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Structure and Role of the Renette Cell and Caudal Glands in the Nematode *Sphaerolaimus gracilis* (Monhysterida)

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Abstract: Ultrastructure of the renette cell and caudal glands was studied in the free-living aquatic nematode *Sphaerolaimus gracilis*. The renette cell occurred posterior to the esophageal-intestinal junction and opened through an ampulla to a ventral pore behind the nerve ring. The caudal gland system of the tail consisted of two gland cells opening through separate pores and 2 to 3 other gland cells of a different type opening through a common pore. The renette cell and the two caudal gland cells were similar and both contained secretory granules, 0.5–1.5 μm in diameter. The material released attached the nematode to the substrate. The renette ampulla was surrounded by a specialized cell, the ampulla cell, which had characteristics of myoepithelium. A plug or valve structure connected to the ampulla cell may regulate the output of the secretory material. The ampulla cell is able to contract and thus is probably under direct neuronal control. Other cells in the renette ampulla region of body cavity were termed supporting cells. Living, cold-relaxed nematodes were attached to sediment particles in the renette pore region and at the tail tip. Release from sediment particles was mechanical at the renette cell discharge site but appeared to be chemical at the caudal gland. In behavioral experiments, nematodes in a water current had the ability to release a thread from the caudal glands while maintaining contact with a sediment particle attached to the tail end. If the thread was strong enough, it also could be used to change location. Nematodes anchored by the thread from the caudal glands to a sediment particle could float in water currents until they attached themselves to another sediment particle with the help of secretions from the renette cells.

Key words: ampulla, ampulla cell, behavior, caudal gland, function, locomotion, morphology, nematode, plug, renette cell, structure, supporting cell, ultrastructure, valve.

The renette cell is a glandular type (3) of secretory-excretory organ (2). It consists of a single gland cell situated near the base of the esophagus (2,3). From the cell there extends anteriorly a renette duct, which usually swells into an ampulla close to the ventral pore (2,3). The caudal gland cells are situated in the posterior body region, usually in the tail (2,3) and open subterminally or terminally at the tail tip (2). Both the renette cell and caudal glands are common in adenophorean nematodes (2,3). Aquatic nematodes with renette and caudal glands share the property of secreting an adhesive material that allows for anchorage in a current of water; currently, this secretory function is attributed only to the caudal glands. Previously, the renette cell was thought to serve as an excretory organ (2,3), a function assigned to it on morphological grounds (2,3). Nematodes in the class Secernentea differ from Adenophorea in lacking a clear renette cell and caudal glands. Ultrastructural studies in *Enoplus communis* Bastian, 1865 and *Monhystera disjuncta* Bastian, 1865 showed that the renette cells produced large amounts of secretory material (2). However, the function of these secretions is uncertain (2). In *M. disjuncta* the secretion of the renette cell is released into the region between the subventral lips (8), but usually the renette cell opens to the ventral side of the body in the region of the esophagus (3).

The function of the caudal glands is currently believed to be attachment of the nematode to a substrate (2,3). Usually the caudal glands share a common orifice at the tail end, which is closed by a spinneret valve (3). This kind of caudal gland system exists in *Chromadorina germanica* (Bütschli, 1874) (5). However, ultrastructural studies have shown (6) that it differs from the gland system of *Theristus caudasaliens* Adams & Tyler, 1980, in which two separate pores occur without a spinneret valve (1). *Sphaerolaimus gracilis* de Man, 1876 has characteristics of both systems.

The purpose of this research was to determine the function of the renette cell

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and caudal glands in *S. gracilis* by means of morphological, ultrastructural, and behavioral studies.

Materials and Methods

Nematodes used for light and electron microscope studies were fixed in 2% glutaraldehyde in cacodylate buffer, and some of the light microscope (LM) material was mounted in glycerol as described previously (7). The behavioral studies were carried out as follows: sediment was taken as core samples and transferred to the laboratory, where the samples were kept in plastic jars at 0 to 4°C. Nematodes were extracted by sieving with cold tap water, and cold-relaxed nematodes were studied in petri dishes. The adhesive points of the body were found by gliding a needle along the body and observing adhesion. A slow current of water was used to determine how water current affects the ability of the renette cell and caudal gland to attach the nematode to sediment particles. Photographs were taken with a Leitz Dialux 20 EB microscope and electron micrographs with a Zeiss EM 900 transmission electron microscope.

Results

Renette cell

General structure: The renette cell of *S. gracilis* was located laterally and posteriorly to the esophageal-intestinal junction (Fig. 1A). The renette cell duct, 2 to 5 μm wide, passed laterally through the body cavity at the level of the esophago-intestinal junction and continued anteriorly in a more ventral position (Fig. 1A). The duct ended posteriorly to the nerve ring and close to the ventral pore (Fig. 1A). Distally, the renette cell duct widened into a renette ampulla 15 to 17 μm long and 10 to 12 μm wide (Figs. 1B;2B,C;4C). The release of the secretion from the renette ampulla was regulated by a plug (Figs. 1B;2B;4C;8–10). The plug and renette ampulla were surrounded and supported by ramifications of the ampulla cell (Figs. 1B;2B,C;4C;5–12). A plug was also identified with LM (Fig. 2B). Measured with LM the plug was 5–8 μm long and 2–3 μm wide (n = 8).

Ultrastructure of cell and cell duct: The renette cell and cell duct were filled with secretory granules, 0.5–1.5 μm in diameter (Figs. 3;4A,B,D;5–12A). The contents of the secretory granules were reticulated (Figs. 3;4A,B,D). The renette cell nucleus was situated at the posterior end of the cell (Fig. 3), where rough endoplasmic reticulum (RER) was more abundant than between the closely packed secretory granules (Figs. 3;4A,B). A few mitochondria lay adjacent to the outer cell membrane (Fig. 3). Secretory granules, Golgi-complexes, and smooth endoplasmic reticulum (SER) were closely connected with each other (Fig. 4A,B), and the SER appeared to develop from RER (Fig. 4B). The cisternae of the granular endoplasmic reticulum were greatly distended by the accumulation of cell products, and vesicles containing RER material were adjacent to SER at one side of the Golgi-complex (Fig. 4B). Mitochondria also were seen at these sites (Fig. 4A,B).

Ampulla cell and supporting cells: A cell was observed closely appressed to the renette ampulla and appeared to be responsible for formation of the plug structure and regulation of the release of the secretion. This cell is termed the ampulla cell. The ampulla cell occurred close to the posterior end of the renette ampulla (Fig. 5), but branched ventrally (Figs. 5,6) with the branches enclosing the renette ampulla both anteriorly (Figs. 11–12) and posteriorly (Figs. 5B;6,7). Small, electron-dense vesicles were synthesized in the ampulla cell (Figs. 5,7). These vesicles were at the base of the plug or valve formation and were assumed to be the material used to form the plug (Figs. 7,8). The plug of the valve is a specialized extracellular structure surrounded and synthesized by the ampulla cell (Figs. 8–10). Ampulla cell branches were connected to each other by desmosome-like junctional complexes (Figs. 8,9,11), which also connected the ampulla cell to the renette ampulla (Fig. 9).

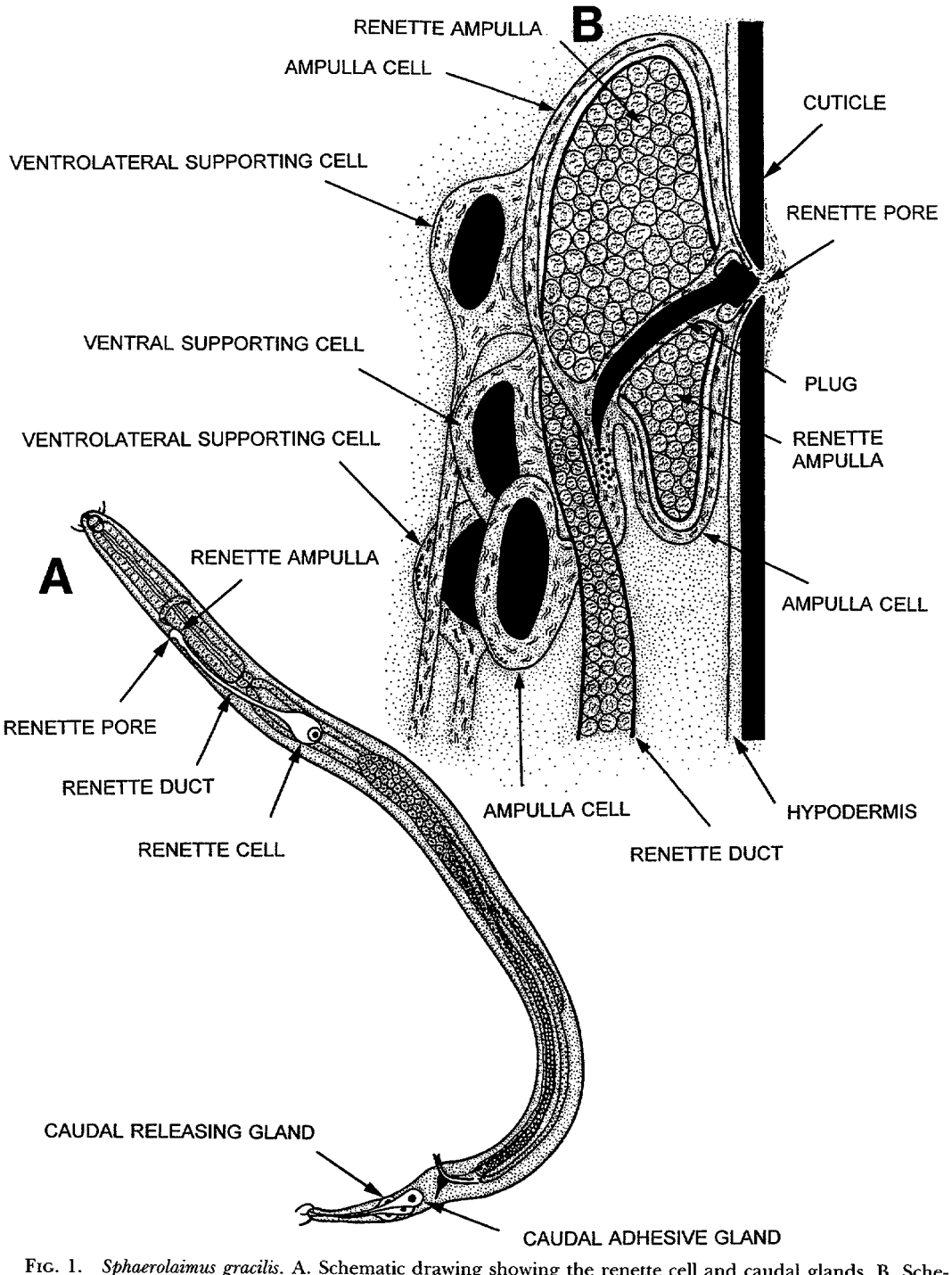

FIG. 1. *Sphaerolaimus gracilis*. A. Schematic drawing showing the renette cell and caudal glands. B. Schematic drawing of the renette pore region.

The rupture of the membranes of the secretory granules of the renette cell released their contents into the extracellular space near the ventral pore (Figs. 9–10). The ampulla cell extended around the ventral pore, forming about a 1-μm-wide

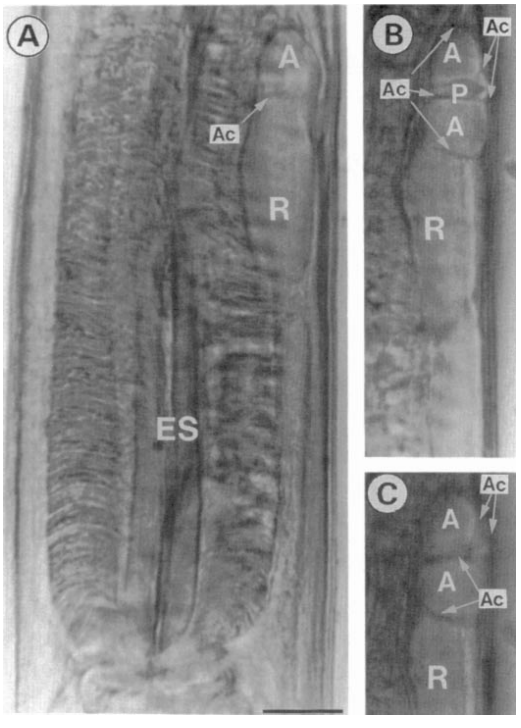

FIG. 2. *Sphaerolaimus gracilis.* Longitudinal photomicrographs of the renette cell duct, distal end. Right dorsolateral view. A. Section to the right of the renette pore showing posterior end of the esophagus (ES), renette cell duct (R), renette ampulla (A), and ampulla cell (Ac) at base of plug. B. Renette ampulla (A) and plug (P) surrounded and supported by the branches of the ampulla cell (Ac). C. Ampulla cell branches (Ac) to the left of the plug and renette pore. Scale bar = 10 μm.

zone free of hypodermal tissue (Fig. 10). Associated with the ventral cord were other specialized cells, termed supporting cells (Figs. 1B;5–12). The ventral supporting cell (VS) enclosed the dorsal side of the ventral cord, including the ampulla cell and the renette cell duct (Figs. 5–8). The other supporting cells, called ventrolateral supporting cells (VLS), were situated in the body cavity between the lateral cords and the ventral cord (Figs. 1B;6–12). These cells are characterized by the cell body lying in the ventral (Figs. 1B;6–12) or lateral chord, a long and wide cell extension in the body cavity between the ventral and lateral chord (Figs. 1B,12), and narrow branches closely connected with each other, the ampulla cell, and the ventral nerve cord (Figs. 9–12). Nerve endings containing neurotransmitter vesicles were observed at the posterior end of the renette ampulla and at the base of the plug formation (Figs. 5B;6,7). The ampulla cell branched into the nerves (Fig. 7).

The ultrastructure of the ampulla cell resembled that of the supporting cells. Both contained numerous meandering microtubules and microfilaments (Figs. 5–12). Typical cytoplasmic organelles included mitochondria, Golgi-complexes, RER, SER, and ribosomes.

Caudal gland apparatus

The tail contained two cells (caudal adhesive gland [CAG]) that were similar to the renette cell and 2 to 3 other cells of different types (caudal releasing gland [CRG]) (Figs. 1A;13,14). One CAG cell occurred more anteriorly than the cell centers of the other CAG cell and the two CRG (Fig. 13). Releasing gland cells (CRG) were characterized by the presence of scattered rough endoplasmic reticulum and large, electron-dense granules (Fig. 13). Two cell ducts of the CAG and at least one

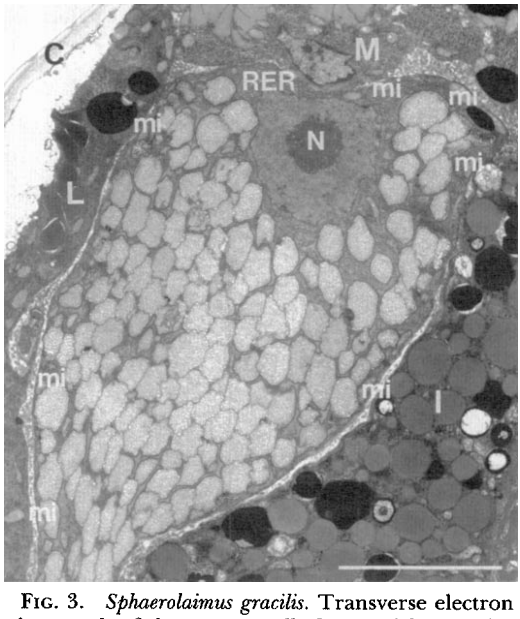

FIG. 3. *Sphaerolaimus gracilis.* Transverse electron micrograph of the renette cell. C = cuticle, I = intestine, L = lateral hypodermal chord, M = muscle cells, mi = mitochondria, N = nucleus of the renette cell, RER = rough endoplasmic reticulum. Scale bar = 5 μm.

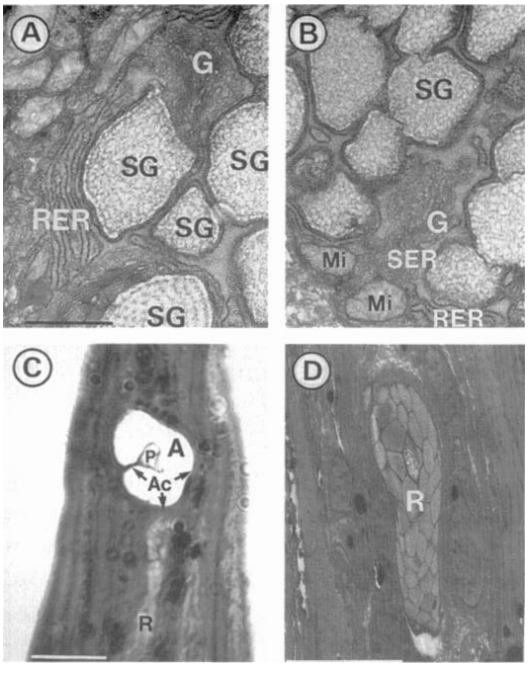

FIG. 4. *Sphaerolaimus gracilis*. A,B) Transverse electron micrographs of the renette cell. Secretory granules (SG), rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), Golgi-complexes (G), and mitochondria (Mi) associated with formation of secretory granules. Scale bar = 1 μm. C) Longitudinal light micrograph showing renette ampulla (A), renette cell duct (R), plug (P), and branches of ampulla cell (Ac). Scale bar = 10 μm. D) Longitudinal electron micrograph of renette cell duct (R) close to the renette ampulla. Scale bar = 5 μm.

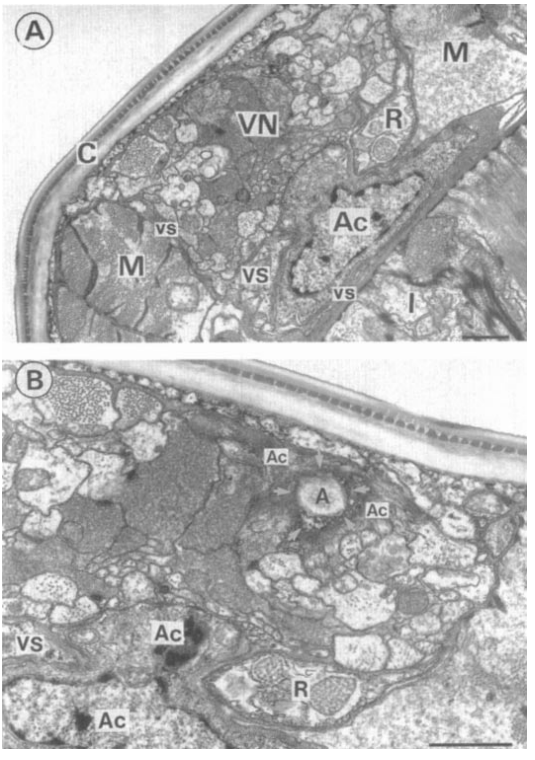

FIG. 5. *Sphaerolaimus gracilis*. Transverse electron micrographs of the ampulla cell. A) Ampulla cell (Ac) branching ventrally. B. Section near (A), showing the posterior end of the renette ampulla (arrowed) and the ventral branch of the ampulla cell. A = renette ampulla, Ac = ampulla cell, C = cuticle, I = intestine, M = muscle cells, R = renette cell duct, VN = ventral nerve cord, VS = ventral supporting cell. Scale bar = 1 μm.

duct of the CRG were present near the tail tip (Fig. 14A). The CRG duct was adjacent to a pair of longitudinal nerves and longitudinal muscles (Fig. 14A). The secretory granules were disrupted at distal ends of both types of ducts (Fig. 14B), and the secretory material was released through two CAG pores and one CRG pore (Fig. 15).

Behavioral studies

Several living, cold-relaxed specimens of *S. gracilis* attached themselves by the tail and renette-pore regions onto sediment particles. As a needle was glided along the nematode body, the needle adhered to the nematode in the region of the renette pore. The needle could be similarly attached to the tail terminus. By attaching the needle to either of these regions, the

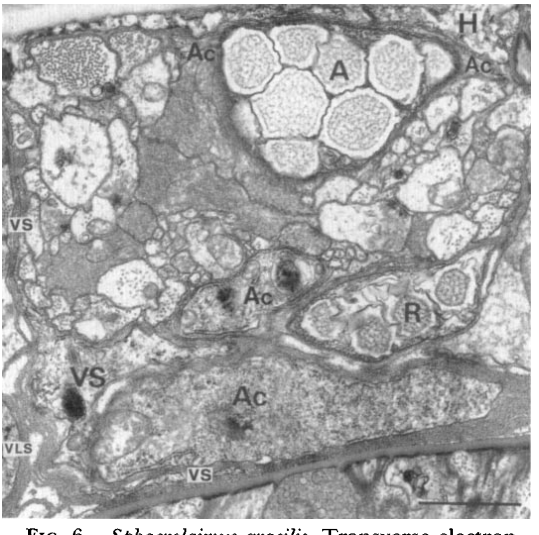

FIG. 6. *Sphaerolaimus gracilis*. Transverse electron micrograph showing renette ampulla (A), ampulla cell (Ac), hypodermis (H), renette cell duct (R), ventral supporting cell (VS), and ventrolateral supporting cell (VLS). Scale bar = 1 μm.

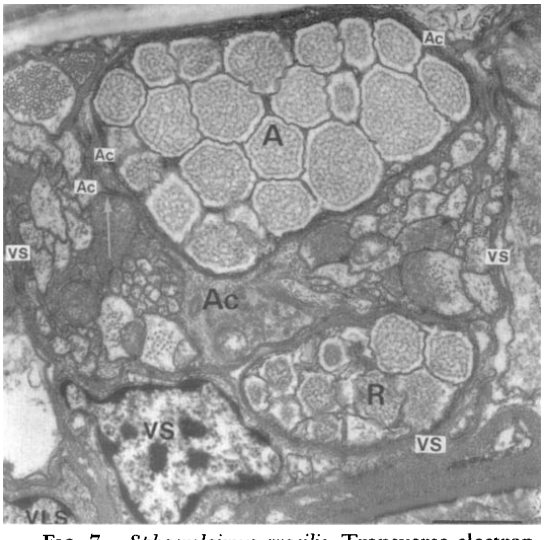

FIG. 7. *Sphaerolaimus gracilis.* Transverse electron micrograph showing electron-dense vesicles in part of the ampulla cell (Ac). Note the vesicles within nerve fibers in close contact with the ampulla cell and the branch of ampulla cell (arrow). (A = renette ampulla, R = renette cell duct with secretory granules, VS = ventral supporting cell with central nucleus, VLS = ventrolateral supporting cell). Scale bar = 1 μm.

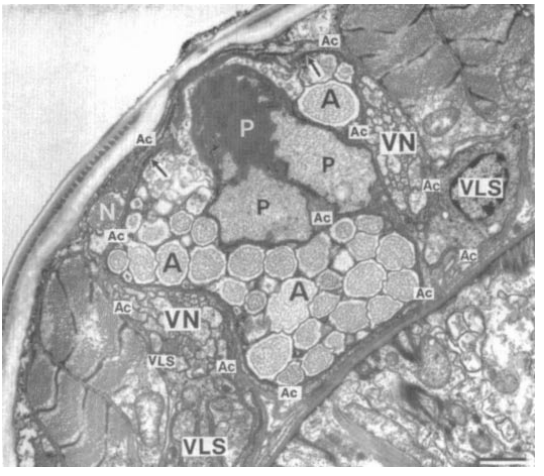

FIG. 9. *Sphaerolaimus gracilis.* Transverse electron micrograph close to the renette pore showing the plug (P). Note the cell junctions (belt desmosomes) between the renette ampulla and ampulla cell (long arrows) and between branches of the ampulla cell (small arrows). Note the secretory granules inside the renette ampulla and released secretory material outside renette ampulla and on the cuticle. A = renette ampulla, Ac = ampulla cell, N = nerve fiber, VN = ventral nerve cord, VLS = ventrolateral supporting cell. Scale bar = 1 μm.

nematodes could be handled. The temperature gradients in the petri dish caused small water currents. When a specimen was taken from the bottom of the petri dish with a needle attached at the renette pore, a sediment particle often remained attached to the tail. When the nematode was kept in the water, the slight current moved the sediment particle several body lengths away from the nematode (Fig. 16). During this time the sediment particle remained anchored to the nematode by a thread released from the caudal glands (Fig. 16).

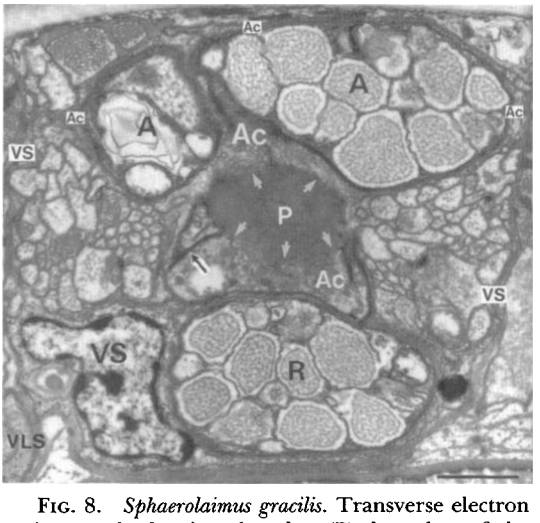

FIG. 8. *Sphaerolaimus gracilis.* Transverse electron micrograph showing the plug (P), branches of the ampulla cell (Ac), renette cell duct (R), ventral supporting cell (VS), and ventrolateral supporting cell (VLS). Note membrane (short arrows) separating plug from ampulla cell and numerous electron-dense vesicles in the ampulla cell. Desmosome-like junctional complexes connect the ampulla cell branches to each other. One is indicated (long arrow). Scale bar = 1 μm.

DISCUSSION

In the Adenophorea, the renette cell and caudal glands have a secretory function (2). By using the posterior tail end, the nematode anchors its body to the sediment (2). Release is thought to be either mechanical or chemical (1,3). Jensen (4) suggested that the renette cell of the marine chromadorid *Ptycholaimellus ponticus* (Filipjev, 1922) secretes tube-building material, whereas Van De Velde and Coomans (8) proposed that secretions in *M. disjuncta* may be used in exodigestive purposes or

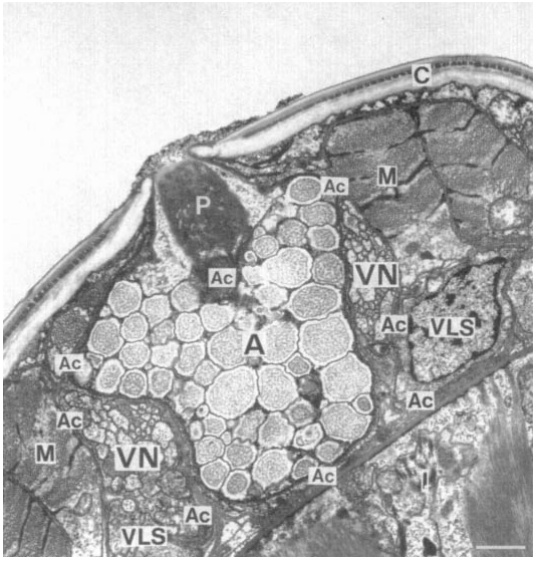

FIG. 10. *Sphaerolaimus gracilis.* Transverse electron micrograph through the renette pore showing plug (P), renette ampulla (A), and ampulla cell (Ac). Note branches of the ampulla cell in close association with ventrolateral supporting cells (VLS) and the ventral nerve chord (VN). C = cuticle, I = intestine, M = somatic muscles. Scale bar = 1 μm.

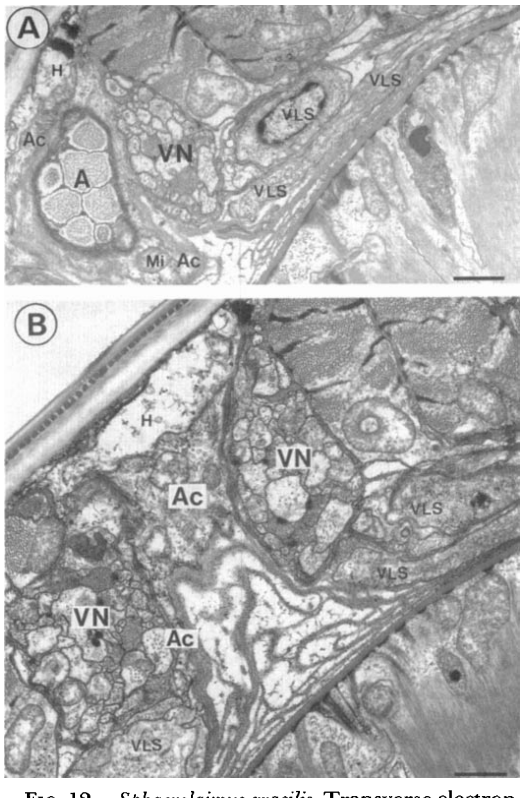

FIG. 12. *Sphaerolaimus gracilis.* Transverse electron micrographs anterior to the renette pore. A) Anterior end of renette ampulla. B) Anterior end of ampulla cell. A = renette ampulla, Ac = ampulla cell, H = hypodermis, Mi = mitochondrion, VLS = ventrolateral supporting cell. Scale bar = 1 μm.

possibly also in tube-building. Comparative ultrastructural studies of the renette cell and caudal glands in the same nematode species are apparently lacking (2). Lippens (5) studied the ultrastructure of the caudal glands and compared different glands in *Chromadorina germanica.* She found that the staining properties and secondary fluorescence of the ventral gland (= renette cell) and caudal glands were similar to each other but different from other glands (5). The fine structure of the renette cell (6,8) and caudal glands (1,5) in different nematode species indicates that these cells are involved in producing large amounts of secretory material (2).

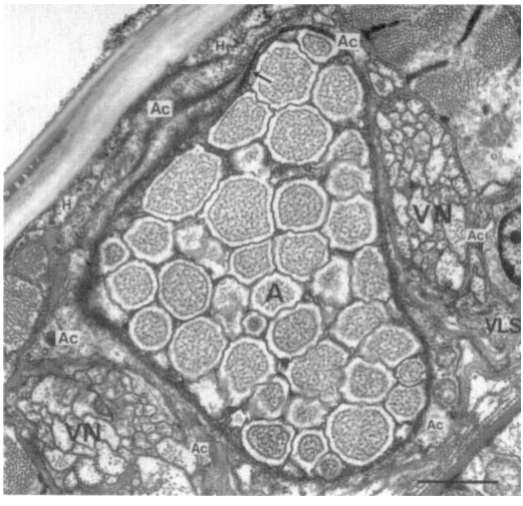

FIG. 11. *Sphaerolaimus gracilis.* Transverse electron micrograph anterior to the renette pore. Note branches of the ampulla cell (Ac) and desmosome-like junctional complex between two branches (arrow). A = renette ampulla region, H = hypodermis, VLS = ventrolateral supporting cell. Scale bar = 1 μm.

Ultrastructurally, the renette cell of *S. gracilis* and that of *M. disjuncta* resemble each other. Each species has the cell in the body cavity behind the esophagus, a narrow duct, and a valve at the end of the duct (8). The secretory granules of these renette cells are similar, having a retriculated content that fills both the cell and its duct. In both of these species, secretory material

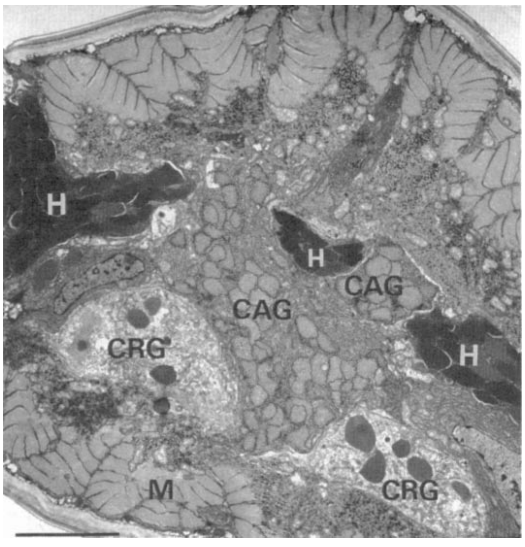

FIG. 13. *Sphaerolaimus gracilis.* Transverse electron micrograph through the tail showing two types of caudal glands. CAG = caudal adhesive gland of the renette cell type, CRG = caudal releasing gland, H = hypodermal chord, M = muscle. Scale bar = 5 μm.

is moved in transport vesicles from the RER-SER system to the Golgi complex. Other common characters are the electron-dense vesicles and filaments at the base of the valve (8). Morphologically, the renette cell complex of *S. gracilis* differs from the corresponding structure of *M. disjuncta* in having the opening at a different position and in lacking a terminal cuticular duct (8). From the cuticular duct of *M. disjuncta,* secretory material is expelled through a pore located between the two subventral lips (8). According to Van De Velde and Coomans (8), secretion may be regulated in the lip region as well, because it was seen in SEM that the pore was open only when the mouth was open (8). In *S. gracilis* the ampulla cell and the plug or valve regulate the output of the material, and the ventral pore has a rigid structure. In *M. disjuncta* the distal end of the renette cell duct was reported to be surrounded by epidermis (= hypodermis) (8). The hypodermis of *S. gracilis* is a narrow layer beneath the cuticle (except for a 1-μm-wide zone around the pore) and does not enclose either the renette ampulla or the renette duct.

Narang (6) studied the ultrastructure of the renette cell in *Enoplus brevis. Enoplus brevis* Bastian 1865 differs from *S. gracilis* and *M. disjuncta* in having the renette cell in the esophageal region and in lacking a valvular apparatus. In *E. brevis* the renette cell duct is embedded for some distance in the matrix layer of the cuticle before opening on the surface, whereas in *S. gracilis* the ventral pore is directly opposite the plug structure and the renette ampulla. In *E. brevis* the ampulla position is more posterior than in *S. gracilis.* In the three adenophorean nematodes (*E. brevis, M. disjuncta, S. gracilis*) the secretory granules of the renette cell appear to be similar, which may mean that their physiological proper-

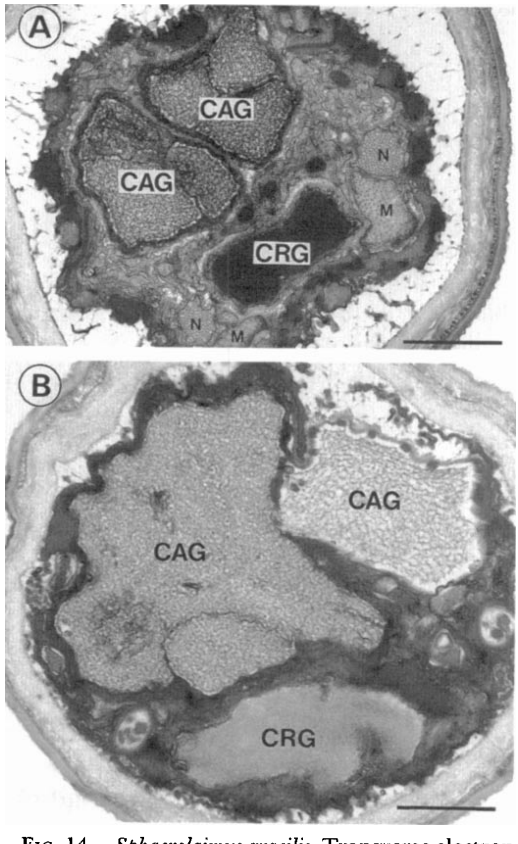

FIG. 14. *Sphaerolaimus gracilis.* Transverse electron micrographs of the ducts of the caudal glands. A) Two ducts of the adhesive cell type (CAG) and one duct of the releasing cell type (CRG). Note two longitudinal muscles (M) and two longitudinal nerves (N). B) Ducts (CAG,CRG) near the tail tip. Note disrupted secretory material and one secretory granule representing both types of material. Scale bar = 1 μm.

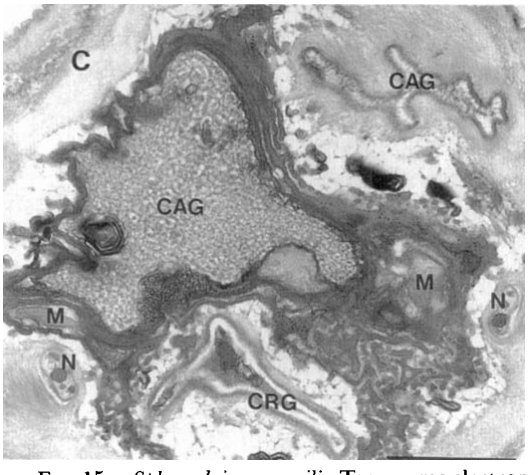

FIG. 15. *Sphaerolaimus gracilis.* Transverse electron micrograph close to the tail tip. Note ducts opening through separate pores. CAG = caudal adhesive gland, CRG = caudal releasing gland, M = muscle fibers, N = Nerves. Scale bar = 1 μm.

ties are also similar (6,8). We suggest that one function of the renette cell in these species is as an adhesive organ.

Electron microscopy of adenophorean caudal glands has been studied by Lippens (5) and Adams and Tyler (1). *Perelepsinonema conifera* Lorenzen 1973 (Desmodorida) differs from the other nematodes studied in having the caudal glands situated preanally (1). Both *P. conifera* and *T. caudasaliens* are thought to have a dual gland system containing three viscid glands and two releasing glands (1). Caudal glands in adenophorean nematodes usually open through a common pore at the tail end, which is provided with a spinneret valve (3). *Theristus caudasaliens* and *S. gracilis* are unique in having separate outlets at the tail terminus and in lacking the spinneret valve (1). The viscid glands of *Theristus caudasaliens* have been considered responsible for attaching the nematode to a substrate (1). Ultrastructurally, the viscid gland differs from that found in *S. gracilis* and is therefore called a caudal adhesive gland in *S. gracilis.* Adhesive granules differ morphologically in *T. caudasaliens* and *S. gracilis.* The other type of gland (releasing gland) produces electron-dense granules, which in the cell and cell duct are larger in *S. gracilis* than in *T. caudasaliens.* Adams

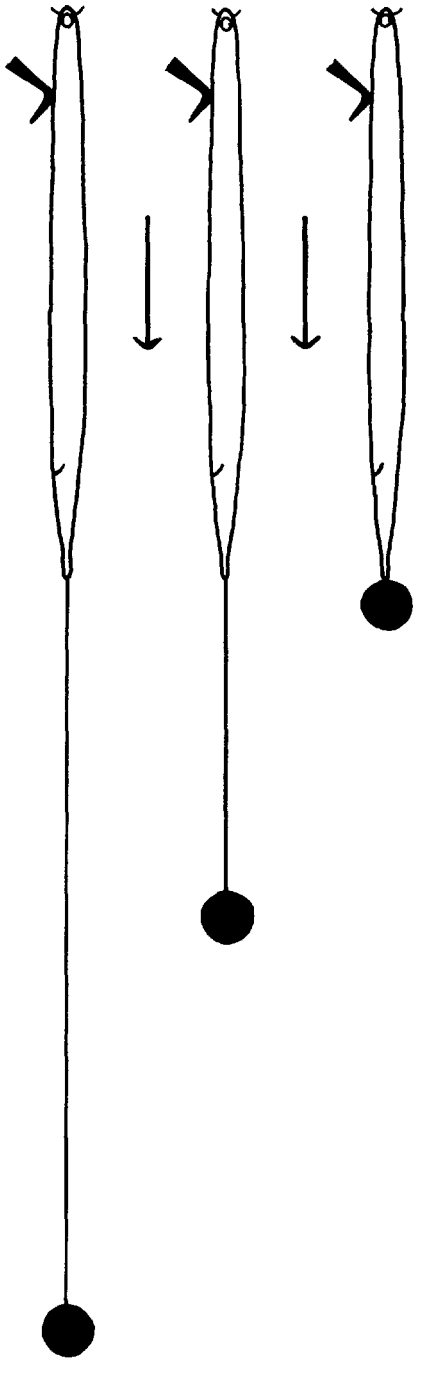

FIG. 16. *Sphaerolaimus gracilis.* Schematic drawing of cold-relaxed specimen demonstrating release of caudal adhesive material. With stationary needle attached to the site renette pore, the nematode is held in the water current (arrows), which pushes the sediment particle backwards; the thread released from the caudal glands is strong enough to keep the nematode attached to the sediment particle.

and Tyler (1) suggested that these glands are used to release the nematode from the substrate. Adhesive granules of *S. gracilis* and *P. conifera,* on the other hand, differ from normal viscid gland granules in being larger and in having a flocculent content (1). The presence of releasing-type glands in these species, however, may indicate the presence of a dual gland system, as suggested by Adams and Tyler (1).

Ultrastructurally, the secretory granules of the renette cell (*M. disjuncta; S. gracilis*) and caudal glands (*Chromadorina germanica; P. conifera,* viscid glands; *S. gracilis,* one type) are strikingly similar (1,5, present study). In the distal ends of the ducts of the renette cell (*M. disjuncta, S. gracilis*) and caudal glands (*C. germanica, P. conifera,* and *S. gracilis*) the secretory granules were found to be disrupted. It is not known whether this disruption is caused by mechanical or enzymatic processes. Our behavioral observations of *S. gracilis* show clearly that this secretory material is used to anchor the nematode to the substrate. Release of the secretion of the renette cell is regulated at least by the plug that is located at the distal end of the renette cell and possibly by the constriction of the branches of the ampulla cell. This secretion release mechanism appears similar to the way myoepithelial cells regulate secretions in the skin glands of vertebrates. The ampulla cell has the characteristics of myoepithelial cells. The ampulla cell with plug formation in *S. gracilis* had not been reported previously in free-living aquatic nematodes. By surrounding secretory cells or their ducts, the myoepithelial cells facilitate secretion in small glands of higher animals. Because of their contractile properties, myoepithelial cells are considered to be specialized smooth muscle cells. Neuronal control of renette cell secretion had been proposed earlier but not demonstrated (8). Nerve endings containing neurotransmitter vesicles are in close contact with the ampulla cell, and the ampulla cell clearly branches into nerves at the base of the plug, which may indicate that in *S. gracilis* the function of the ampulla cell is under neuronal control.

The supporting cells of *S. gracilis* are closely related to the mesenchymal cells of the vertebrate connective tissue. Similar cells also were found supporting the lateral chords anterior to the ampulla region in *S. gracilis.*

In conclusion, these studies showed that both the renette cell and one type of caudal gland are involved in attaching *S. gracilis* to a substrate, and that these cells may function in conjunction with each other. In releasing a thread from the caudal gland and floating in a water current, *S. gracilis* evidently has the ability to change locations, and the renette cell appears to allow *S. gracilis* to anchor at a new site. These studies clearly showed that cold-relaxed, living specimens of *S. gracilis* were attached to the substrate at both ends of the body, anteriorly by secretions of the renette cell and posteriorly by secretions of the caudal glands. Our results also indicate that in *S. gracilis,* the anterior part of the body is released from the substrate mechanically and the posterior part is released chemically.

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