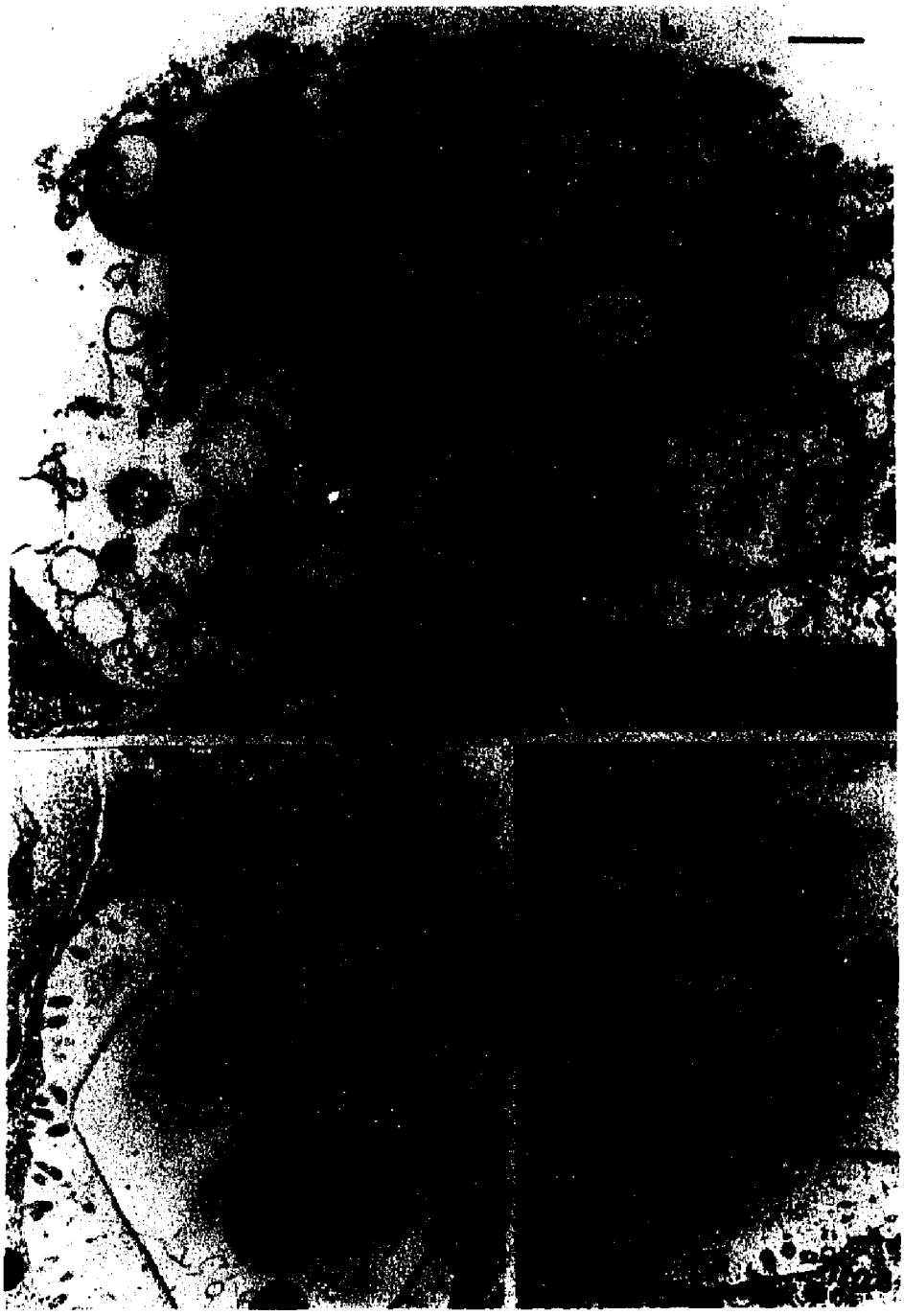

Fig. 29. Transverse section of the intestine of Diplolaimella sp. fed on Dunaliella salina for 2 days showing algal food debris characteristic of this food species in the lumen. Note the typically "grainy" lysosomes characteristic of an algal diet; ad = algal cell debris, gl = glycogen, lu = lumen, ly = lysosome. X 20,500. Bar is 0.5 µm.

Fig. 30. Longitudinal section of the intestine of C. germanica fed on Amphora acutioscula for 4 days showing food cell debris in the lumen; ad = algal cell debris, gl = glycogen, lu = lumen, SIV = stage IV inclusion. X 17,000. Bar is 1 µm.

Fig. 31. The bacterium D5-2 used as food; b = bacterial cell. X 20,000. Bar is 0.5 µm.

Fig. 32. Semi-longitudinal section of the intestine of C. germanica after exposure to bacterial food D5-2 for 2 hr showing one intact cell in the lumen; b = bacterial cell, lu = lumen. X 17,000. Bar is 0.5 µm.

Fig. 33. The alga Amphora acutioscula used as food. Note the thick sculptured cell envelope; ce = cell envelope, ch = chloroplast. X 6000. Bar is 1 µm.

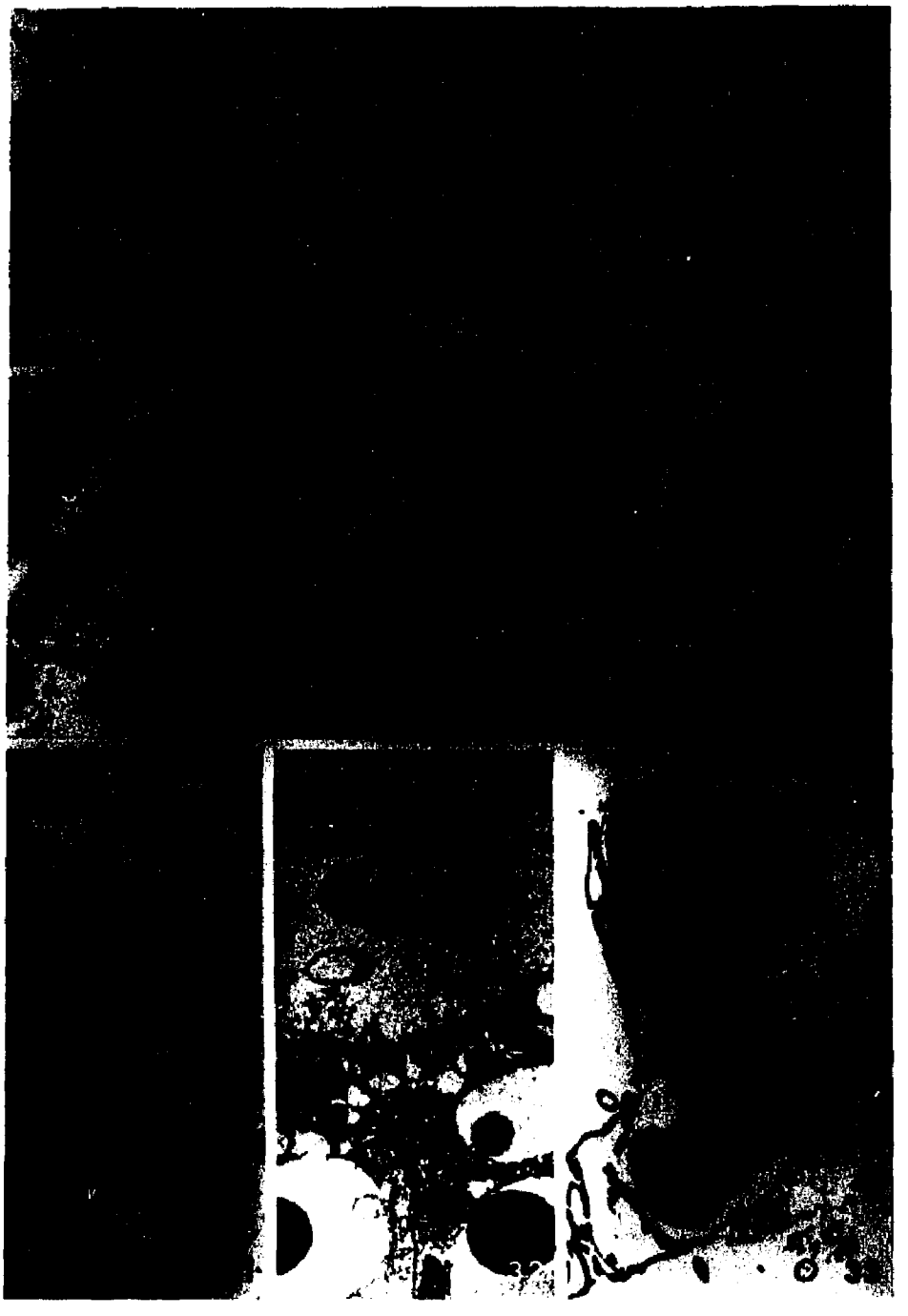

Fig. 34. Longitudinal section of the intestine of Diplolaimella sp. showing amoeba "resting" on the fibrillar matrix. There are undigested and partially digested bacteria nearby; am = amoeba, b = bacterial cell, bd = bacterial cell debris, fm = fibrillar matrix, i = intestine, lu = lumen, n = nucleus. X 17,000. Bar is 1 µm.

Fig. 35. Oblique section of the intestine of Diplolaimella sp. showing amoebae in the intestinal lumen. The arrow points to a pseudopod. Note the condensed globules, parallel sheets of fibrils, and vesicles characteristic of the fibrillar matrix; am = amoeba, fm = fibrillar matrix, i = intestine, lu = lumen. X 12,400. Bar is 1 µm.


fm

Fig. 36. Portion of a whole mount of C. germanica with indigo product in the intestinal cells and lumen indicating nonspecific esterase activity confined to this region and its absence in the esophagus; eb = esophageal bulb, i = intestine. X 860. Bar is 10 µm.

Fig. 37. Portion of a whole mount of Diplolaimella sp. showing the reaction product of nonspecific esterase activity in the intestine; e = esophagus, i = intestine. X 750. Bar is 10 µm.

Fig. 38. Longitudinal section of the intestine of Diplolaimella sp. showing the remains of Dunaliella sp. and several intact bacteria; ad = algal cell debris, b = bacterial cell, fm = fibrillar matrix, i = intestine, lu = lumen. X 16,700. Bar is 1 µm.

Fig. 39. Semi-longitudinal section of the intestine of Diplolaimella sp. fed on Dunaliella sp. as a regular diet food with algal and bacterial cells intact in the lumen. Note the "grainy" inclusions characteristic of algal diets and the less electron-dense inclusions further from the luminal cell borders than the lysosomes; a = algal cell, b = bacterial cell, ch = chloroplast, in = inclusion, fm = fibrillar matrix, lu = lumen, ly = lysosome. X 11,390. Bar is 1 µm.

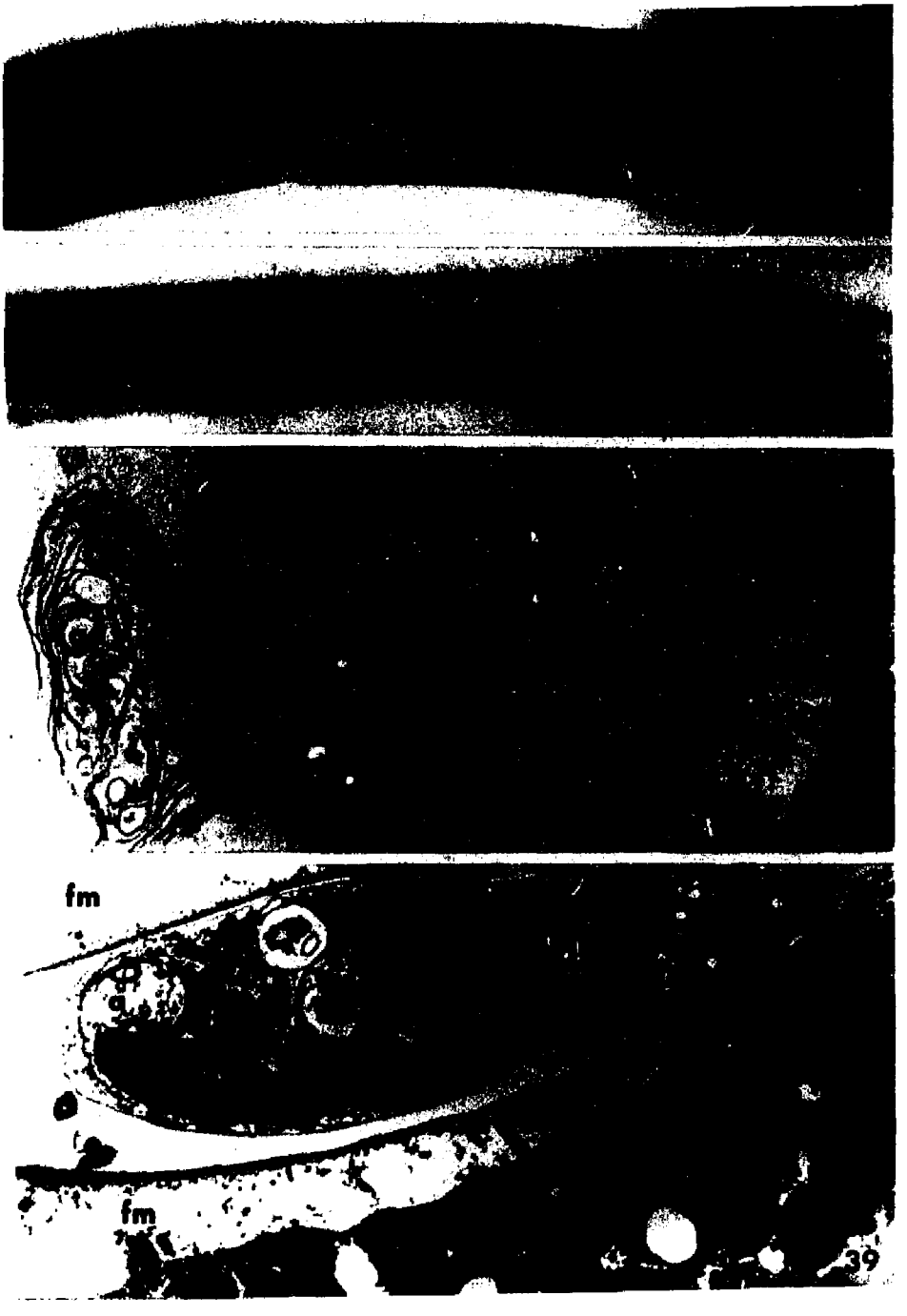
fm
fm
39

Fig. 40. Longitudinal section of the intestine of Diplolaimella sp. fed on Chlorococcum sp. for 4 days with intact starch granules from the food cells and other food cell debris characteristic of this alga scattered in the lumen; ad = algal cell debris, am = amoeba, b = bacterial cell, i = intestine, lu = lumen, sg = starch granule. X 11,700. Bar is 1 µm.

Fig. 41. A view of Chlorococcum sp. showing the starch granules, chloroplasts, and typical cell envelope of this alga; ce = cell envelope, ch = chloroplast, sg = starch granule. X 14,000. Bar is 1 µm.

Fig. 42. Longitudinal section of the intestine of C. germanica fed on Chlorococcum sp. for 4 days showing characteristic starch granules scattered in the lumen. Arrows point to fibrillar material associated with the intestinal cell borders in the lumen; i = intestine, lu = lumen, sg = starch granule, SIV = stage IV inclusion. X 17,000. Bar is 0.5 µm.


lu
sg
41
42

REFERENCES

Barka, T. and P. J. Anderson. 1962. Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. J. Histochem. Cytochem. 10:741-749.

Bird, A. F. 1971. The structure of nematodes. Academic Press, New York. 318 pp.

Braun, V., V. Bosch, K. Hantke, and K. Schaller. 1974. Structure and biosynthesis of functionally defined areas of the Escherichia coli outer membrane. Ann. N. Y. Acad. Sci. 235:66-82.

Burstone, M. S. and J. E. Folk. 1956. Histochemical demonstration of aminopeptidase. J. Histochem. Cytochem. 4:217-226.

Calow, P. and J. B. Jennings. 1974. Calorific values in the Phylum Platyhelminthes: the relationship between potential energy, mode of life and the evolution of entoparasitism. Biol. Bull. 147:81-94.

Chitwood, B. G. and M. B. Chitwood. 1950. An introduction to nematology. University Park Press, Baltimore. 213 pp.

_____ and D. G. Murphy. 1964. Observations on the two marine monhysterids--their classification, cultivation, and behavior. Trans. Amer. Micros. Soc. 83:311-329.

_____ and R. W. Timm. 1954. Free-living nematodes of the Gulf of Mexico. Fish. Bull. U. S. Fish Widl. Serv. 55:313-323.

Colam, J. B. 1971a. Studies on gut ultrastructure and digestive physiology in Rhabdias bufonis and R. sphaerocephala (Nematoda: Rhabditida). Parasit. 62:247-258.

_____. 1971b. Studies on gut ultrastructure and digestive physiology in Cosmocerca ornata (Nematoda: Ascaridida). Parasit. 62:259-272.

_____. 1971c. Studies on gut ultrastructure and digestive physiology in Cyathostoma lari (Nematoda: Strongylida). Parasit. 62:273-283.

Coull, B. C. 1970. Shallow water meiobenthos of the Bermuda platform. Oecologia 4:325-357.

Duncan, A. F., F. Schiemer, and R. J. Klekowski. 1974. A preliminary study of feeding rates on bacterial food by adult females of a benthic nematode, Plectus palustris De Man 1880. Polskie Arch. Hydrobiol. 21:249-258.

Dystra, M. J. and H. C. Aldrich. 1976. Cytochemistry of the cell coat in slime molds. J. Cell Biol. 70:709a.

Fedorko, M. E. and R. F. Levine. 1976. Tannic acid effect on membrane of cell surface origin in guinea pig megakaryocytes and platelets. J. Histochem. Cytochem. 24:601-605.

Feldman, M. R. 1972. Fine structural studies of the nematode Leidynema appendiculata (Leidy 1850). Trans. Amer. Micros. Soc. 91:337-347.

Fenchel, T. 1969. The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated protozoa. Ophelia 6:1-182.

Gerlach, S. A. and M. Schrage. 1971. Life cycles in marine meiobenthos. Experiments at various temperatures with Monhystera disjuncta and Theristus pertenuis (Nematoda). Mar. Biol. 9:274-280.

Ghuysen, J. M. 1968. Use of bacteriolytic enzymes in determination of wall structure, and their role in cell metabolism. Bact. Rev. 32:425-464.

_____, D. J. Tripper, and J. L. Strominger. 1966. Enzymes that degrade bacterial cell walls. In Methods in enzymology: complex carbohydrates, E. F. Neufeld and V. Ginsberg (eds.), vol. 8. Academic Press, New York, pp. 685-699.

Holt, S. J. 1958. Studies in enzyme histochemistry. Proc. R. Soc. Lond. B. 148:465-532.

Hopper, B. E. and S. P. Meyers. 1966a. Aspects of the life cycle of marine nematodes. Helg. wiss. Meeres. 13:444-449.

_____ and _____. 1966b. Observations on the bionomics of the marine nematode, Metoncholaimus sp. Nature 209:889-900.

_____, J. W. Fell, and R. C. Cefalu. 1973. Effect of temperature on life cycles of nematodes associated with mangrove (Rhizophora magle) detrital system. Mar. Biol. 23:293-296.

Hutchinson, G. E. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? Amer. Nat. 93:145-159.

Hylleberg, J. 1976. Resource partitioning on basis of hydrolytic enzymes in deposit-feeding mud snails (Hydrobiidae). Oecologica 23:115-125.

Ivester, M. S. and B. C. Coull. 1975. Comparative study of ultrastructural morphology of some mouthparts of four haustoriid amphipods. Can. J. Zool. 53:408-417.

_____ and _____. 1977. Niche fraction studies of two sympatric species of Enhydrosoma (Copepoda, Harpacticoida). Mikrofauna Meers. 61:In Press.

Jennings, J. B. 1972. Feeding, digestion and assimilation in animals. Macmillan and Co., New York. 244 pp.

_____ and A. Deutsch. 1975. The occurrence and possible adaptive significance of β-glucuronidase and arylamidase ("leucine aminopeptidase") activity in two species of marine nematodes. Comp. Biochem. Physiol. 52A:611-614.

Lackey, J. B. 1961. Bottom sampling and environmental niches. Limnol. Oceanogr. 6:271-279.

Lee, D. L. 1965. The physiology of nematodes. W. H. Freeman and Co., San Francisco. 154 pp.

_____ and H. J. Atkinson. 1976. Physiology of nematodes. Macmillan Press Ltd., London. 218 pp.

Lee, J. J., M. E. McEnery, E. M. Kennedy, and H. Rubin. 1975. A nutritional analysis of a sublittoral diatom assemblage epiphytic on Enteromorpha from a Long Island salt marsh. J. Phycol. 11:14-49.

_____, J. H. Tietjen, and J. R. Garrison. 1976. Seasonal switching in the nutritional requirements of Nitocra typica, a harpacticoid copepod from salt marsh aufwuchs communities. Trans. Amer. Micros. Soc. 95:628-637.

_____, _____, R. J. Stone, W. A. Muller, J. Rullman, and M. McEnery. 1970. The cultivation and physiological ecology of members of salt marsh epiphytic

communities. Helg. wiss. Meers. 20:136-156.

Lippens, P. L. 1974. Ultrastructure of a marine nematode, Chromadorina germanica (Buetschli, 1874) II. Cytology of lateral epidermal glands and associated neurocytes. Z. Morph. Tiere 79:283-294.

Luft, J. 1971. Ruthenium red and violet: 1. Chemistry, purification, methods of use for electron microscopy and mechanism of action. Anat. Rec. 171:346-368.

Mackie, W. and R. D. Preston. 1974. Cell wall and intercellular region. In Algal physiology and biochemistry, W. D. P. Stewart (ed.). University of California Press, Los Angeles, pp. 40-85.

Man, J. G. de. 1880. Tijdschr. ned. dierk. Ver. 5:1-104.

Marchant, R. and W. L. Nicholas. 1974. An energy budget for the free-living nematode Pelodera (Rhabditidae). Oecologia 16:237-252.

McIntyre, A. D. 1969. Ecology of marine meiobenthos. Biol. Rev. 44:245-290.

Meyers, S. P., W. A. Feder, and K. M. Tsu. 1963. Nutritional relationships among certain filamentous fungi and a marine nematode. Science 141:520-522.

_____, B. E. Hopper, and R. Cefalu. 1970. Ecological investigations of the marine nematode Metoncholaimus scissus. Mar. Biol. 4:43-47.

Nicholas, W. L., A. Grassia, and S. Viswanathan. 1974. The efficiency with which Caenorhabditis briggsae (Rhabditidae) feeds on the bacterium, Escherichia coli. Nematologica 19:411-420.

O'Colla, P. S. 1962. Mucilages. In Physiology and biochemistry of algae, R. A. Lewin (ed.). Academic Press, New York, pp. 337-356.

Ott, J. and F. Schiemer. 1973. Respiration and anaerobiosis of free-living nematodes from marine and limnic sediments. Neth. J. Sea Res. 7:233-243.

Pamatmat, M. M. 1968. Ecology and metabolism of a benthic community on an intertidal sandflat. Int. Revue ges. Hydrobiol. 53:211-298.

Pearse, A. G. E. 1961. Histochemistry, theoretical and applied. Churchill Ltd., London. 998 pp.

Perkins, E. J. 1958. The food relationships of the microbenthos, with particular reference to that found at Whitstable, Kent. Ann. Mag. Nat. Hist. 1:64-77.

Quintarelli, G., M. Bellocci, and R. Geremia. 1973. On phosphotungstic acid staining IV. Selectivity of the staining reaction. J. Histochem. Cytochem. 21:155-160.

Rapport, D. J. and J. E. Turner. 1977. Economic models in ecology. Science 195:367-373.

Rubin, H. and J. J. Lee. 1976. Informational energy flow as an aspect of the ecological efficiency of marine ciliates. J. theor. Biol. 62:69-91.

Sato, T. 1967. A modified method for lead staining of thin sections. J. Electronmicroscopy 16:133-136.

Schiemer, F. and A. Duncan. 1974. The oxygen consumption of a freshwater benthic nematode, Tobrilus gracilis (Bastian). Oecologia 15:121-126.

Schoener, T. W. 1974. Resource partitioning in ecological communities. Science 185:27-38.

Sellner, B. W. 1976. Survival and metabolism of the harpacticoid copepod Thompsonula hyaenae fed on different diatoms. Hydrobiol. 50:233-238.

Sheader, M. and F. Evans. 1975. Feeding and gut structure of Parathemisto gaudichaudi (Guerin) (Amphipoda, Hyperiidea). J. mar. biol. Ass. U. K. 55:641-656.

Sheffield, H. G. 1964. Electron microscope studies on the intestinal epithelium of Ascaris suum. J. Parasitol. 50:364-379.

Shepard, N. and N. Mitchell. 1976. Simultaneous localization of proteoplycan by light and electron microscopy using toluidine blue O. A study of epiphysial cartilage. J. Histochem. Cytochem. 24:621-629.

Smith, D. S. 1968. Insect cells: their structure and function. Oliver and Boyd Ltd., Edinburgh. 372 pp.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastr. Res. 26:31-43.

Swedmark, B. 1964. The interstitial fauna of marine sands. Biol. Rev. 39:1-42.

Tietjen, J. H. 1967. Observations of the ecology of the marine nematode Monhystera filicaudata Allgen, 1929. Trans. Amer. Micros. Soc. 86:304-306.

_____. 1969. The ecology of shallow water meiofauna in two New England estuaries. Oecologia 2: 251-291.

_____. 1971. Ecology and distribution of deep-sea meiobenthos off North Carolina. Deep Sea Res. 18:941-957.

_____ and J. J. Lee. 1972. Life cycles of marine nematodes: influence of temperature and salinity on the development of Monhystera denticulata Timm. Oecologia 10:167-176.

_____ and _____. 1973. Life history and feeding habits of the marine nematode, Chromadora macrolaimoides Steiner. Oecologia 11:303-314.

_____ and _____. 1975. Axenic culture and uptake of dissolved organic substances by the marine nematode, Rhabditis marina Bastian. Cah. Biol. Mar. 16:685-693.

_____ and _____. 1977. Feeding behavior of marine nematodes. In Ecology of marine benthos, B. C. Coull (ed.). University of South Carolina Press, Columbia, pp. 21-35.

Ugolev, A. M. 1965. Membrane (contact) digestion. Physiol. Rev. 45:555-595.

Ward, A. R. 1973. Studies on the sublittoral free-living nematodes of Liverpool Bay. I. The structure and distribution of the nematode populations. Mar. Biol. 22:53-66.

Warwick, R. M. 1971. Nematode associations in the Exe estuary. J. mar. biol. Ass. U. K. 51:439-454.

_____ and J. B. Buchanan. 1970. The meiofauna off the coast of Northumberland I. The structure of the nematode population. J. mar. biol. Ass. U. K. 50:129-146.

Webb, M. G. 1956. An ecological study of brackish water ciliates. J. Anim. Ecol. 25:149-175.

Wieser, W. 1953. Die Beziehung zwischen Mundhohlengestalt, Ernahrungswiese und Vorkommen bei freillebenden marienen Nematoden. Ark. Zool. 4:439-484.

_____. 1960. Benthic studies in Buzzards Bay. II. The meiofauna. Limnol. Oceanogr. 5:121-137.

_____ and F. Schiemer. 1977. The ecophysiology of some marine nematodes from Bermuda: seasonal aspects. J. exp. mar. Biol. Ecol. 26:97-106.