Journal of Nematology 25(4):616–624. 1993.

Ultrastructure of Coelomocytes in *Sphaerolaimus gracilis* de Man, 1876 (Nematoda)

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Abstract: The structure of coelomocytes in the adenophorean aquatic nematode *Sphaerolaimus gracilis* de Man 1876 was studied with light and electron microscopes. Acid phosphatase and catalase activities were demonstrated by electron microscopy. Two pairs of coelomocytes occurred laterally posterior to the esophagointestinal junction. The anterior pair of the coelomocytes, with the renette cell and gonad, lay in either the left or the right lateral side of the body. The posterior pair of coelomocytes was in the opposite side of the body, usually posterior to the renette. A long, thin, cell-extension-like structure appeared to originate from the coelomocytes. Coelomocytes were characterized by specialized organelles (CC-organelle) and large vacuoles. The CC-organelle contained crystalline structures like those in peroxisomes. Acid phosphatase and catalase activities were detected in the matrix of CC-organelles and catalase activity in the vacuoles. It was assumed that vacuoles originate from the CC-organelles. Coelomocytes showed pinocytotic activities, and numerous vesicles were observed between the cell membranes and the vacuoles.

Key words: acid phosphatase, catalase, CC-organelle, coelomocyte, enzyme, nematode, *Sphaerolaimus gracilis*, ultrastructure.

Coelomocytes are highly specialized cells that occur in fixed positions in the body cavity of many nematodes (1,5). They vary in size, form, and number, but currently they are thought to have a similar, yet unresolved function (1,5). Knowledge of the ultrastructure of coelomocytes, which provides evidence of function and relationships, has been derived largely from their study in animal parasites, particularly members of the genus *Ascaris* (3,9,13,14). Large parasites, like *Ascaris* and *Strongylus*, have large branched coelomocytes. The surface of the branches is covered by numerous cytoplasmic swellings. Unbranched, ovoid coelomocytes are typical for small nematodes, such as members of Adenophorea (5).

From taxonomic studies of aquatic nematodes in Finland, coelomocytes were observed for the first time in *Penzancia velox* (Bastian, 1865) and the predaceous species, *Sphaerolaimus gracilis* (Turpeenniemi, unpubl.). Ready availability of specimens of *S. gracilis* provided an adequate source of material for detailed studies of these cells. The purpose of this study was to characterize the fine structure of the coelomocytes occurring in *S. gracilis* and to evaluate by enzymatic methods their metabolic role.

Material and Methods

Nematodes were collected with a Kajak-Hakala core sampler from the Bothnian Bay, in the northern part of the Baltic Sea. The study area and the nematode species composition are recorded by Schiemer et al. (18). Specimens were extracted from sediment using 0.5-, 0.2-, 0.1-, and 0.04-mm mesh sieves. Live nematodes were fixed at room temperature in 4% formalin buffered by TAF. For light microscopy (LM), specimens were processed to glycerol using two methods. Part of the material was pipetted into a 79:1:20 solution of 96% ethanol, glycerol, and distilled water in 5-ml glass tubes. The tubes were closed with parafilm and left at room temperature. The rest of the material was transferred from formalin into a 1:3:3 solution of glycerol–water–ethanol and stored for some duration in vials at 40 C. Permanent slide mounts were studied using a Wild M20 microscope.

For electron microscopy (TEM), live nematodes were fixed in 2.5% glutaraldehyde in cacodylate buffer, postfixed in OsO_4 and dehydrated in an acetone series at 4 C. A final 100% acetone treatment was

Received for publication 29 March 1993.

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I thank Professor H. Hyvärinen for valuable discussions and Drs. R. V. Anderson and I. J. Holopainen for comments on the manuscript and their assistance with the English language.

at room temperature. Specimens were embedded in epon, and ultrathin sections were cut with an LKB ultramicrotome with a diamond knife and studied in a Zeiss EM 900 transmission electron microscope. To demonstrate acid phosphatase in coelomocytes, the method of de Jong (7) was used. Cytochemical staining for catalase was performed with 3,3′-diaminobenzidine (DAB) and H_2O_2 (8). The tails were severed to facilitate penetration of chemicals.

Results

Two pairs of coelomocytes occurred posterior to the esophagointestinal junction. The anterior pair of coelomocytes, together with the renette cell and the gonad, were either in the left or right lateral side of the intestine in the nine females and nine males examined. Of these 18 specimens, three females and four males had the renette, gonad, and the anterior pair of coelomocytes in the left side; the other specimens had them in the right side. The first pair of coelomocytes was 1.5 body diameters posterior to the end of the esophagus. The distance between the pairs of coelomocytes was two body diameters. The posterior pair of the coelomocytes was mostly posterior to the renette. Usually the cells in one pair were well separated from each other. However, in one fixed, actively feeding specimen, the coelomocytes in each pair were abutting.

With TEM, the coelomocyte was ovular in transverse (Fig. 1) and longitudinal sections. One coelomocyte was 16.3 μm wide (Fig. 1), and another was 25 μm long. Occasionally coelomocytes were observed with a thin cell extension (Fig. 2A,B). The cytoplasm of coelomocytes contained the usual cellular components of rough (RER) and smooth (SER) endoplasmic reticula, Golgi bodies, and mitochondria (Figs. 4A,B,5A). An extensive network of SER and a bundle of about 35 microtubules was

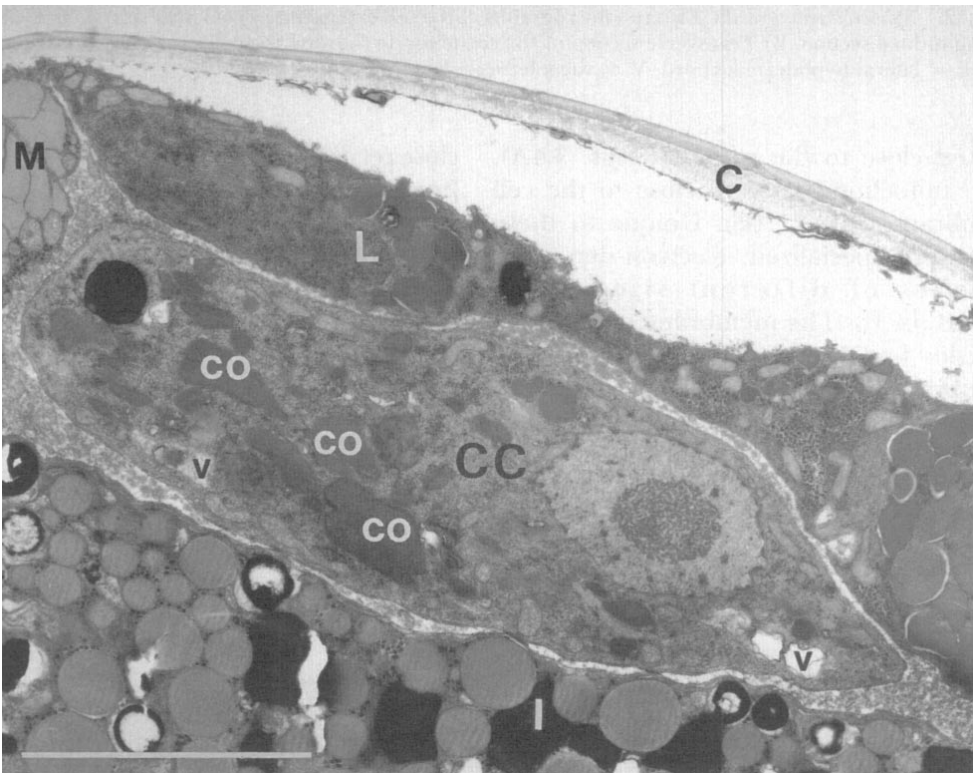

FIG. 1. *Sphaerolaimus gracilis*. Electron micrograph in cross section of the coelomocyte (CC). Note CC-organelles (CO) and vacuoles (V) in the cytoplasm. Lateral hypodermal chord is separated from the cuticle (C), apparently due to polymerization. (I = intestine, L = lateral hypodermal chord, M = muscle cells; scale bar = 5 μm.)

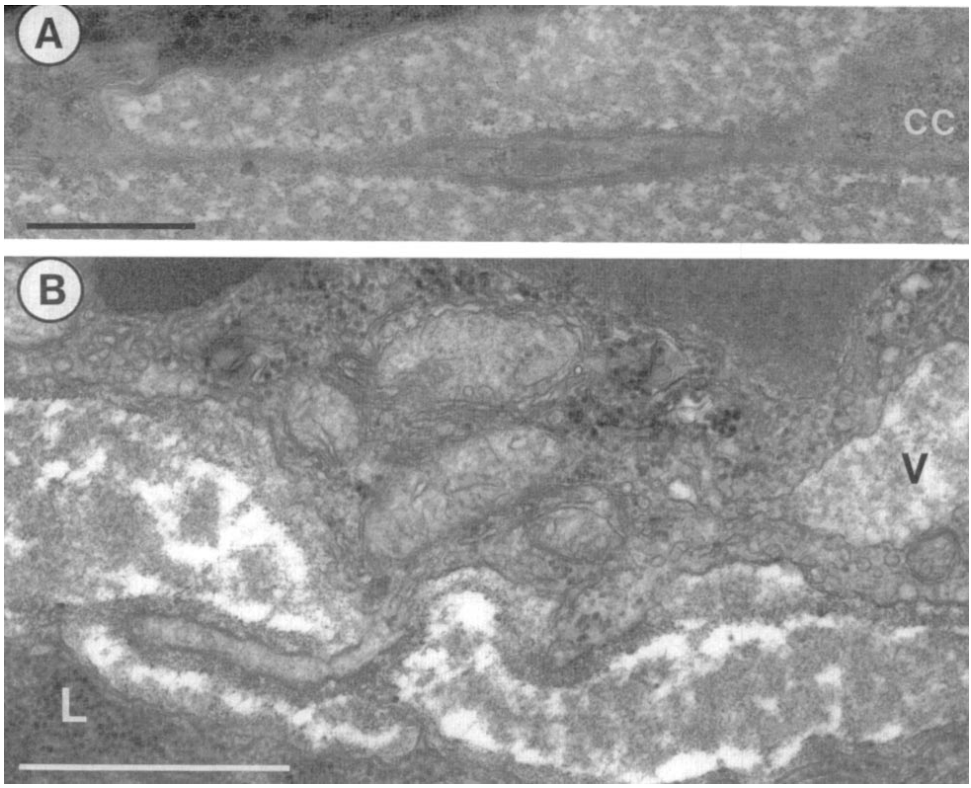

FIG. 2. *Sphaerolaimus gracilis*. Electron micrographs of the cell extensions of coelomocytes. A) Cell extension in longitudinal section. B) Transverse section of the coelomocyte showing the cell extension. (CC = coelomocyte, L = lateral hypodermal chord, V = vacuole; scale bar = 1 μm.)

located close to the nucleus (Figs. 3,4A). Most mitochondria were close to the cell membrane (Figs. 1,4B). Unique to these cells were specialized, electron-dense organelles of different sizes (Figs. 1,4A,B,5A,B). The membrane of these organelles had the characteristics of a cell membrane. The matrix often exhibited a homogenous and electron-dense texture (Fig. 4A) and contained two different cores. The more regular of the cores was composed of tubules ca. 10 nm in diameter (Fig. 6C,D). Substructure of the other core, seen only in longitudinal section, was formed of parallel, slightly sinuous pairs of lines separated by about 5 nm (Figs. 4A,5A,6B,D). Minute granules were arranged in apposition along these lines. A clear vacuole was inside some of the specialized organelles (Fig. 4A,B). Many organelles were large, some being 4 μm in diameter (Fig. 5A). Organelles having a close relationship with vacuoles are termed, herein, coelomocyte organelle (CC-organelle) because they seem to be unique to nematodes. The matrix of the CC-organelles showed both acid phosphatase and catalase activities (Fig. 5A,B). Catalase also was detected inside the vacuoles (Fig. 5B). Like the CC-organelles, vacuoles showed considerable structural heterogeneity. Large granular vacuoles contained clear membrane-bounded vacuoles (Fig. 1,4A,B) and electron dense bodies without a surrounding membrane (Fig. 4B). Flow of pinocytotic vesicles was observed from the cell surface to the large vacuole (Fig. 6A).

DISCUSSION

The fine structure of the cytoplasm of coelomocytes in *S. gracilis* is, in general, like that in *Ascaris* (3,13,14), *Phocanema decipiens* (2) and *Caenorhabditis elegans* (20).

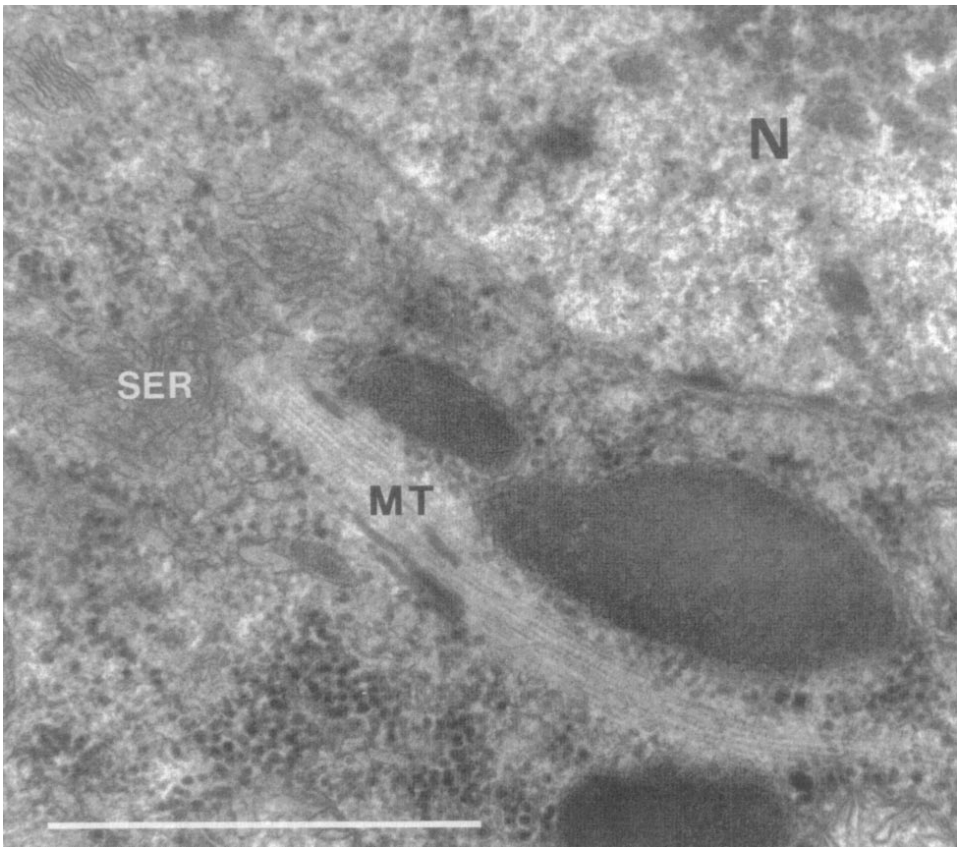

FIG. 3. *Sphaerolaimus gracilis.* Transverse section of a coelomocyte. Note large network of smooth endoplasmic reticulum (SER) adhering to one end of a bundle of microtubules. (MT = bundle of microtubules, N = nucleus; scale bar = 1 μm.)

The intracellular fiber system of branched coelomocytes of *A. suum* (3,13) or the extracellular fiber system of the coelomocyte in *P. decipiens* (2), however, are not present in *S. gracilis.*

There is not enough knowledge of the morphology of coelomocytes in nematodes for systematic comparison. Members of the Secernentea contain both unbranched and branched coelomocytes (5). Large vertebrate parasites like *Ascaris* and *Strongylus* have branched coelomocytes (5), whereas medium-sized invertebrate parasites belonging to the *Thelastomatidae* (4,17) and small bacterial-feeding rhabditids like *Caenorhabditis elegans* (20) have unbranched coelomocytes. Small adenophorean nematodes appear to have rounded coelomocytes (5). The large size of the coelomocytes and their numerous branches bearing many cytoplasmic swellings increase cellular surface area and metabolic efficiency in *Ascaris.* These characteristics are important because of the large size of this nematode. If coelomocyte structure is correlated with nematode size, coelomocytes may have functional limits. Because the number of coelomocytes is usually four (5,16), with a maximum of six (1), these cells cannot properly function if the size of the nematode exceeds certain limits. A reasonable suggestion is that small coelomocytes in small or medium-sized nematodes are functionally analogous with branched coelomocytes present in large nematodes.

Based on morphological criteria, the chemical nature of the cytoplasm in *S. gracilis* coelomocytes resembles that of *Phocanema decipiens.* In *P. decipiens,* histochemistry revealed that the coelomocytes con-

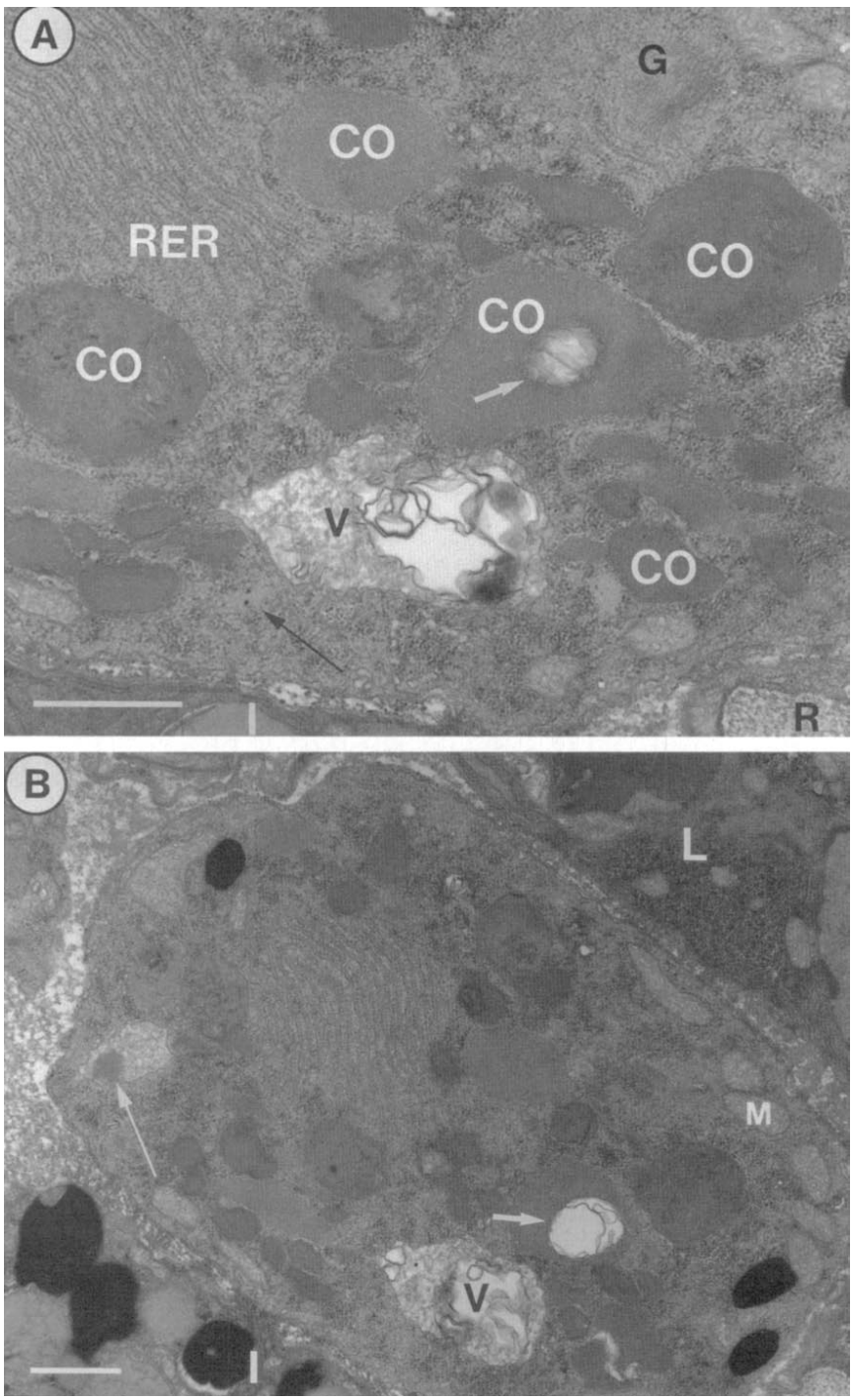

FIG. 4. *Sphaerolaimus gracilis*. Electron micrographs of a coelomocyte. Transverse sections. A) Showing numerous CC-organelles, a vacuole, RER, and Golgi body. Note bundle of microtubules (long arrow) in cytoplasm close to vacuole and small vacuole in a CC-organelle (short arrow). B) Section near (A), containing mitochondria beneath the plasma membrane and a vacuole containing electron dense area (long arrow). Note vacuole inside CC-organelle (short arrow). (CO = CC-organelle, G = Golgi body, I = intestine, L = lateral hypodermal chord, M = mitochondria, R = renette cell, RER = rough endoplasmic reticulum, V = vacuole; scale bar = 1 μm.)

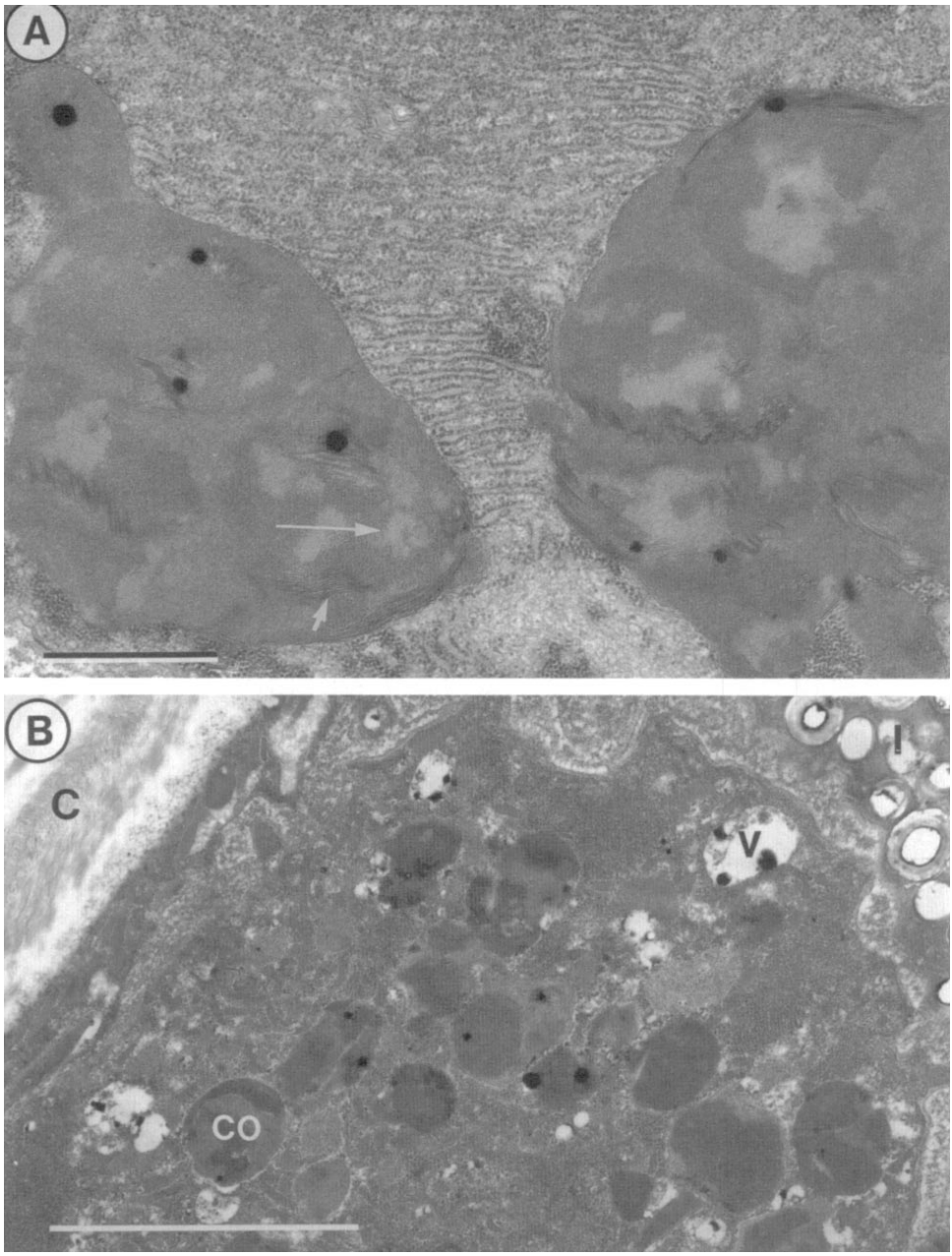

FIG. 5. *Sphaerolaimus gracilis.* Longitudinal sections of coelomocytes showing acid phosphatase and catalase activities. A) Acid phosphatase activity in two CC-organelles. Note electron-lucent areas, one showing crystal substructure; a more regular core (long arrow). A less regular core is marked with a short arrow. Scale bar = 1 μm. B) Catalase activity in coelomocyte. Scale bar = 5 μm. (Black areas have enzyme activity, CO = CC-organelle, C = cuticle, I = intestine, V = vacuole.)

tain few carbohydrates and no fat droplets (2). In addition, coelomocytes are not involved in the molting process (2). Histochemical investigations in *Parascaris equorum* showed that peroxidase activity is high in the cytoplasmic swellings of the coelomocytes (12). Hurlaux (12) thought that coelomocytes act as respiratory centers based on the presence of oxidative enzymes. Without histochemistry or cy-

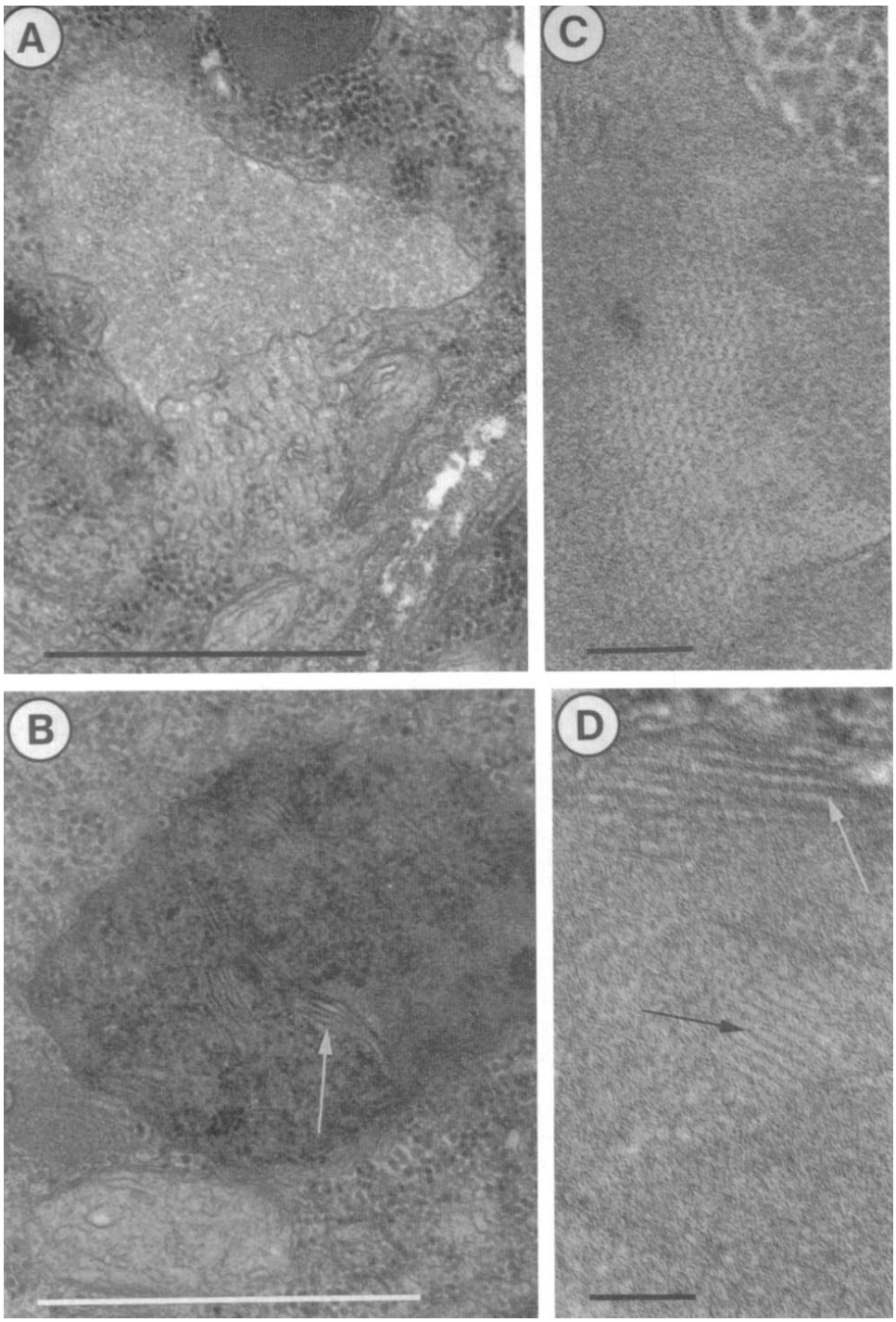

FIG. 6. *Sphaerolaimus gracilis*. Electron micrographs of coelomocytes. A) Numerous vesicles between vacuole and cell membrane. The vesicle stream continues into plasma membrane of neighboring section. Scale bar = 1 μm. B) CC-organelle showing the general structure of a less regular core (arrow). Scale bar = 1 μm. C) High magnification of one type of crystal (a more regular core). Scale bar = 0.1 μm. D) A less regular core (white arrow) and a more regular core (black arrow) in the CC-organelle. Note a double-line substructure in the lines of the more regular core. Scale bar = 0.1 μm.

tochemistry it cannot be determined if CC-organelles are the same organelles as the electron dense bodies (EDB) (3) or the secretory granules (13) reported in coelomocytes of *A. suum*. However, based on morphological comparison, it is likely that the structures in *A. suum* are similar to the CC-organelles of *S. gracilis*. Evidence indicates that there is a distinct population of lysosomes and peroxisomes in the coelomocytes of *A. suum*. Because CC-organelles contain both acid phosphatase and catalase activities, they are neither lysosomes nor peroxisomes (6).

Morphologically, the cores in CC-organelles are related to peroxisomal cores (10) and have polytubular substructure (11). Small tubules (primary tubules) are arranged around a space (secondary tubule) characteristically in a given species (10). In finely polytubular cores, small tubules encircle a space of about 10 nm in diameter in ratios of 6:1 and 12:1 (10). In coarsely polytubular cores, small primary tubules encircle a large central space in a 10:1 pattern (10). Longitudinally, the narrow and wide spacings alternate (6,10). Both these cores are typical of CC-organelles, and the finely polytubular core appears to have 6:1 pattern. The small granules associated with the narrow spacings (primary tubules) in the coarsely polytubular core type in *S. gracilis* appear to be unique.

It has been shown that the core of peroxisomes is associated with urate oxidase (6,10,11,19). The core of CC-organelles possibly is also composed of urate oxidase, because of similar structure to peroxisomes (10). Urate oxidase catalyzes the oxidation of uric acid to allantoin (15). In some marine invertebrates, purines are degraded to uric acid, allantoin, allantoic acid, urea, and then ammonia and carbon dioxide (15). The presence of catalase in CC-organelles indicates that oxidase(s) must also be present, producing H_2O_2. The catalase observed in the inner, granular margins of the vacuoles indicates oxidation and a close relationship between CC-organelles and the vacuoles. The morphology of coelomocytes indicates that they do not have a secretory function and therefore cannot be classified as hypodermal glands.

Coelomocytes clearly are involved in the metabolism of body cavity fluid, and CC-organelles take part in this process. The urate oxidase-like cores in the CC-organelles indicate that these cells may be associated with purine degradation. Because of the abundance of CC-organelles in coelomocytes and the oxidative and hydrolytic functions of these organelles, coelomocytes may be important centers of intermediary metabolism in nematodes.

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