Fine Structure of the Esophagus of
Pratylenchus penetrans

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Abstract: The fine structure of the esophagus of Pratylenchus penetrans is described. The gland lobe is syncytial and contains two types of nuclei: three large nuclei with little chromatin, and more numerous smaller nuclei with large amounts of chromatin. Some of the smaller nuclei are associated only with glandular tissue, whereas others are part of nerve cells within the esophagus. Clusters of free ribosomes, rough endoplasmic reticulum, and numerous mitochondria occur in the lobe region where the secretory granules are formed. No Golgi bodies were observed. On the basis of these observations, possible differences in the mechanism of secretory granule formation between plant-parasitic nematodes are discussed. Several other minor differences between the fine structure of other plant-parasitic nematodes previously examined and that of P. penetrans are also noted. Key Words: ultrastructure.

In describing the fine structure of the feeding apparatus of Pratylenchus penetrans (Cobb) Filipj. & Shuurm.-Stekh., Chen and Wen (4) showed some details of the procorpus, median bulb, and isthmus. The objective of the current study was, through the study of serial sections, to complete the description of the P. penetrans esophagus, including the glands.

MATERIALS AND METHODS

Young, mature female P. penetrans which had been cultured monoaxically by the method of Krusberg (7) were examined. This culture, which was supplied by W. F. Mai, Cornell University, originated from a population in a cherry orchard in western New York State. Processing for electron microscopy was as described by Kisiel et al. (6). Sections were cut at 50-90 nm with a diamond knife on an LKB Ultratome Type 4801A. Sections were stained first with uranyl acetate and then with lead citrate; then they were examined and photographed with a JEM-7 or a JEM T-7 electron microscope operated at 80 KV or a Philips 200 electron microscope operated at 60 KV.

Serial cross sections were taken of the esophagus of two nematodes, and the sections from an area 6 μm in depth were placed on each grid. Serial longitudinal sections of the esophageal area were taken in the same manner from two nematodes. In addition, micrographs were made of random cross or longitudinal sections from 15 P. penetrans.

RESULTS

The basic structure of the P. penetrans esophagus is shown in Fig. 1.

Esophageal glands: The esophageal glands of the genus Pratylenchus were described as being located in one compact lobe (9). In P. penetrans, the gland lobe is syncytial, as is that reported for males of Heterodera glycines (1). The glands contained two types of nuclei. First, there is a set of three large nuclei (primary nuclei), readily seen by light microscopy. These nuclei are irregular in shape, show only a small amount of chromatin (Fig. 5), and contain a prominent nucleolus. Second, there are a number of smaller, elongate nuclei (secondary nuclei) located contiguous to the basement membrane of the gland (Fig. 4, 5). These nuclei contain a large amount of chromatin in the glands and do not have a nucleolus. A small amount of cytoplasm and dense masses of ribosomes, enclosed by a membrane, surround these nuclei.

The posterior portion of the gland contains abundant mitochondria, parallel strands of rough endoplasmic reticulum (RER), and what are apparently newly formed secretory granules (Fig. 3). In this region, the granules appear as homogenous
masses of varying electron density and are not surrounded by a membrane (Fig. 3). Golgi bodies were not observed. The mitochondria in this area are surrounded by a single layer of ribosomes which appear to be in contact with the entire periphery of the outer mitochondrial membrane. Some areas contain strands of randomly arranged RER which have a daedaloid appearance (Fig. 3, 4, 5). Smaller loops and circles of ribosomes attached to endoplasmic reticulum membranes were also observed. Some areas of the lower lobe contain clusters of free ribosomes (Fig. 2).

Portions of the lobe contain many mitochondria in which a portion or all of the cristae and mitochondrial plasm have been replaced by myelinated structures (Fig. 4). Small lipid droplets are present, mostly near the periphery of the gland.

The esophageal lumen connects with the lumen of the intestine in the anterior portion of the gland lobe. This junction is marked with desmosomes, but no evidence of an esophago-intestinal valve was observed (Fig. 5, 6).

**Isthmus:** As the glands pass into the isthmus, they form three processes, each defined by a double membrane (Fig. 9). These structures are dorsal gland processes and the two subventral gland processes.

Within the isthmus and posterior to the valve of the median bulb, the esophageal lumen is triradiate and cuticularized (Fig. 5, 9, 10), as it is in *Ditylenchus dipsaci* (10). The isthmus is covered by a basement membrane, with half-desmosomes spaced evenly along the inner side of the membrane. Prominent filaments, described from other organisms as tonofilaments (5), arise from these half-desmosomes and penetrate the tissue comprising the walls of the isthmus (Fig. 8).

Cross sections through the isthmus, halfway between the esophago-intestinal junction and the median bulb, exhibit much detail. Within the lumen of each of the three gland processes are secretory granules, occasionally free ribosomes, and microtubules which are located near the periphery of the lumen (Fig. 7, 9). The internal structure of the isthmus is complex (Fig. 9). For each of the three processes, the enclosing membrane is joined by a desmosome near the junction of two of the triradiate lumen segments. Between each process is a membrane-delineated area. Each of these membranes is attached to one side of the tip of a triradiate lumen segment by a desmosome. Thus, in a cross section of the isthmus, nine desmosomes are seen contiguous to the lumen. Two of the membrane-delineated areas between the gland processes are simple and contain only microtubules. The third area contains a nerve chord and is seen as a complex of membrane-enclosed areas which are filled with microtubules and, occasionally, mitochondria (Fig. 9).

The two subventral gland processes terminate in ducts which join the esophageal lumen immediately posterior to the metacorpus valve, as described by Chen and Wen (4).

**Metacorpus:** Six radial muscle cells, attached in pairs to the outer wall of the esophageal valve, radiate outwards to the basement membrane, where the myofilaments are attached to the membrane by
FIG. 2-4. 2). Cross section through the posterior region of the esophageal gland lobe which shows clusters of free ribosomes (C---)) and mitochondria (M) encircled by rough endoplasmic reticulum (E). 3). Cross section through the esophageal gland lobe (→) showing numerous newly formed secretory granules (S). The granules in this region are homogeneous. Rough endoplasmic reticulum (E), Mitochondria (M) associated with the endoplasmic reticulum. 4). Cross section through the esophageal gland lobe (→). Many mitochondria in this region are degenerating, as indicated by the presence of myelinated structures (My---)). Secondary nuclei (n---)), Rough endoplasmic reticulum (E), Secretory granules (S).

FIG. 5-7. 5). Cross section through the anterior region of the esophageal gland lobe (→). This section shows a portion of a primary nucleus (N---)) and two secondary nuclei (n---)). The rough endoplasmic reticulum (E), mitochondria (M), secretory granules (S---)), and esophageal lumen (L---)) are also indicated. 6). Longitudinal section through the anterior region of the gland lobe showing the esophago-intestinal junction as marked by desmosomes (D---)) formed at the termination of the wall of the esophageal lumen (L---)). 7) Longitudinal section through the anterior region of the isthmus and the posterior metacorpus (Me). The angle of cut is such that to one side of the isthmus the outer wall (Wi) is shown, whereas on the other side one of the gland processes (GP) containing secretory granules (S-..->) is seen. The myofilaments of the radial muscles (R) are seen within the metacorpus.

FIG. 8-10. 8). Longitudinal section through the isthmus. This micrograph, which is an enlargement of Figure 7, clearly shows the tonofilaments (T---)) terminating in half-desmosomes (D---)) within the wall of the isthmus. One of the gland processes (GP) containing secretory granules (S---)), and the esophageal lumen (L---)) are also seen. 9). Cross section through the isthmus. Three gland processes are seen, the dorsal gland process (dGP) and the two subventral gland processes, each of which contains secretory granules (S---)). The lumen (L) posterior to the valve of the metacorpus is triradiate. Nine desmosomes (D---)) form the junctional complexes where the membranes surrounding the gland processes and the areas intermediate to the gland processes join the wall of the lumen. Two of these intermediate areas contain only microtubules (Mi), whereas the third area contains a nerve chord (Nc). 10). Cross section through the posterior region of the metacorpus. Three gland processes (GP) are evident, but one is smaller (GPl). The nerve chord (Nc), myofilaments of the radial (R) muscles, triradiate lumen (L).

FIG. 11-12. 11). Cross section through the anterior region of the metacorpus. The lumen (L) anterior to the median bulb valve is cylindrical. Also shown are the dorsal esophageal gland (Gp), two types of secretory granules, homogeneous (Sh---)) and granular (Sg---)), and the myofilaments of the radial muscle cells (R). 12). Cross section through the procorpus showing the posterior area of the ampulla (A). The ampulla in this region does not partially encircle the membrane complex (Mc---)) surrounding the lumen (L). Two nuclei (N---)) of the esophagus are seen, as well as abundant mitochondria (M) and ribosomes (Ri---)) which encircle the ampulla and lumen, and granular (Sg---)) and homogeneous (Sh---)) secretory granules.

FIG. 13-14. 13). Cross section through the procorpus (→), anterior to the section in Figure 12. This region contains abundant packed mitochondria (M) and ribosomes (R---)). Granular (Sg---)) and homogeneous (Sh---)) secretory granules, and the lumen (L) is well defined. Ampulla (A), secretory granules (S---)).
half-desmosomes (Fig. 7, 10). Desmosomes are present where the muscle cell membranes attach to the outer wall of the valve, as can be seen in cross section. The areas between the muscle bands differ in composition at different levels of the bulb. Near the posterior portion of the metacorpus, two of the areas lying between the muscle bands contain packed mitochondria, whereas the third area contains a nerve chord (Fig. 10). The dorsal gland process lies between two muscle bands and passes through the entire metacorpus contiguous to the esophageal lumen and the metacorpal valve (Fig. 11). At midbulb, the areas between the muscle bands contain packed mitochondria, ribosomes, and large lipid droplets. The muscle cell nuclei lie at this level. The nerve chord lies contiguous to the basement membrane in this region. The nerve chord was not observed at the anterior end of the metacorpus.

**Procorpus:** The esophageal lumen anteriad to the valve of the median bulb is cuticularized and cylindrical (Fig. 11-14) as it is in *D. dipsaci* (10).

Immediately posteriad to the junction of the dorsal esophageal gland process with the esophageal lumen, the lumen is surrounded by a network of membranes (Fig. 16). These membranes, which are joined to the lumen wall by 8-9 half-desmosomes, encompass a small number of microtubules, ribosomes, and, occasionally, a mitochondrion. In this region, the ampulla of the dorsal gland process partially encircles this membrane complex (Fig. 16). The central core of esophageal tissue is enclosed by a layer which contains packed mitochondria, lipid droplets, and, at some levels, 1-3 nuclei. As in other parts of the esophagus, the procorpus is covered by a basement membrane.

The ampulla terminates in a duct which joins the esophageal lumen about 2 μm posteriad to the base of the stylet. The ampulla is sac-like, about 5 μm in width near the anterior end and narrower posteriad (Fig. 15). The anterior portion of the ampulla is covered by a refractive material which, in longitudinal section, is seen to comprise 2-4 clearly defined layers (Fig. 15). Posteriad, the gland process is enclosed by a double membrane. Secretory granules fill the

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**FIG. 15-16.** Longitudinal section through the procorpus showing the layers of refractive material (→) which cover the anterior region of the ampulla (A). 16. Cross section through the procorpus just posterior to where the dorsal gland joins the lumen. The ampulla (A) partially encircles the membrane complex (Mc→) surrounding the lumen (L). Microtubules (Mi→) are arranged near the inner membrane of the ampulla. Desmosomes (D→) of the membrane complex, secretory granules (S→).
ampulla, and these granules vary in texture from homogeneous to granular (Fig. 12, 13). A single membrane encloses the granular bodies. Microtubules arranged parallel to the longitudinal axis of the nematode occur at regular intervals near the inner membrane of the ampulla (Fig. 16).

**DISCUSSION**

The position of the secondary nuclei in the esophageal lobe and the types of tissue associated with them suggest that they represent two sets of nuclei of different function. Those nuclei in the anterior portion of the lobe are in association with the nerve process that extends through the isthmus, an indication that they are nuclei of cell bodies. Nuclei located in the posterior of the lobe are surrounded by gland tissue and are part of the glandular syncytium.

The ribosome clusters in the gland lobe are most interesting. Formations of this type have not been reported from the gland of any other plant-parasitic nematode thus far studied (2, 3, 10). Another difference is the apparent absence of Golgi bodies in the gland lobe in *P. penetrans*. Bird (3) concluded that the secretory granules of *Meloidogyne javanica* were made up of ribosomes in the gland lobe in a process governed by the Golgi bodies and RER. He supports this interpretation by experimental evidence that the secretory granules consist mostly of ribonucleic acid, and that ribosome-like particles result when the granules are broken down to form the stylet exudate (3).

In *P. penetrans*, the close association of the ribosome clusters and the secretory granules suggests that the ribosome clusters are assembly areas which may represent a precursor stage of secretory granule formation. If this interpretation is correct, then functionally the ribosome clusters are somewhat analogous to the condensing vacuoles in the glands of mammals. Condensing vacuoles are now known to be areas where proteins are concentrated to levels found in secretory granules (8). However, in higher animals (5), and apparently within the esophageal glands of the Heteroderidae, which are the most carefully examined group of plant-parasitic nematodes (2, 3, also Baldwin, personal communication), the Golgi complex plays a primary role in the concentration and packaging of secretory granules. Since Golgi bodies are probably absent in *P. penetrans*, secretory granule formation would proceed by different mechanisms than in organisms where the Golgi complex is present.

Myelinated structures similar to those within the mitochondria of some gland areas are reported as being abundant within tissues of senescing nematodes (6). In the current study where only young nematodes were examined, we interpret this observation as evidence of rapid turnover of the mitochondria in the highly active glandular tissues.

The separation of the gland lobe into three processes within the isthmus is interpreted as evidence that, during the course of evolution, the basal portions of three glands in *P. penetrans* had fused. The observation of tonofilaments within the isthmus walls is believed to be the first report of these structures from nematode tissues. Presumably, these structures add strength and elasticity to the walls of the isthmus.

**LITERATURE CITED**

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Life Cycle, Pathogenicity, Histopathology, and Host Range of Race 5 of the Barley Root-Knot Nematode

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Abstract: The optimum temperature for development of race 5 of Meloidogyne naasi was 26 C. A life cycle was completed in 34 days. Growth of sorghum was suppressed when inoculated with M. naasi. Observations of M. naasi-infected sorghum roots demonstrated that roots were penetrated just behind the root cap; giant cells were generally initiated either in the procambial region or in very young phloem. When giant cells developed in the cortex, corresponding areas of the vascular system did not have an endodermis, pericycle, or phloem fibers. Nineteen plant species were tested for suitability as hosts for race 5 of M. naasi. Reproduction occurred on 11 of 12 monocotyledenous hosts and none of 7 dicotyledenous hosts. Reproduction often occurred without gall development. Key Words: Meloidogyne naasi, sorghum, barley.

The occurrence of Meloidogyne naasi Franklin has been reported in the United States (1, 5, 6, 8, 13) and in several European countries (3, 4, 7, 9). Michell et al. (10, 11) demonstrated that the species contained at least five races separable by hosts. Race 5, from Kansas, was the only race to reproduce on sorghum. The life cycle, pathogenicity, histopathology, and host range of race 5 of M. naasi are described in this paper.

MATERIALS AND METHODS

Inoculum for all experiments was reared on 'RS 610' hybrid grain sorghum [Sorghum bicolor (L.) Moench] in a greenhouse. Larvae were collected within 48 h after infected roots were placed in a low-volume spray mist chamber. Experimental plants were grown in Haynie loam soil (45% silt, 40% sand, 15% clay). Experiments were fertilized as needed with a 20-20-20 soluble fertilizer.

Life Cycle: Sorghum seeds were surface sterilized with 50 µg/ml chlorohexidine acetate for 5 min (12), rinsed in sterile distilled water five times, and placed on sterilized, moist filter paper in petri plates for 3 days. Seedlings were transplanted to silica sand (ca. 5mm diam) when they were 2.5-3.0 cm tall.

After they had been surface sterilized (as described for seeds), 50-75 larvae were pipetted on and about each seedling root. All test seedlings were grown at 30 C for 48 h; then they were washed from the sand. Three seedlings with slightly swollen roots were transplanted into steam sterilized soil in each 15x15-cm pot. Plants were placed randomly in growth chambers set to maintain soil temperatures at 22, 26, 30, and 34 C (±1 C) with a 12-h photoperiod at 23,672 - 26,900 lux.

Six plants grown at each temperature were processed every 2 days. Soil was washed from the roots, which were then stained in acid fuchsln-lactophenol and cleared in lactophenol. Specimens were dissected from stained tissues and the developmental stages of M. naasi determined. A life cycle was considered complete when hatched second stage larvae were found in the egg masses. A similar experiment was conducted with 'Meimi' barley, Hordeum vulgare L., with