Fine Structure of the Esophagus of Males of Sarisodera hydrophila (Heteroderoidea)

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Abstract: The fine structure of the esophagus, including procorpus, metacorpus, isthmus, gland lobe, and esophago-intestinal junction, is examined in males of Sarisodera hydrophila. A cuticle-lined lumen extends most of the length of the esophagus, broadens to form a pump chamber in the metacorpus, and posteriorly is continuous with junctional complexes among four esophago-intestinal cells. These four cells are partially enveloped by the gland lobe which basically consists of three gland cells, one dorsal and two subventral. Each gland cell has an anterior process which opens into the lumen of the esophagus through a cuticle-lined duct. The dorsal gland joins the lumen in the anterior portion of the procorpus, whereas ducts of the subventral glands terminate at the base of the metacorpus pump chamber. The subventral glands are predominant in the posterior portion of the gland lobe and are partially ensheathed by a narrow portion of the dorsal gland which extends to within 5 μm of the posterior terminus of the gland lobe. Contents of the dorsal gland include primarily electron dense granules, although rough endoplasmic reticulum (RER) is predominant posteriorly. Secretory granules within the subventral glands vary in morphology and are evenly distributed throughout the two cells among other organelles, including RER and a large Golgi apparatus. Innervation of the esophagus includes nerve processes which originate from several perikarya (cell bodies) located in the anterior portion of the gland lobe. The esophagus of males of S. hydrophila is compared with that of other Heteroderoidea, Heterodera glycines and Meloidogyne incognita. Key words: comparative morphology, digestive glands, nonfeeding, secretion.

pierced were processed for comparison. Post-fixation was with 1% osmium tetroxide, and embedding was in Spurr's epoxy (15). Specimens were sectioned with a glass or diamond knife on a Porter-Blum MT2-B ultramicrotome and stained with uranyl acetate and lead citrate (3). Sections from more than 35 specimens were examined using Hitachi HU-12 and H-600 transmission electron microscopes.

OBSERVATIONS

The esophagus of males of *S. hydrophila* is composed of a procorpus, metacorpus, isthmus, and a glandular lobe which ventrally overlaps the anterior portion of the intestine (Fig. 1). The esophagus is enclosed by basal lamina which is most pronounced in the region of the metacorpus (Fig. 6). A cuticle-lined lumen extends most of the length of the esophagus, excluding the posterior half of the gland lobe. In a portion of the metacorpus, the lining is thickened and modified as a pump chamber, often called a valve (Figs. 1, 6). Further posteriorly, in the mid region of the gland lobe, the esophagus, including the cuticle-lined lumen, joins the intestine. The lobe is about 60 μm long and is primarily composed of one dorsal and two subventral gland cells. A cytoplasmic process extends from each of the three gland cells and joins the lumen of the esophagus anteriorly through a cuticulized duct. The junction of orifice of the dorsal gland occurs in the procorpus 3–5 μm posterior to the stylet knobs, whereas the two subventral gland ducts each occur at the base of the metacorpus pump chamber (Fig. 1).

The procorpus is composed essentially of elongate epithelial cells. Generally, six occur in a given cross section and surround a complex of tissues, which includes the round esophageal lumen, and the dorsal gland process (Figs. 2–5). Anteriorly, the three largest of the six cells, in dorsal and subventral positions, are noncontractile portions of the stylet protractor muscle cells (Fig. 2). Nuclei of epithelial cells of the procorpus typically occur within a narrow process from each cell, and very little cytoplasm occurs between the plasmalemma (cell membrane) and the nuclear membrane (Fig. 5).

The dorsal gland is prominent throughout the procorpus, although it broadens slightly at the anterior terminus forming an ampulla (Fig. 3). Contents of the process and ampulla are similar to those in the lobe region and include membrane-bound secretory granules which are .6 μm or less in diameter (Figs. 3, 7, 8, 17). The matrix of the granules is generally homogeneous (Fig. 7), although in the ampulla of some individuals the core of the granule is less electron dense than the periphery (Figs. 3, 8). The cuticle-lined dorsal gland duct originates posteriorly within the ampulla and is tetraradiate in cross section (Fig. 3). It is external to the plasmalemma of the gland cell and lined with a thin layer of cuticle, although the cuticle is interrupted at the apices of the radii. Anteriorly, the duct emerges from the dorsal gland ampulla, is round in cross section, and its orifice joins with the esophageal lumen (Figs. 1, 4). Two nerve processes occur adjacent to the dorsal gland process (Fig. 2) and extend the length of the procorpus; their perikaryons (cell bodies) occur in the metacorpus.

The metacorpus is only slightly broader in diameter than the procorpus (Figs. 1, 6). Anteriorly in the metacorpus, the esophageal lumen is round, but in the posterior half the lumen becomes triradiate and includes the broad pump chamber which has a heavily cuticularized lining (Figs. 1, 6). The metacorpus includes three pairs of radial muscle cells. One pair attaches to the dorsal and one to each of the subventral positions between radii of the chamber. The muscle cells of each pair diverge peripherally and attach at ventral and subdorsal positions on the inner wall of the metacorpus. Three epithelial cells, nerve elements, and the dorsal gland ampulla are accommodated subventrally and dorsally between the muscle pairs. The dorsal gland process is often reduced in diameter in the metacorpus; posteriorly, two prominent subventral gland processes extend to the level of the base of the pump chamber (Figs. 1, 12). These processes join the esophageal lumen through cuticularized ducts which posteriorly are tetraradiate in cross section and are similar to that of the dorsal gland (Figs. 3, 9). In the subventral glands these ducts are frequently distended with secre-
tory products of the gland, sometimes resulting in a large extracellular vesicle (Fig. 10).

The isthmus region primarily consists of the three gland processes which surround the triradiate lumen (Figs. 11, 12). Anteriorly, each gland process is associated with a small cord of nerve processes (Fig. 12), but posteriorly those associated with the subventral glands migrate dorsally. Further posteriorly, the perikaryons of all the processes are closely associated with the dorsal gland (Fig. 17). Anteriorly, the isthmus is ensheathed by muscles, and radii of non-glandular tissue occur between each of the processes (Fig. 11). In the region of the nerve ring and posteriorly, the dorsal gland process becomes reduced in size. The subventral gland processes are predominant and contact one another so that their parallel plasmalemmas appear to form a single unit (Fig. 12).

Secretory granules of the subventral gland processes are similar to those located posteriorly in the gland lobe. Typically they are roughly spherical with a well-defined membrane and relatively dense periphery (Figs. 13, 17). Variations include homogeneous contents (Fig. 14) or irregular shape with flocculent contents (Fig. 15). Frequently, crystalline material occurs within granules (Fig. 16). Generally, secretory granules of the subventral glands are larger (1.0 μm or less in diameter) and less electron dense than those of the dorsal gland.

As the esophagus broadens into the gland lobe, the subventral glands are no longer equal in diameter when viewed in cross section. The smaller gland is positioned centrally, and the larger gland is adjacent and latero-ventral. The remainder of the gland lobe includes the dorsal gland, perikaryons, and the irregularly shaped esophageal lumen with associated tissue (Fig. 17).

The esophageal lumen is modified from round in the procorpus to triradiate in the valve and isthmus and irregularly shaped in the gland lobe (Figs. 2, 5, 6, 10, 11, 12, 17). Posteriorly as it approaches the junction with intestine, the cuticle lining terminates and is continuous with membranes which resemble junctional complexes (Figs. 18–20). The membranes are modified plasmalemmas of two pairs of esophago-intestinal cells. The outer pair of esophago-intestinal cells partially encloses the inner pair; anteriorly, the four cells are enclosed by a sheath cell (Fig. 18). In some cases, extracellular spaces distended with flocculent material occur between the inner pair of esophago-intestinal cells, but no continuous lumen was observed (Fig. 19). Posteriorly, the intestine includes a small indistinct lumen. The lumen includes evaginations of the plasmalemma lining which might be interpreted as poorly defined microvilli (Fig. 21).

The dorsal gland nucleus frequently occurs at about the level of esophago-intestinal cells (Fig. 20), although the position of gland nuclei is variable among specimens. For example, in some cases the dorsal gland nucleus occurs further posteriorly at about the same level as the anterior-most subventral gland nucleus. The dorsal gland nucleus, like those of the subventral glands, is about 3.5 μm in diameter, is irregular in shape with little peripheral chromatin, and possesses a large spherical nucleolus (Figs. 20, 24). Unlike subventral gland nuclei, the dorsal gland nucleus apparently is not associated with a large Golgi complex; adjacent cytoplasm primarily includes dense secretory granules and rough endoplasmic reticulum (RER).

Further posteriorly, the dorsal gland is reduced to a narrow process which partially ensheaths the remainder of the gland lobe (Fig. 22). This sheath includes primarily RER as well as a few mitochondria and terminates about 5 μm from the base of the gland lobe. The predominant component of the posterior portion of the gland lobe is the more ventrally positioned subventral gland. The smaller more centrally located subventral gland terminates about 3 μm from the base of the lobe. Secretory granules of the subventral glands are distributed throughout the posterior portion of the glands (Fig. 22), and the cytoplasm also includes RER and a few small mitochondria. A large Golgi complex is distributed throughout the glands and sometimes extends into processes of the isthmus. However, dictyosomes are most abundant in the region of each gland nucleus (Figs. 23, 24).
In some individuals, the cytoplasm of subventral glands includes vacuolated regions, and separating plasmalemmas may be discontinuous (Figs. 12, 17, 22).

DISCUSSION

The esophagus of males of *S. hydrophila* generally resembles that of *H. glycines* but is particularly distinct from *M. incognita*. Males of *M. incognita* lack a dorsal gland but retain a reduced ampulla without secretory granules (4). In *S. hydrophila* and *H. glycines*, the three esophageal gland processes each join the esophageal lumen through a single cuticularized duct, but in *M. incognita*, each duct branches into three separate channels. An intestinal caecum occurs in males of *M. incognita* but is absent in *S. hydrophila* and *H. glycines* (4).

Secretory granules of subventral glands of *S. hydrophila* are more or less evenly distributed throughout the glands, whereas in *H. glycines*, secretory granules are concentrated anteriorly and RER predominates posteriorly. In *M. incognita*, a specialized region consisting of RER occurs posteriorly in the gland lobe.

The gland lobe of males of *S. hydrophila* is composed of three distinct gland cells. In *H. glycines*, plasmalemmas separating glands were not observed in any individuals, so that the cells appeared to be amalgamated. However, in males of *M. incognita* fixed by identical procedure, subventral glands were clearly delimited by plasmalemmas (4). In some individuals of *S. hydrophila*, plasmalemmas separating subventral glands are discontinuous. Examination of approximate serial sections in such individuals suggests that these membranes are relatively sensitive to mechanical injury, but on the basis of the present study, autolysis cannot yet be ruled out. In males of *H. glycines*, absence of plasmalemmas separating subventral glands is consistent among specimens. Dissolution of membranes might occur in conjunction with atrophy of digestive glands, since the glands are probably not functional in nonfeeding males. Similar to *H. glycines*, the gland lobe of *P. penetrans* was described as syncytial (9), and Bird (5) suggested that subventral glands in females of *M. javanica* appeared to fuse. These observations might be better understood by examining development of the digestive system of males and comparison with feeding stages. Furthermore, comparisons using various additional methods designed for rapid fixation of tissue also will be useful.

Organelles observed in esophageal glands of *S. hydrophila* suggest secretion by a process which is similar to glands of many other organisms (7). Typically, synthesis originates between membranes of RER, and products are subsequently refined and packaged as granules in Golgi cisternae (11). Variation in morphology and particularly electron density of secretory granules in *S. hydrophila* may reflect stages of further modification and condensation of contents. In addition, electron dense peripheral zones or crystalline structures within granules may suggest sequestering of specific proteins (e.g., enzymes). Contents of condensed granules apparently pass from the gland cell to extracellular space of the duct by exocytosis at the apices of the tetraradial base where the cuticle lining is interrupted. Frequently, in subventral glands of *S. hydrophila*, this process apparently results in distended ducts with large extracellular vesicles. Curiously, we have not observed similar structures associated with the dorsal gland duct in *S. hydrophila* or with any of the ducts in *H. glycines* or *M. incognita* (Baldwin, unpublished observations). Contents of granules of subventral glands in *H. glycines* and *M. incognita* were homogeneous, in contrast to the variable forms in *S. hydrophila*. These differences in structure of granules as well as variation among species in distribution of granules and organelles (discussed below) might reflect fundamental cytochemical distinctions in feeding forms. The host responds to secretion of digestive glands of *S. hydrophila* by formation of a single mononucleate cell (10), whereas the host response to *H. glycines* is a multinucleate syncytium.

The relative abundance of organelles varies among the species examined and differences also occur between the dorsal and subventral glands. In *S. hydrophila*, RER is less abundant than in *H. glycines*. *Meloidogyne incognita* has large amounts of RER, but the Golgi complex includes few dictyosomes (Baldwin, unpublished obser-
similar cords extend from the anterior end of the esophagus to the terminal bulb (I). Heteroderoidea probably occur throughout the metacorpus and isthmus of the esophagus. The three nerve cords observed in the metacorpus and isthmus of \textit{S. hydrophila} or \textit{H. glycines}, although RER is particularly abundant posteriorly including the sheath portion of the dorsal gland of \textit{S. hydrophila}.

Few studies of changes which occur in secretory granules or organelles of digestive glands in starved or nonfeeding stages of invertebrates have been made. Generally, synthesis proceeds in spite of fasting, even when there is cessation of discharge, although the amount of RER may eventually be diminished (7). Apparently, abundant secretory granules in digestive glands of males of Heteroderoidea do not necessarily indicate feeding. On the other hand, the absence of a continuous lumen among esophagointestinal cells does not necessarily preclude feeding, since separating junctional complexes may function as a valve (4,12,14). A poorly defined lumen occurs in the anterior portion of the intestine in \textit{S. hydrophila} and \textit{H. glycines} but was not observed in \textit{M. incognita} (4).

Innervation of the procorpus and metacorpus of \textit{S. hydrophila} is similar to that of \textit{H. glycines} and \textit{M. incognita}, but nerve processes in the isthmus of \textit{S. hydrophila} primarily originate from a group of perikaryons associated with the dorsal gland in the anterior portion of the gland lobe. In \textit{H. glycines}, similar perikaryons occurred throughout the isthmus as well as a few in the gland lobe. In contrast, nerve processes of \textit{M. incognita} were confined to the periphery or the dorsal side of the isthmus, but their perikaryons apparently occurred among adjacent ganglia outside the esophagus. The three nerve cords observed in the metacorpus and isthmus of Heteroderoidea probably occur throughout the Secernentea. In \textit{Caenorhabditis elegans}, similar cords extend from the anterior end of the esophagus to the terminal bulb (1).

Comparison of the esophagus and specifically the digestive glands in males with feeding juveniles and females might lead to the discovery of "degenerate" characters linked to the nonfeeding status, such as the atrophy of the dorsal gland in males of \textit{M. incognita}. Such characters would reasonably be considered derived and might exhibit a pattern among Heteroderoida which could be useful in testing interpretations of phylogeny. The possible significance of distinctive characteristics of the subventral glands in males of \textit{S. hydrophila} may be best elucidated by further comparison with additional genera of Heteroderoidea.

\section*{LITERATURE CITED}

Fig. 1. Diagram illustrating the male esophagus of *S. hydrophila*. Scale units are in micrometers (μm) and indicate distance from anterior end of nematode.

Fig. 2. Cross section through anterior procorpus showing noncontractile portion of stylet protractors (SP) (level 53, Fig. 1). Arrowheads indicate boundary of procorpus. DGP = dorsal gland process; EL = esophageal lumen; NP = nerve-processes.

Fig. 3. Cross section through dorsal gland ampulla with dorsal gland duct (DGD) (level 50, Fig. 1). SG = secretory granules; EL = esophageal lumen.

Fig. 4. Cross section slightly posterior to joining of esophageal lumen (EL) and lumen of dorsal gland duct (DGD) (level 48, Fig. 1).

Fig. 5. Cross section through procorpus with nuclei (N) of epithelial cells (level 65, Fig. 1). DGP = dorsal gland process; EL = esophageal lumen.

Fig. 6. Cross section of metacorpus including triradiate pump chamber (PC) with cuticle lining (level 108, Fig. 1). Arrowheads indicate radial muscles. BL = basal lamina; DGP = dorsal gland process.

Fig. 7. Secretory granule of dorsal gland with homogeneous matrix.

Fig. 8. Secretory granule of dorsal gland with core less dense than periphery.

Fig. 9. Cross section through subventral gland ducts (SvD) (level 110, Fig. 1). EL = esophageal lumen.

Fig. 10. Cross section through distended subventral gland duct (SvD) (level 111, Fig. 1). EL = esophageal lumen. Scale as in Fig. 9.

Fig. 11. Cross section through anterior portion of isthmus (level 117, Fig. 1). Arrowheads indicate muscles which ensheath isthmus. EL = esophageal lumen.

Fig. 12. Cross section through isthmus in region of nerve ring (NR) showing dorsal (DGP) and subventral gland processes (SvP) (level 138, Fig. 1). Arrowheads indicate plasmalemma boundaries of adjacent subventral glands. EL = esophageal lumen; NP = nerve-processes.

Fig. 13. Secretory granule of subventral gland with core less dense than periphery.

Fig. 14. Secretory granule of subventral gland with homogeneous matrix. Scale as in Fig. 13.

Fig. 15. Secretory granule of subventral gland with poorly defined boundary and flocculent contents. Scale as in Fig. 13.

Fig. 16. Secretory granule of subventral gland containing crystalline material. Scale as in Fig. 13.

Fig. 17. Cross section through anterior portion of gland lobe including esophageal lumen (EL), dorsal (DG) and subventral glands (SvG) (level 158, Fig. 1). Arrowheads indicate plasmalemma boundaries of glands. Pk = nuclei of perikaryons.

Fig. 18. Cross section through anterior portion of inner pair of esophago-intestinal cells (I1 and I2), outer pair of esophago-intestinal cells (O1 and O2), and sheath cell (S) (level 162, Fig. 1). JC = junctional complexes.

Fig. 19. Distended extracellular space between plasmalemma boundaries (arrowheads) of inner pair of esophago-intestinal cells (level 164, Fig. 1). JC = junctional complexes; Nu = nucleolus; Pk = nuclei of perikaryons; RE = rough endoplasmic reticulum.

Fig. 20. Cross section through gland lobe at level of dorsal gland nucleus (DN) and inner pair of esophago-intestinal cells (I1 and I2), including nuclei (N) (level 165, Fig. 1). JC = junctional complexes; Nu = nucleolus; Pk = nuclei of perikaryons; RE = rough endoplasmic reticulum.

Fig. 21. Cross section through intestinal lumen (Lu) with poorly defined evaginations (Ev) of surrounding cells (level 190, Fig. 1).

Fig. 22. Cross section through posterior portion of gland lobe in which subventral glands (SvG) are predominate and dorsal gland (DG) is reduced to a narrow sheath (level 165, Fig. 1). Arrowheads indicate plasmalemma boundaries of glands. RE = rough endoplasmic reticulum.

Fig. 23. Cross section showing two dictyosomes of Golgi (Go) in subventral glands.

Fig. 24. Cross section through posterior most subventral gland nucleus (level 187, Fig. 1). Arrowheads indicate nuclear membrane. Go = dictyosomes of Golgi; Nu = nucleolus.
Esophagus of Sarisodera: Baldwin 291

2.2

DG

SvG

2.3

Go

Nu

2.4

Go

1.0μm

0.5μm