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Ultrastructure of Esophageal Gland Secretory Granules in Juveniles of *Heterodera glycines*[1]

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Abstract: Ultrastructural observations of the feeding sites of soybean cyst nematode juveniles 3 days after inoculation of soybean roots revealed the presence of feeding tubes in the host cell syncytium. Feeding tubes, which were extruded from the stylet tips, were formed by products of secretory granules that originated in the dorsal esophageal gland and accumulated in the ampulla of the gland extension. Granules traversing the space between the gland cell and the ampulla were regulated in their movement by two sets of sphincter-like muscles located anterior and posterior to the metacorpus pump chamber. Sections through the sphincter muscles revealed obliquely arranged fibers, which in a contracted mode caused microtubules in the gland extension to be tightly packed and devoid of granules.

Key words: esophageal gland, feeding tube, *Glycine max*, *Heterodera glycines*, host–parasite relation, secretory granule, sphincter muscle, soybean, soybean cyst nematode, syncytia, ultrastructure

The dorsal and subventral glands of plant-parasitic tylenchid nematodes secrete granules (1). These granules were observed in the root-knot nematode *Meloidogyne javanica*, and Bird (2) stated that secretions of the subventral glands appeared to be associated with egg hatch and host penetration. Granules in the subventral glands disappeared within 1–3 days after penetration during which time there was a threefold increase in the size of the subventral and dorsal glands. Video-enhanced light microscopy has shown the secretion granules of cyst nematodes to move en masse from the gland cells to the ampullae located at the anteriad extensions of the cells (20; Endo, unpubl.). Once the nematode has established a feeding site, the dorsal gland secretions move through the ampulla and stylet into the host cell and form feeding tubes. Feeding tubes have been reported for cyst (19), root-knot (15,19), and reniform nematodes (11–13). Secretion granules in the dorsal and subventral glands of infective juveniles are initially spheroid and electron dense but change in size and density after host penetration. Their products are apparently released during salivation and feeding. This report describes changes in ultrastructural morphology of secretory granules during early stages of cyst nematode infection, sphincters that control movement of secretory granules through the gland cells, and the exudation of gland products into host cells.

Materials and Methods

Second-stage juveniles (J2) of *Heterodera glycines* were obtained from egg masses and cysts collected from infected soybean roots grown in clay pots containing field soil infested with soybean cyst nematodes. Seedlings of susceptible 'Lee' and resistant 'Pickett' soybeans were grown in moistened vermiculite. Water suspensions of J2 were pipetted onto newly formed secondary roots which were then coated with fine-textured vermiculite to retain the J2 in the vicinity of the roots. Groups of five seedlings were placed in 10-cm-d clay pots containing moist vermiculite and covered with plastic film to retain moisture during penetration. After 24 hours, roots were washed and the plants were transferred to clean vermiculite. At 1, 3, 4, and 6 days after inoculation, roots were washed with tap water to remove adhering particles of vermiculite. Infected regions of the secondary roots were cut into 2–4-mm-long segments while immersed in buffered 3% glutaral-

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dehyde (13,18) supported on a sheet of dental wax, then transferred to vials. Specimens were fixed, rinsed, and postfixed with osmium tetroxide in 0.05 M phosphate buffer (pH 6.8). Fixation for 1.5 hours was followed by rinsing in six changes of buffer over a period of 1 hour, then postfixed in 2% osmium tetroxide for 2 hours, dehydrated in an acetone series, and infiltrated with a low-viscosity embedding medium (17). Silver-gray sections of selected root segments were cut with a diamond knife and mounted on noncoated 48 × 194 μm (75 × 300-mesh) copper grids. Sections were stained with 2% aqueous uranyl acetate (10 minutes) and lead citrate (5 minutes). Thin sections were viewed with a Philips 400-T electron microscope operating at 60 kV with a 20-μm objective aperture.

Results

Within 3 days after inoculation of soybean roots, *Heterodera glycines* juveniles induce syncytia from host cells adjacent to their feeding sites (Figs. 1, 3). The syncytium has dense cytoplasm due to an increase in ribosomes and endoplasmic reticulum (ER) which contrasts with the usual vacuolate condition of the parenchymatous pericyclic cells and adjacent tissue at the protoxylem poles. Host cell changes appear to be associated with nematode secretions which emanate from the stylet orifice after a feeding site is established (Fig. 2). The roughly circular to oblong bodies apparent within the cytoplasmic matrix (Fig. 3) may be sections through sinuous feeding tubes that are extruded into the host cell during stylet thrusts and feeding. Initially, the outer boundary of the feeding tube is membranous with irregular filaments inside. In Figure 1, the nematode stylet associated with the secretion bodies is retracted and may reenter at or near the same site. The hole in the cell wall caused by the stylet is filled with an electron-dense feeding plug that probably serves to retain the integrity of the newly established syncytium as a feeding site (6). The multiple sites of cell wall dissolution (Fig. 4) are directly related to early stages of syncytium formation that allow for continuity of cytoplasm among affected cells.

Procorpus: In contrast to the ultrastructure of the infective vermiform J2 of *H. glycines* (7), the nematode at 3 days after inoculation has a greatly enlarged ampulla filled with moderately large electron-dense secretion granules near the dorsal esophageal gland valve (Fig. 5). Slightly posteriad, the ampulla contains the same type secretion granules, but a major portion of the space is occupied by enlarged low-density granules about three times larger than the anteriorly located granules (Fig. 6). Further posteriad, some of the secretion granules within the ampulla become enlarged and closely spaced. The contents of the enlarged granules may be of uniform density or have inclusions of irregular clusters of electron-dense material (Fig. 7).

Procorpus–metacorporeal valve: At the junction of the procorpus and the metacorpus, the dorsal gland extension is encircled by a collar of obliquely oriented muscle fibers. Upon contraction, these muscles form a sphincter around the dorsal gland extension (Figs. 8, 9). Within the dorsal gland extension are many tightly packed microtubules that are shown in cross (Fig. 8) and longitudinal sections (Fig. 9). A pair of neural processes lie within the infolding of the dorsal gland extension membrane and the membrane of the surrounding muscle fibers (Fig. 8).

Posteriad to the procorpus–metacorporeal valve, the secretory granules again appear and resemble those in the anterior region of the ampulla (Fig. 9). While some granules may appear enlarged, they do not expand to the extent shown in the basal portion of the ampulla and procorpus. They closely resemble the secretion granules present in the cytoplasmic matrix of the dorsal esophageal gland cell where secretory granules are assembled by the Golgi apparatus.

Subventral gland ampullae: The secretion granules in the ampullae of the subventral esophageal gland are considerably smaller and more dense than those in the dorsal

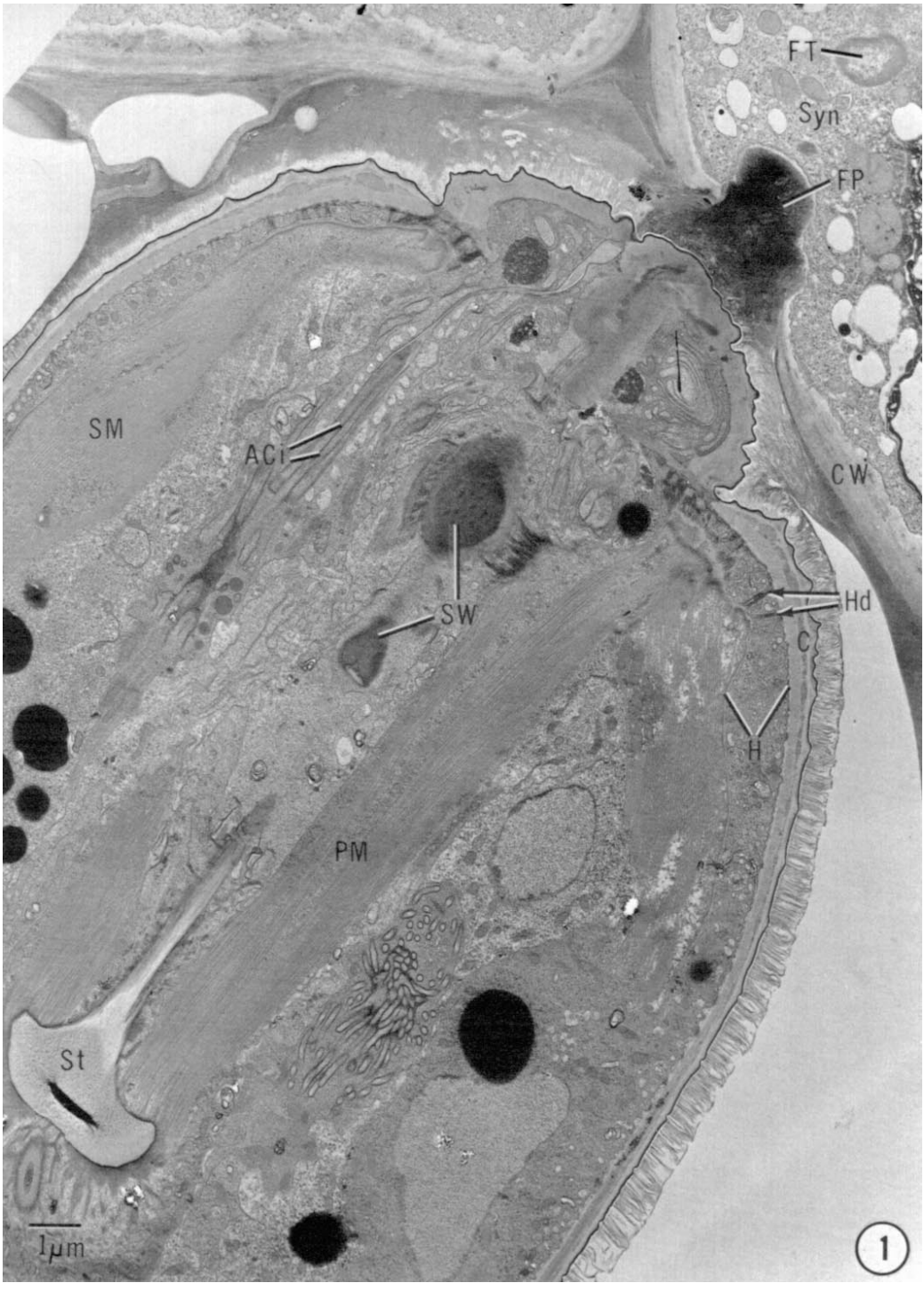

FIG. 1. Longitudinal section of J2 juvenile of *Heterodera glycines* at feeding site, 3 days after inoculation of Lee soybean roots. The stylet (St) is retracted and a feeding plug (FP) fills the stylet entry point. The ovate body in the host cytoplasm is a secretory product of the nematode that may function as a feeding tube (FT). The nematode shows an early stage of growth as shown by the thickening of the hypodermis (H) and stretching of the hemidesmosomes (Hd). The cuticle (C) has a uniform layer of fibriller material oriented perpendicular to the surface. ACi, amphidial cilia; CW, cell wall; PM, stylet protractor muscles; SM, somatic muscles; SW, stomatal wall; Syn, syncytium.

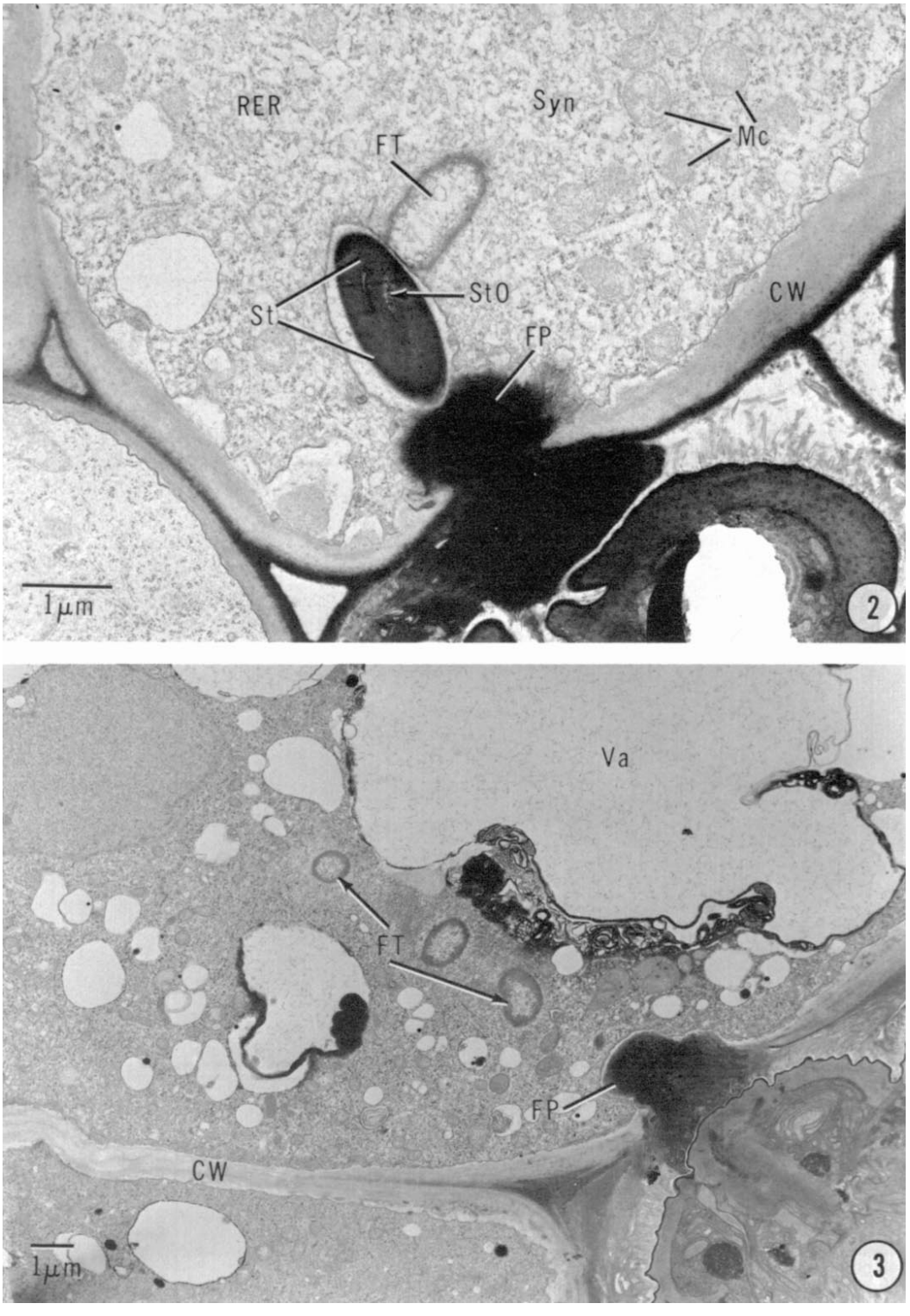

FIG. 2. Longitudinal section through the lip region of a nematode 3 days after inoculation. Close to the stylet tip (St), cut tangentially, is a feeding tube (FT) with a filamentous internal matrix. The cytoplasm of the initiated syncytium (Syn) contains large concentrations of rough endoplasmic reticulum (RER), and mitochondria (Mc). The cell wall (CW) near the feeding site shows signs of proliferation. FP, feeding plug; StO, stylet orifice.

gland cell or its extension and ampulla (Figs. 6, 11–13). These secretion granules differ in morphology from those observed in the subventral gland cell and its extension in the infective, vermiform J2 of *H. glycines* (7). There is a similarity between subventral and dorsal gland extensions in that both contain an abundance of longitudinally oriented microtubules.

Isthmus–metacorporeal valve: Similar to the anterior terminus of the metacorpus, a collar of obliquely oriented muscle fibers surrounds the dorsal (Fig. 10) and subventral gland extensions at the junction of the posterior region of the metacorpus and the isthmus (Figs. 10, 12). In a contracted mode, the muscles cause a narrowing of the passageway of the dorsal and subventral gland extensions (Fig. 12, inset). Secretion granules occur in either end of the gland extensions and continuity between sectors of the gland extensions is retained by the cytoplasmic microtubules (Fig. 10).

Posterior to the nerve ring of the nematode, the dorsal gland extension widens and merges with the dorsal gland cell (Fig. 14). Numerous secretory granules appear clustered in the dorsal gland near the esophageal lumen and are continuous with the irregularly oriented, dilated, rough endoplasmic reticulum. The subventral gland extensions are located on either side of the lumen of the esophagus and are characterized by having relatively few organelles compared to dorsal gland cytoplasm (Fig. 14). As observations were made posteriorly and through the enlarged dorsal gland, the secretion granules were fewer and more scattered within the gland cell. The secretory granules of the subventral extension were small and relatively electron dense.

Discussion

The presence of circular outlines of feeding tubes within the host cytoplasm near the feeding site coincides with the findings of Rumpenhorst (15) who illustrated the abundance of strand-like secretions emanating from the stylet of sedentary nematodes, such as cyst and root-knot nematode species. The results of the present study and those of previous investigations (6) are consistent with the concept that secretions in the form of feeding tubes are integral parts of host–parasite interactions and probably correspond to the short-term (resistant) and long-term (susceptible) relationship of the soybean cyst nematode with soybean roots (5).

These responses of soybean roots to infection by *H. glycines* were shown in light microscope (4,5) and ultrastructural studies in both susceptible and resistant plants (8,14). Among the initial responses of the host cells to nematode infection was the reduction in size of a central vacuole and an increased density of the cytoplasm in the form of smooth and rough endoplasmic reticulum interspersed with mitochondria, plastids, and moderately sized vacuoles. The break in cell walls with their rounded ends appeared to be consistent with the concept that cell wall openings are induced by a chemical rather than a mechanical process (3,8–10).

The circular particles in the syncytial cytoplasm near the nematode feeding site represent sections of an undulating feeding tube extending from the stylet orifice (Fig. 3). The secretion granules in the dorsal gland ampulla vary in density and size and may represent stages of degradation related to the release of their contents. The secretion granules at the anterior part of the ampulla appear to be similar to those within the gland cells. Granules in the posterior part of the ampulla, however, show a gradual enlargement, reduction in electron density, and, in some cases, an accumulation of residue within an enlarged ir-

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FIG. 3. Section of syncytium stimulated by nematode shown in Figure 1. Series of ovate secretion structures in the cytoplasm that may be oblique sections through a sinuous feeding tube (FT) extending into the host cell. The first cell that was stimulated shows evidence of accumulation of dense cytoplasm with many ribosomes that are free or associated with ER, plastids, and mitochondria. Size of vacuoles (Va) depends on the cytoplasmic density. CW, cell wall; FP, feeding plug.

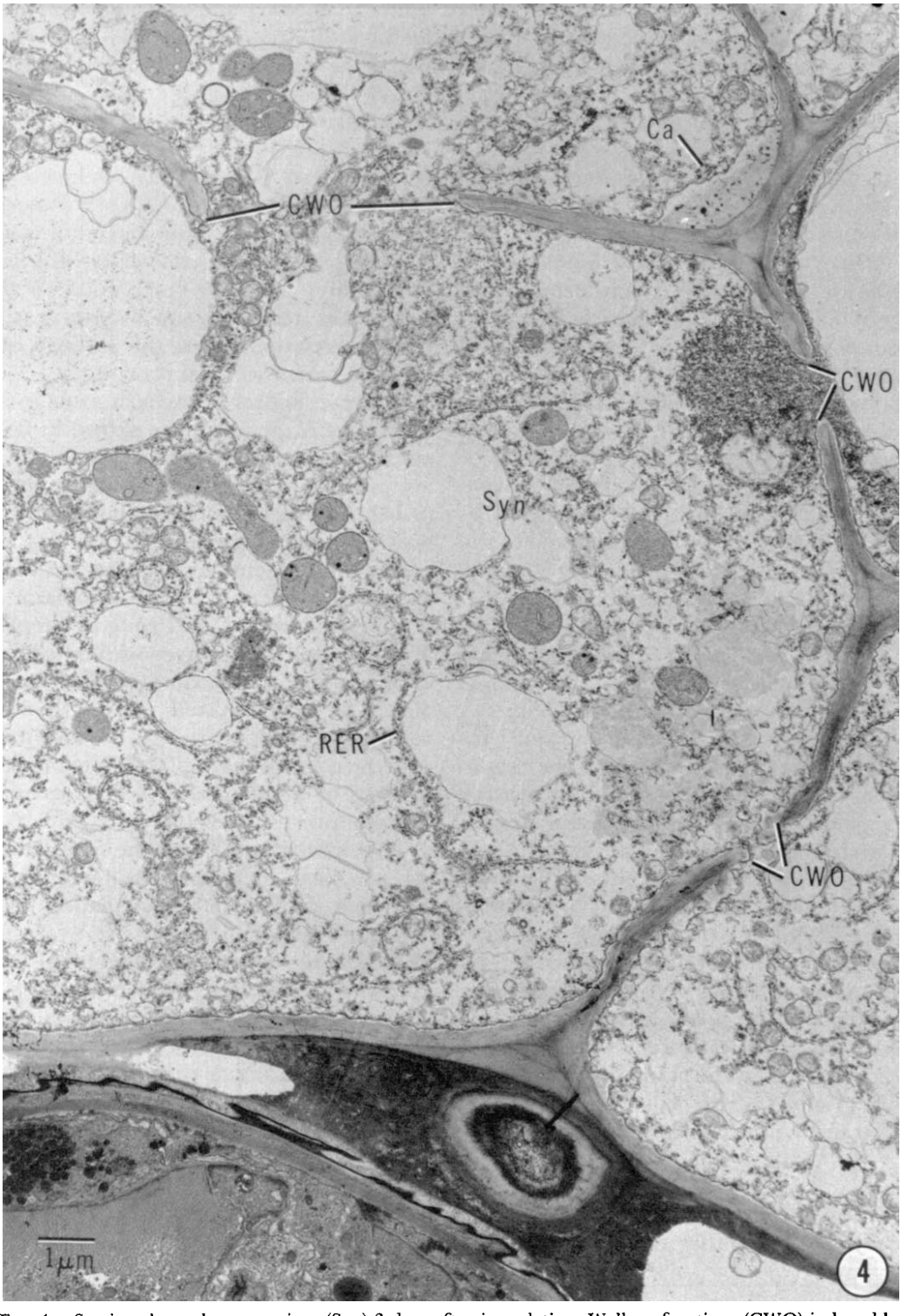

FIG. 4. Section through a syncytium (Syn) 3 days after inoculation. Wall perforations (CWO) induced by nematode feeding results in cytoplasmic continuity and multinucleate condition. Callose-like material (Ca) is deposited along intact and fragmented cell walls. RER, rough endoplasmic reticulum.

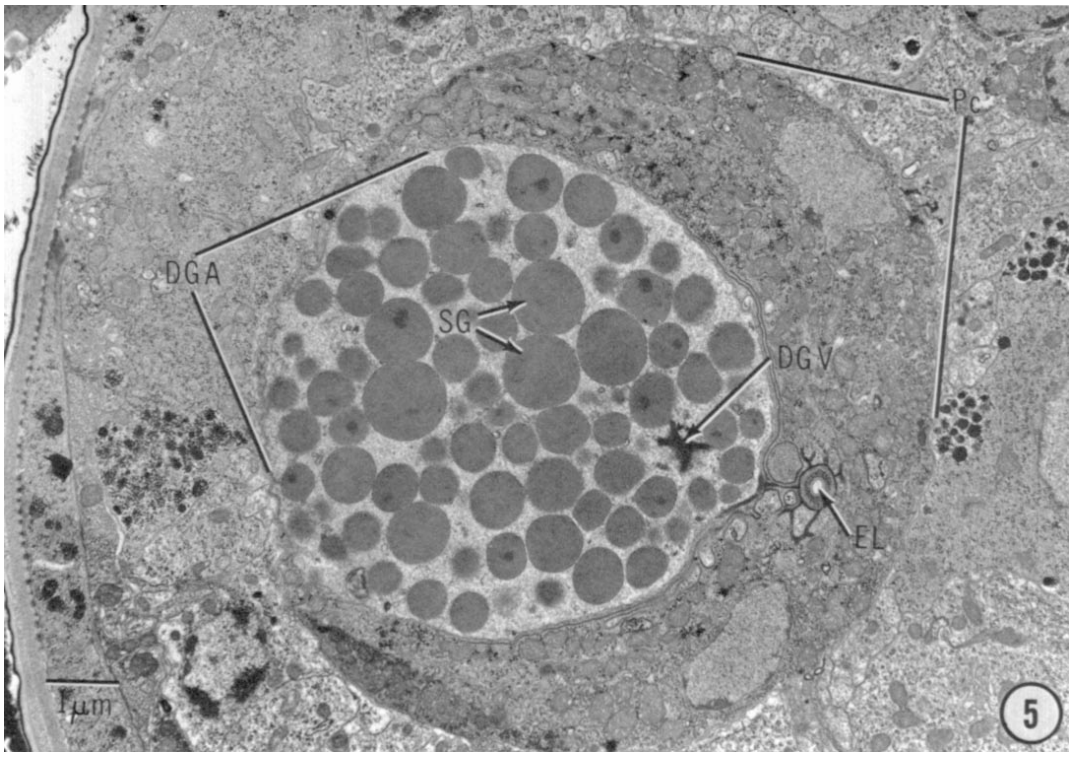

FIG. 5. Transverse section through the dorsal gland ampulla (DGA) of a *Heterodera glycines* juvenile 3 days after inoculation. The ampulla is distended with many secretion granules (SG). The adjacent procorpus tissue (Pc) with many mitochondria and much rough ER is associated with the stylet protractor muscle system. The dorsal esophageal gland valve (DGV) lies within the ampulla cytoplasm close to the esophageal lumen (EL).

regular membrane. During salivation, the dense secretory granules appear to break down and their contents combine with the intergranule matrix of the nematode ampulla. Eventually, the contents may be extruded into the host to form the feeding tubes or released as fluid to intermingle with the host cytoplasm. Rumpenhorst (15) suggested that the same feeding tubes can be assumed to be ducts into which plant cells secrete special products which are directed into a feeding ampulla of the host. The nematode is thought to feed on the contents of this ampulla. However, Wyss and Zunke (20) could not detect such an ampulla and proposed that in observations of living nematodes, nutrients are withdrawn directly from the feeding tubes. Direct attachment of the feeding tube to the stylet tip was documented previously for *Rotylenchulus reniformis* in cotton, *Heterodera schachtii* in *Raphanus sativus*, and *H. glycines* in soybean (Fig. 2) (13,19).

The sphincter-like valve reported by Shepherd et al. (16) in *Aphelenchoides blastophthorus* also has been shown to occur in infective J2 of *H. glycines* (7). In the current study, the valve of the dorsal gland extension was closed, and gland secretions were observed anteriorly and posteriorly to it. The dorsal gland extension has been illustrated as an open channel (7). In vivo observations of the secretory granules that traverse this region were made with video-

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FIG. 6. Transverse section through the dorsal gland ampulla (DGA) shows two types of spherical secretion granules. The dense, relatively small granules (SG) are similar to granules at the extreme anterior of the ampulla and in the central component of the dorsal gland itself, low density secretion granules (LDSG) are found in the dorsal gland extension and the posteriad region of the ampulla. They have filamentous or flocculent contents. Pc, procorpus; GlR, glycogen rosettes.

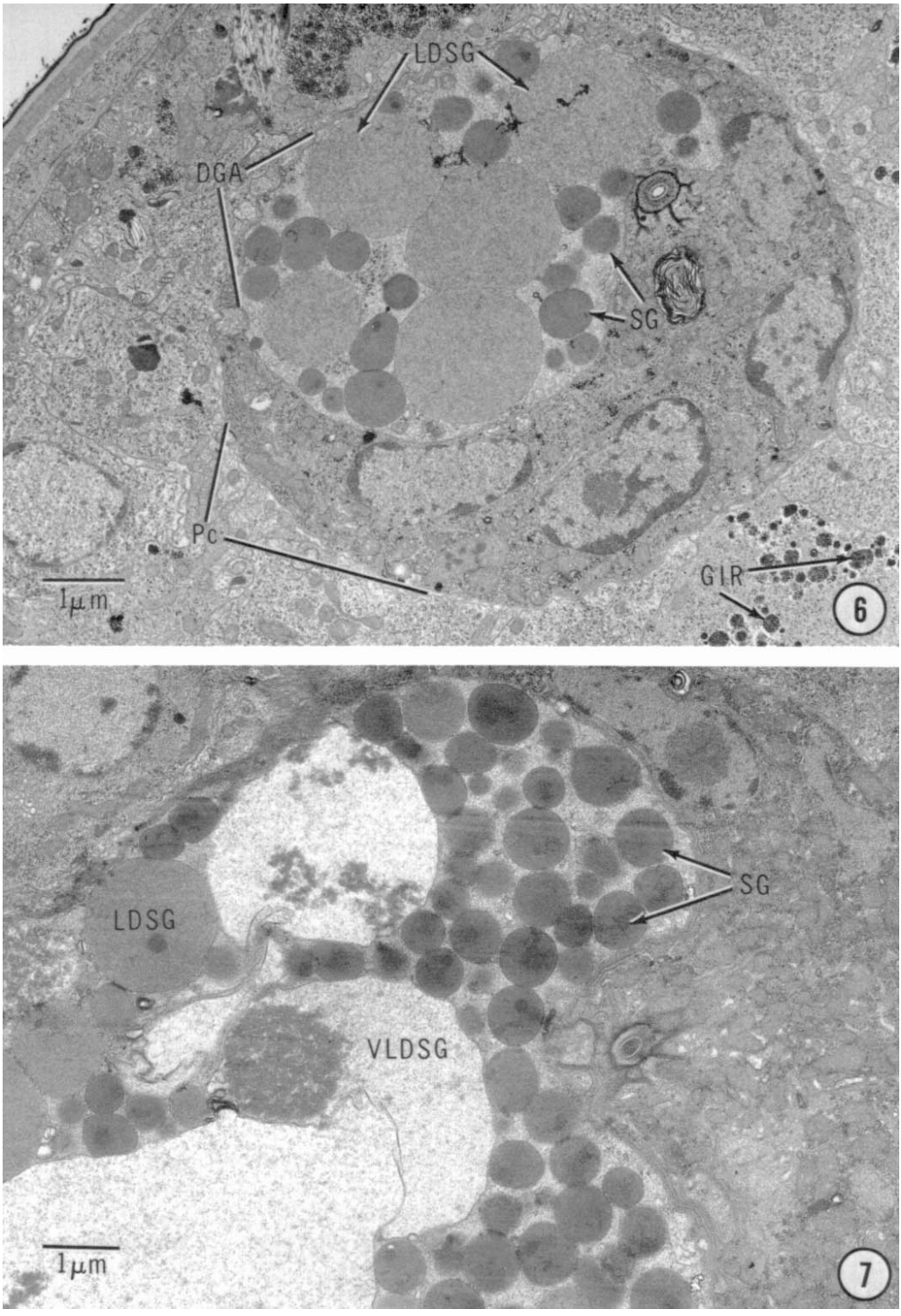


FIG. 7. Longitudinal section through dorsal gland ampulla showing diversity of granule size, shape, and content. Small secretion granules (SG) are electron dense and relatively uniform in texture. Enlarged very low density secretion granules (VLDSG) apparently result from distension of the plasma membrane and contain fine textured, filamentous, flocculent material. LDSG, low density secretion granules.

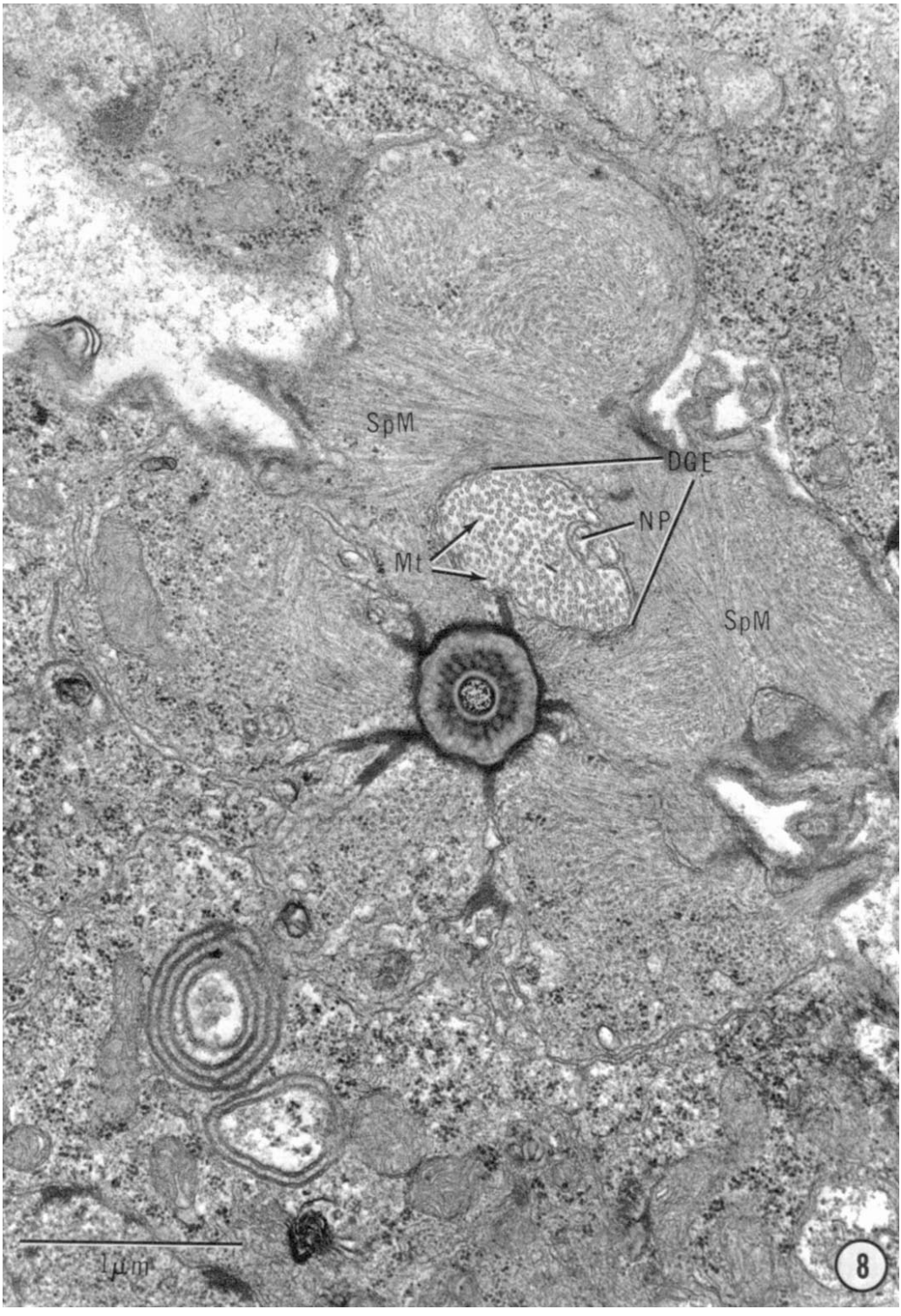
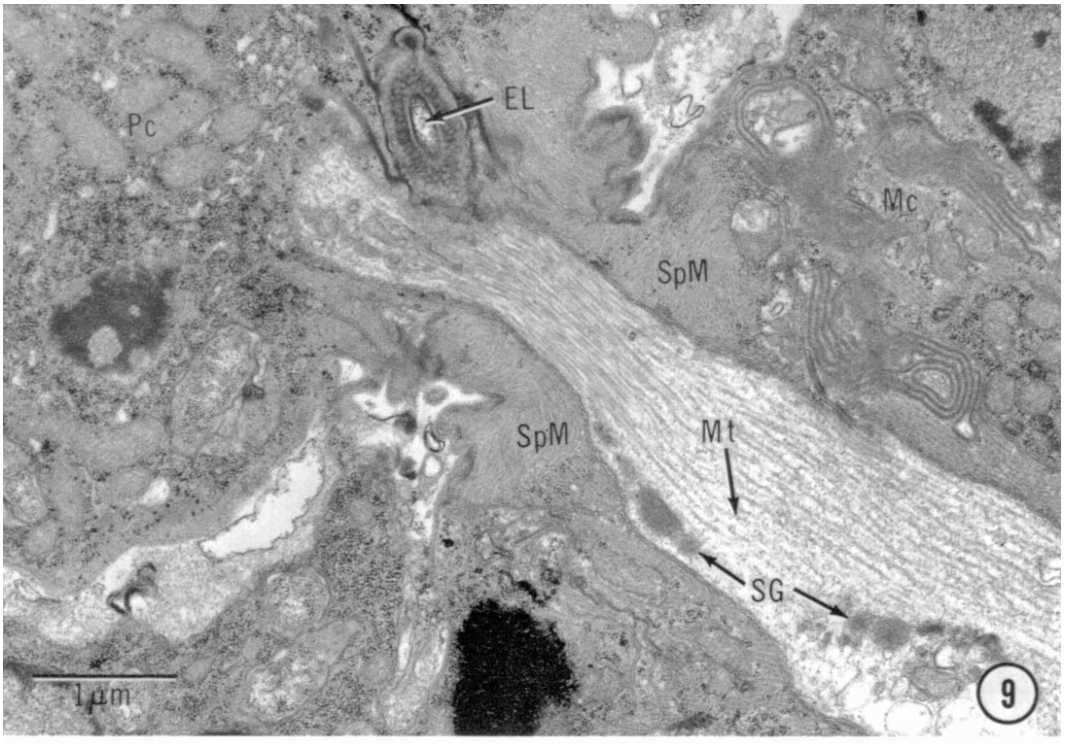


FIG. 8. Enlarged view of the procorpus–metacorpus valve shows closely packed microtubules (Mt) and neural process (NP) within the dorsal gland extension (DGE) surrounded by a network of sphincter muscle elements (SpM). These and the compressed gland extension are supported by membrane junctions that also adhere to the esophageal lumen wall.



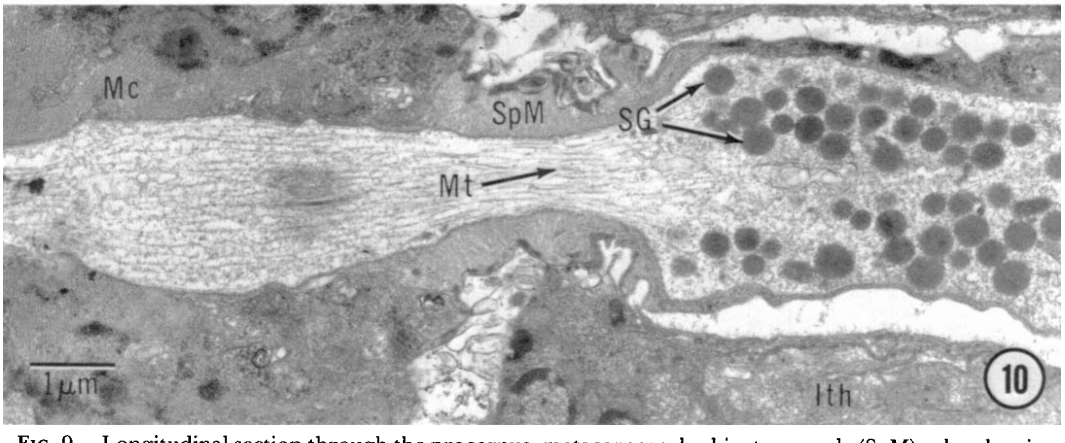


FIG. 9. Longitudinal section through the procorpus–metacorporeal sphincter muscle (SpM) valve showing tighly packed microtubules (Mt) that apparently control the movement of secretion granules (SG), but allow for cytoplasmic continuity within the dorsal gland extension. Mc, metacorpus; Pc, procorpus; EL, esophageal lumen.

FIG. 10. Longitudinal section through isthmus–metacorporeal sphincter valve showing accumulation of secretion granules (SG) in the dorsal gland extension. Note absence of secretion granules in region of valve and dorsal gland extension that traverses the metacorpus (Mc). SpM, sphincter muscle; Mt, microtubules; Ith, isthmus.

enhanced light microscopy (unpubl.). A recent video film (20) showed a similar sphincter muscle of *Heterodera schachtii* in *Raphanus sativus* var. *oleiformis* roots. Furthermore, nematode salivation and food ingestion appeared to be coordinated between the operation of the dorsal gland sphincter valve and the tetraradiate valves of the subventral glands. Secretory granules or their contents appeared to accumulate in the subventral gland ampullae and were released into the central-esophageal lumen just posterior to the base of the metacorpus pump chamber.

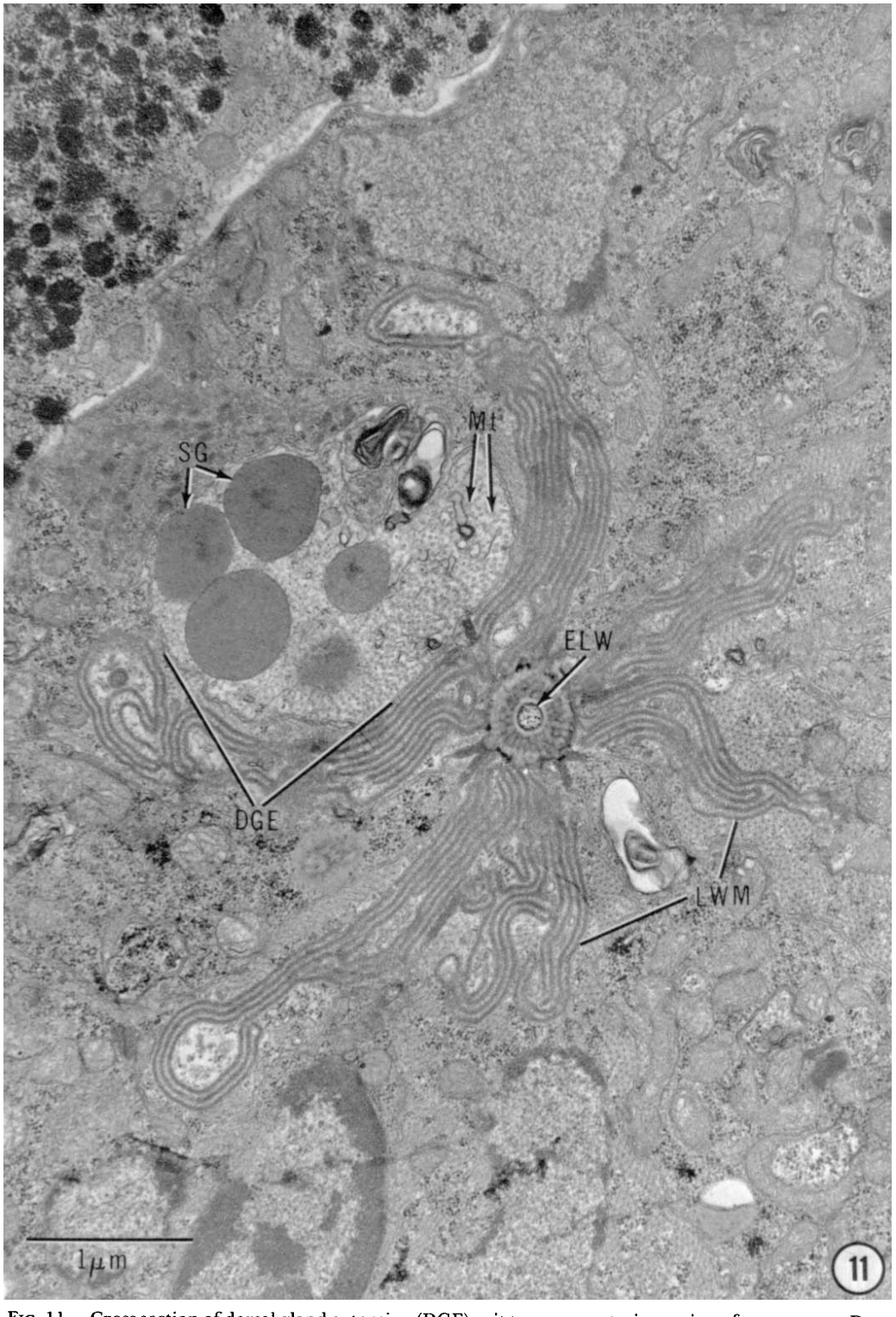


FIG. 11. Cross section of dorsal gland extension (DGE) as it traverses anterior region of metacorpus. Dense, spherical secretory granules (SG) in gland extension are similar to those observed in the dorsal gland cell. The esophageal lumen wall (ELW) and border of the dorsal gland extension are surrounded by a network of membranes (LWM). These lumen wall-associated membranes lie close to the muscle elements of the metacorpus. Mt, microtubules.

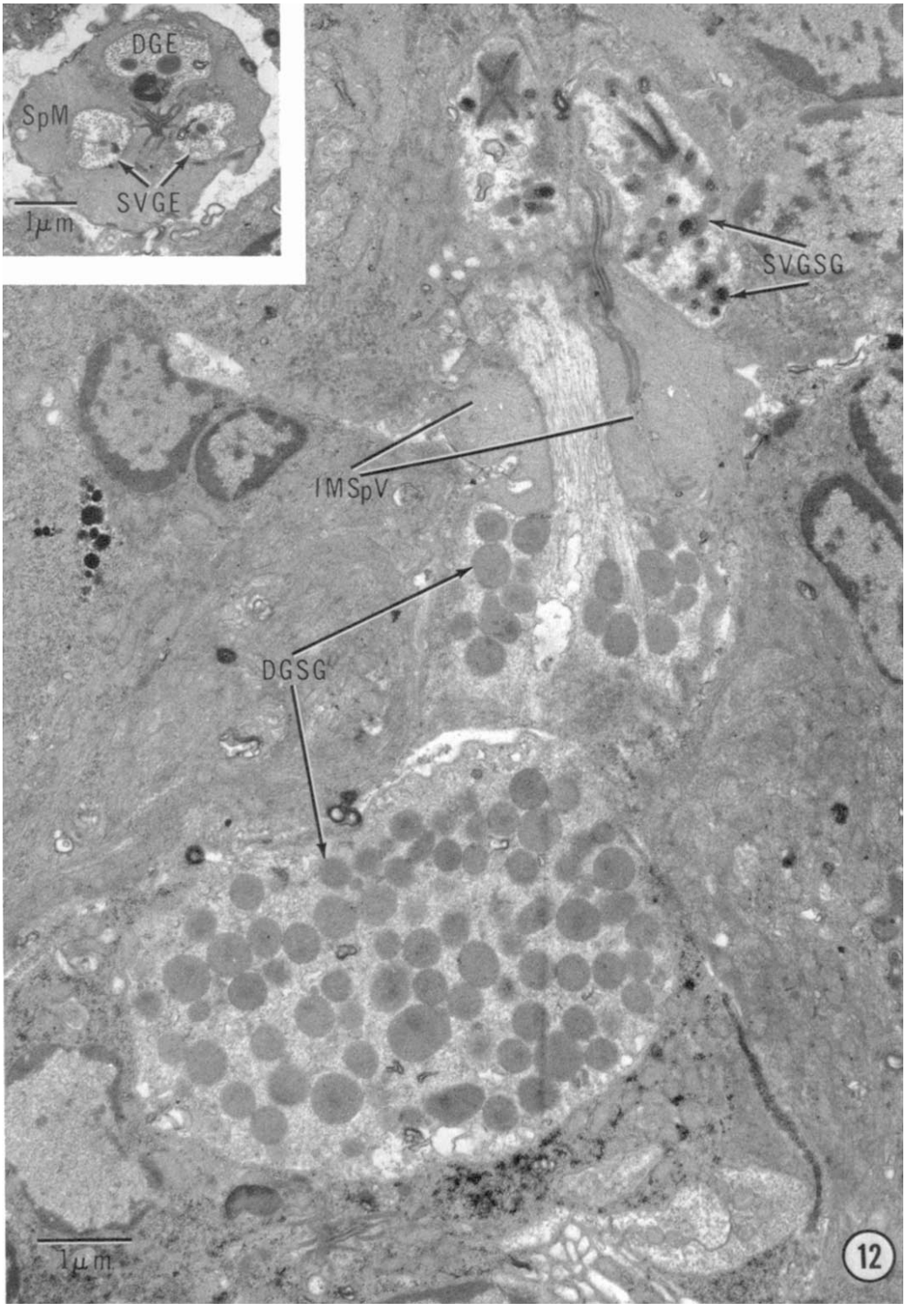


FIG. 12. Tangential–longitudinal section through the base of the metacorporeal pump and the isthmus of a J2 at 3 days after inoculation showing the difference in secretion granule morphology between subventral and dorsal gland extensions. The sphincter-like muscle serves as an isthmus–metacorporeal valve (IMSpV) and appears to regulate the movement of the secretion granules within the dorsal and subventral gland extensions. DGSG, secretory granules, dorsal gland; SVGSG, secretion granules, subventral glands. Inset) Cross section through sphincter-like muscle (SpM) region at the base of metacorpus of a J2 juvenile 3 days after inoculation. DGE, dorsal gland extension; SVGE, subventral gland extension.

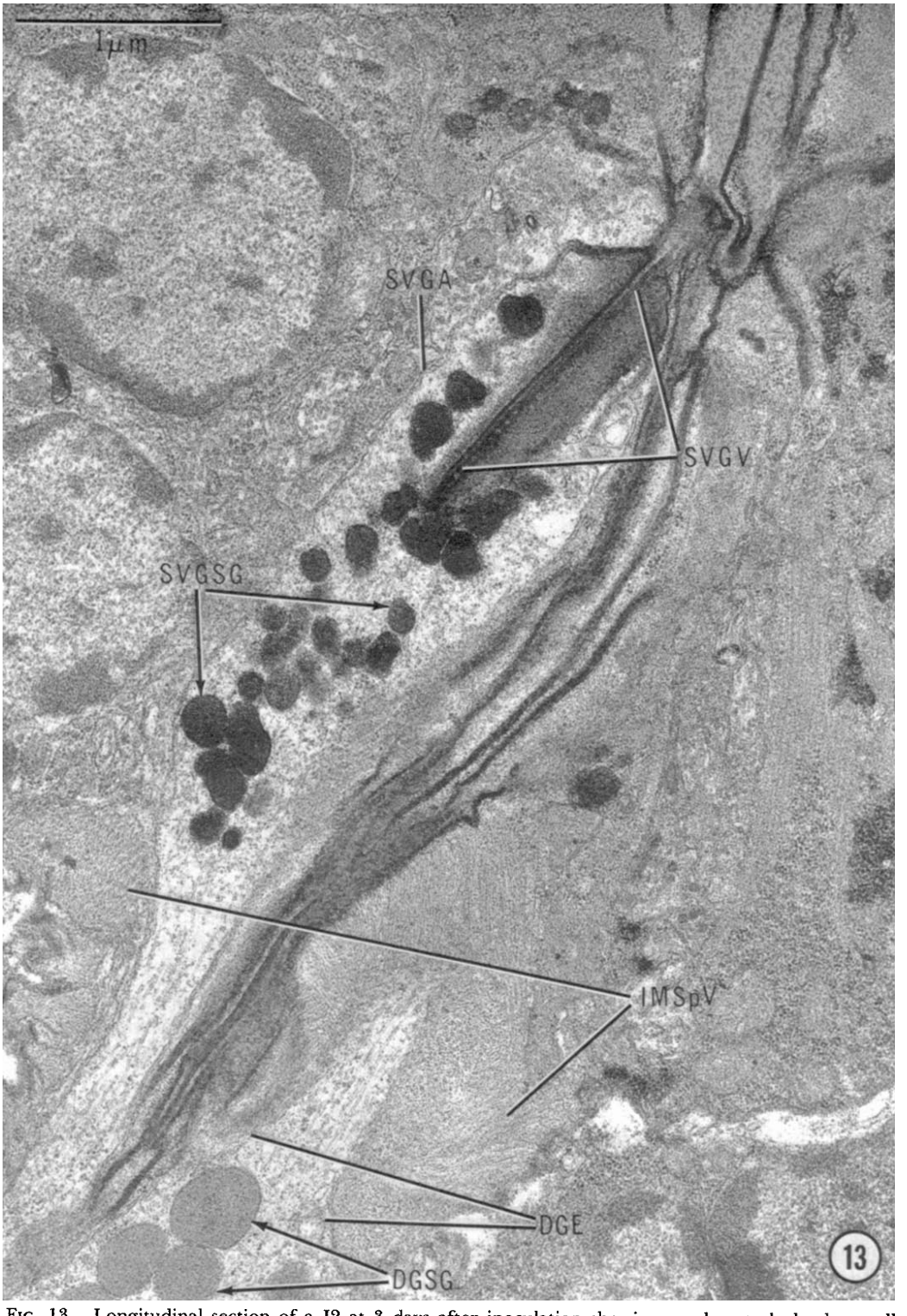


FIG. 13. Longitudinal section of a J2 at 3 days after inoculation showing a subventral gland ampulla (SVGA) with closed subventral gland valve (SVGV) and small, dense irregular shaped secretion granules (SVGSG). Posterior metacorpus sphincter valve appears closed restricting the movement of the secretion granules of the dorsal gland (DGSG). Note similarity of these granules to those shown in cross section of a different specimen (Fig. 7). DGE, dorsal gland extension; IMSpV, isthmus–metacorporeal valve.

FIG. 14. Cross section through anterior glandular region showing dorsal gland (DG) with accumulation of secretion granules (DGSG) and subventral gland extensions (SVGE) containing widely dispersed cytoplasm. A portion of the triradiate lumen (TLE) is shown in a longitudinal plane.

Whereas the sphincter-valve muscle in the posterior part of the metacorpus had been reported (7), its role during the feeding process had not been explained. The sphincter muscle seems to be activated to control the apparent microtubule-associated flow of secretory granules from the dorsal esophageal gland and the subventral glands.

Whereas the dorsal gland is very active in terms of the production of secretory granules 3 days after inoculation, the subventral glands appear to be inactive. The granules are small and electron dense, in contrast to their variable morphology in infective J2 (7). These observations are consistent with the observation made by Bird (2) who reported that in root-knot nematodes, granules accumulated in ducts of the subventral esophageal glands shortly before hatching and appeared to be associated with the penetration of the egg shell and cell wall and that the granules disappeared within 1–3 days of entry into the host. Similarly in *H. glycines,* 3 days after inoculation, the subventral gland secretions are not functional or are in a low part of a cycle of secretory granule production. The greatly enlarged sac-like structure observed in the posteriad region of the procorpus of *H. glycines* 3 days after inoculation may be comparable to the "vacuole" or "vesicle" observed in the esophageal wall tissue in front of the metacorpus of *Heterodera schachtii* during its feeding on *Raphanus oleiformis* when observed with video-enhanced light microscopy (20,21).

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