Electron Microscopy of the Stomatostylet and Esophagus of Criconemoides curvatum[1]

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Abstract: The stomatostylet of *Criconemoides curvatum* consists of three parts: tooth cone, shaft, and knobs. The tooth cone constitutes the outer conical covering and inner lining of the anterior half of the stylet lumen. The tooth cone is easily separated from the shaft by treating an isolated stylet with 0.5% sodium hypochlorite. The posterior half of the shaft is cylindrical, tapering anteriorly to form the shaft extension, wedged between the inner and outer tooth cone. The shaft extension extends to the stylet lumen orifice, which is subterminal and ventral. Six ducts enter the shaft through the junction between the shaft and knobs. They extend anteriad toward the tip of the shaft extension. Cytoplasmic connections between the ducts and the cells surrounding the stylet occur near the junction between the shaft and the basal knobs. Ribosome and membranous structures are observed in these ducts. The esophagus of the adult female consists of a fused procorpus and metacorpus with a large valve possessing thickened cuticular walls at the anterior and posterior ends. The dorsal esophageal gland reservoir is composed of many honeycomb-like compartments made up of two types of differing electron density. The subventral esophageal glands, however, consist of only one type of granules. Both dorsal and subventral esophageal glands open into the esophageal lumen through trachea-like branched multiple canals. *Key Words:* stylet, esophageal glands, ultrastructure.

The stylets of *Criconemoides xenoplax* Raski and *C. macrodorum* Taylor have been investigated with the light microscope, respectively by Seshadri (8) and de Grisse (4). They have shown that the Criconemoides stylet possesses six minute flattened pores symmetrically spaced around the stylet lumen: one dorsal, one ventral, and four sublateral. From observations of Andrassy (1) and Goody (5), the morphology of the *Criconemoides* stylet is generally similar to other tylenchid stylets. Accordingly, the tylenchid stylet is composed of three parts: the tooth cone, which is anterior; the shaft, which is the cylindrical middle part; and the basal knobs which constitute the posterior portion. A study by Chen and Wen (3) on the feeding apparatus of *Pratylenchus penetrans* (Cobb 1917) Filip and Schuurm. —Stekh., 1941, confirmed the 3-part structure of the tylenchid stylet. With electron

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Received for publication 26 January 1976.

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microscopy, however, Chen and Wen (3) showed that the composition and morphology of these parts of the stylet differ from the description by Andrassy (1) and Goody (5). The tylenchid stomatostylet is apparently a rather complicated structure. This report presents details of the ultrastructure of the feeding apparatus of *Criconemoides curvatum* Raski.

MATERIALS AND METHODS

Criconemoides curvatum was obtained from soils collected from turf plots at Rutgers University and extracted by centrifugal flotation (6).

For light-microscope studies, stylets of *C. curvatum* were individually isolated for detailed observation. The stylets were excised by crushing the nematodes under a cover slide and removing them from the nematode bodies. The tooth cone of each isolated stylet was separated from the shaft by treating with 0.5% sodium hypochlorite, a method modified from Wright (1). Observations were made with bright-field and phase-contrast light microscopes.

In electron-microscope studies, the procedures for killing, fixation, dehydration, infiltration, embedding, sectioning, and staining were the same as those described previously (10).

OBSERVATIONS

Stylet: The stylet of *C. curvatum* consists of 3 parts: tooth cone, shaft, and knobs (Figs. 1, 2, 3). The orifice of the stylet lumen is subterminal and opens ventrally (Fig. 2).

The tooth cone appears to constitute more than 2/3 of the stylet, yet it is not a simple "cone." It is solid at the extreme tip of the stylet but is soon split, just below the tip, by a wedge-shaped shaft extension (anterior portion of the shaft) to form two layers (Fig. 1-A,B,C). As a result, the tooth cone becomes separated by the shaft into an outer ring, forming the cover, and an inner ring, forming the lining of the stylet lumen for the anterior 2/3 portion of the stylet. The interlocking between shaft and tooth cone is discernible by light-microscope observation when the stylet is excised (Fig. 4). Sodium hypochlorite treatment of the isolated stylet separates the tooth cone from the shaft and the knobs (Fig. 4 B) and reveals a gradual but sharply pointed shaft extension toward the anterior end of the stylet (Fig. 4-C). The isolated tooth cone therefore has, between the inner and outer layers (Fig. 4-B), a hollow space that the shaft extension ordinarily occupies.

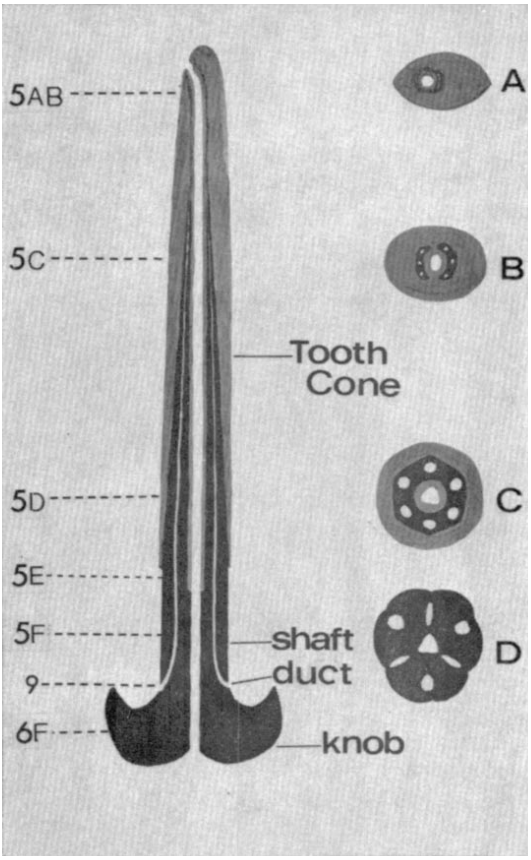

FIG. 1. A schematic drawing of the stomatostylet of *Criconemoides curvatum,* showing the complex interlocking structures between the tooth cone and the anterior portion of the shaft. The six ducts running through the shaft are shown both longitudinally and transversally at different levels. Numbers 5AB—6F on the left indicate approximate levels of Figs. 5 and 6.

When the stylet is placed in the sodium hypochlorite the tooth cone will dissolve in the solution gradually, whereas the shaft and knobs will maintain their structures. In electron micrographs (Fig. 3), the texture of the tooth cone appears to be cuticularized, and is different from the shaft and knobs.

The shaft (Fig. 4-C) extends from the basal knobs to the tip of the stylet. The lower of posterior portion of the shaft is not covered by the outer tooth cone, and it

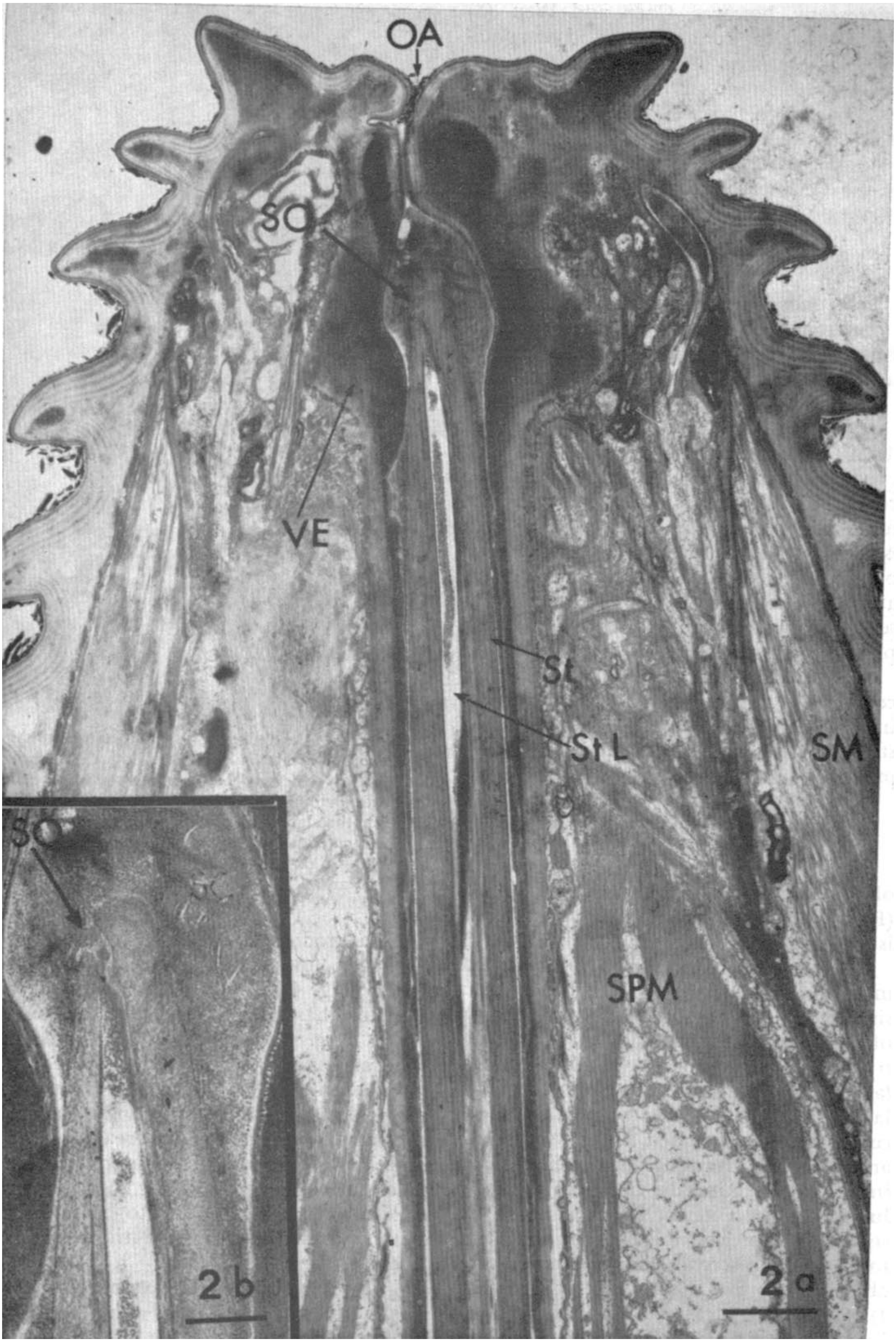

FIG. 2. a) Longitudinal section of anterior region of the stomatostylet of *Criconemoides curvatum.* OA, oral opening; SO, stylet opening; VE, vestibule extension; St, stylet; St L, stylet lumen; SM, somatic muscle; SPM, stylet protractor muscles.

b) Enlarged view showing subterminal opening of the stylet (SO). Bar represents 0.5 μm.

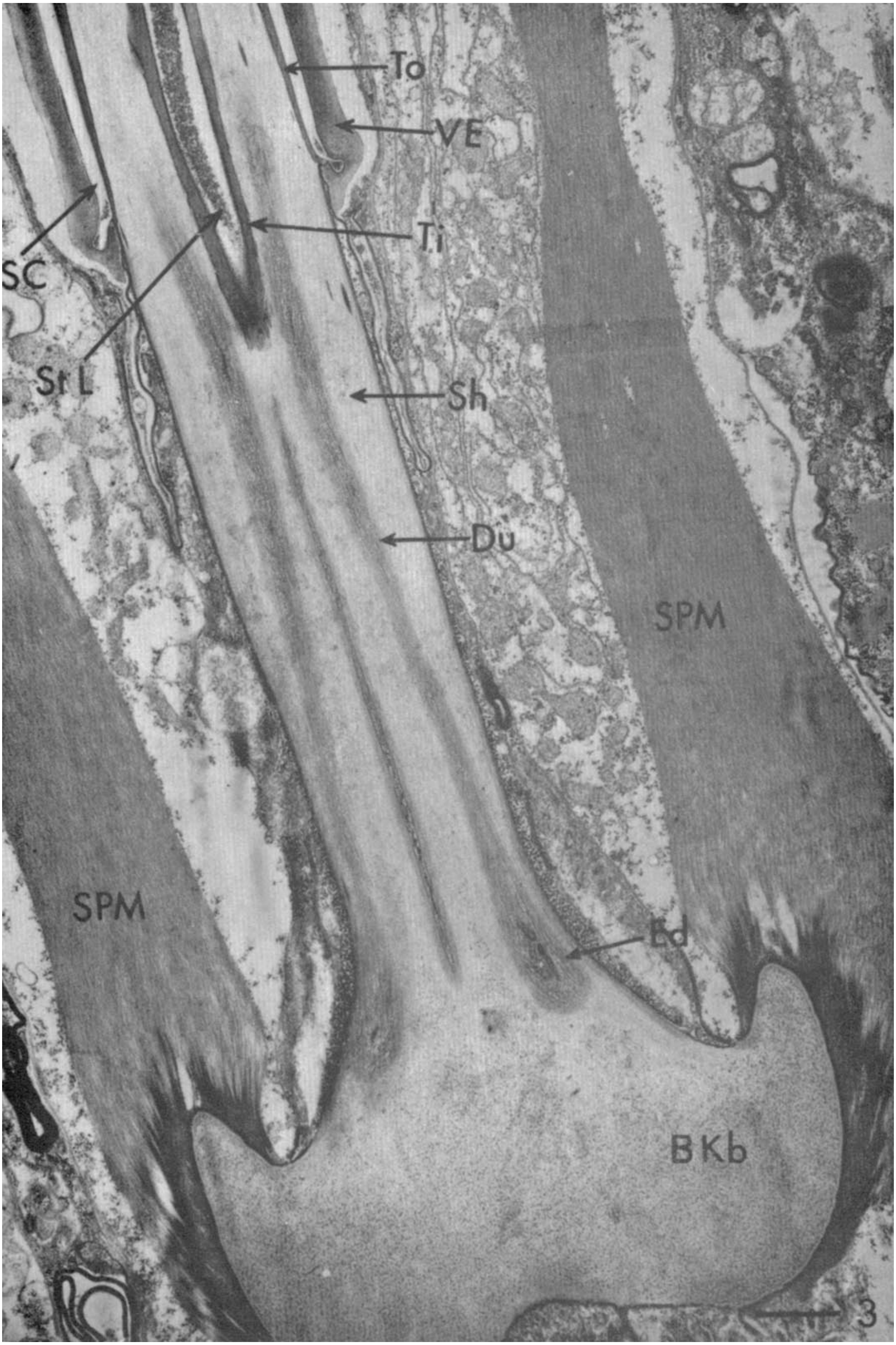

FIG. 3. Longitudinal section of posterior region of the stomatostylet of *Criconemoides curvatum*. SC, stylet chamber; St L, stylet lumen; VE, vestibule extension; To, outer tooth cone; Ti, inner tooth cone; Sh, shaft; Du, duct of shaft; Ed, duct end; B Kb, basal knobs; SPM, stylet protractor muscles. Bar represents 0.5 μm.

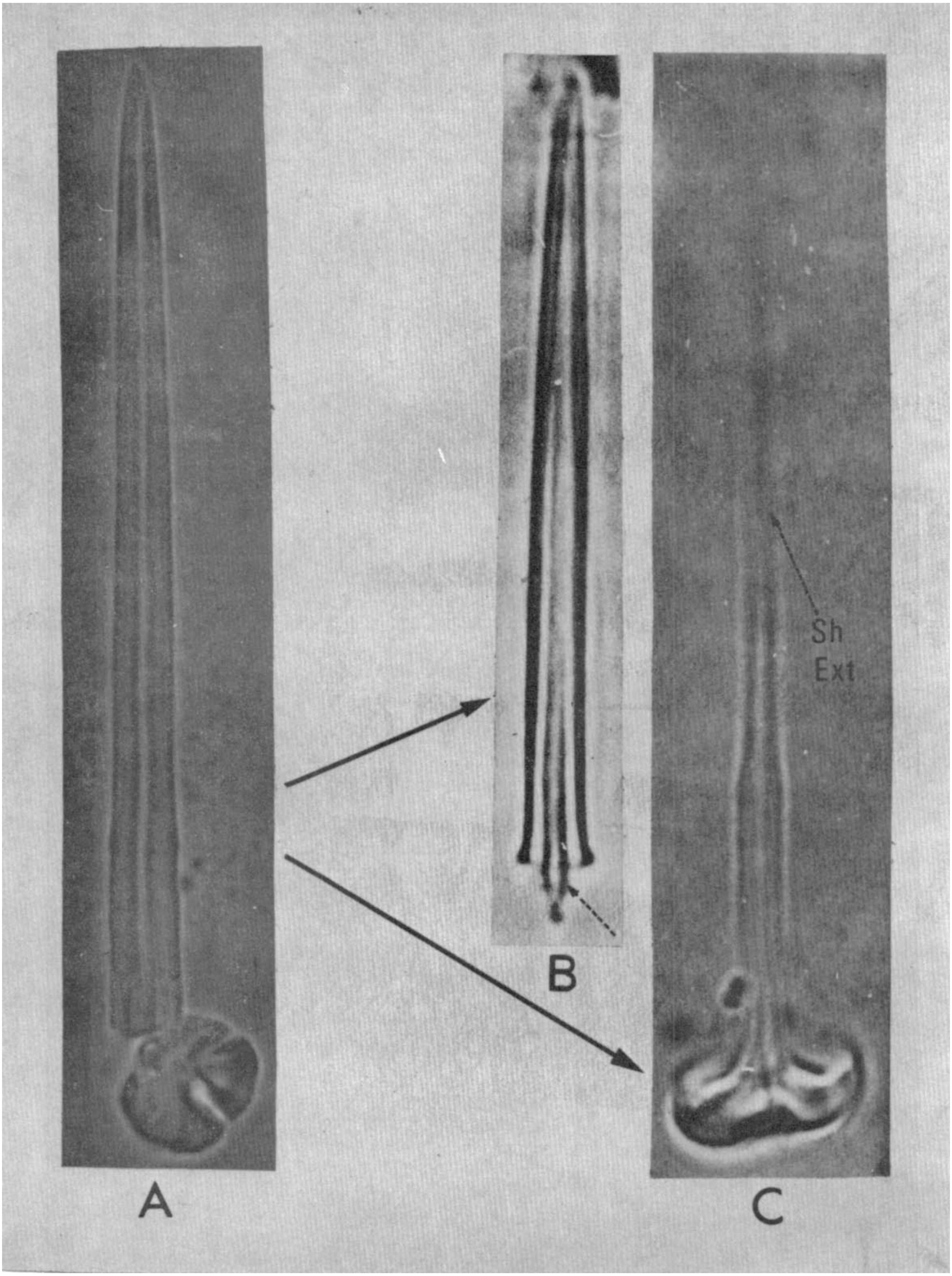

FIG. 4. Stylet components of *Criconemoides curvatum.*

A) An intact stylet before separation.

B) The tooth cone is separated from the shaft. Note that the inner lining (dash-line arrow) of the tooth cone is longer than the outer covering.

C) Showing shaft and basal knobs without the tooth cone. The shaft consists of a posterior cylindrical portion and the long anterior tapering tip (shaft extension).

is cylindrical (Fig. 3, 4). The upper portion (shaft extension) tapers anteriorly and inserts between the outer and inner layers of the tooth cone, forming an interlocking complex that is the anterior 2/3 of the stylet. In cross-sections, the shaft portion at the stylet tip appears to be an irregularly shaped electron-translucent ring (Fig. 5-A,B). The shaft gradually enlarges posteriorly to form two kidney-shaped parts, each containing three ducts (Fig. 5-C). The shaft portion further enlarges posteriorly to become a hexagonal ring with six evenly distributed ducts, one dorsal, one ventral, and four sublateral (Fig. 5-D). The diameter of the shaft reaches its maximum at a point where the outer layer of the tooth cone no longer covers the shaft (Fig. 5-E). Near the basal knobs, the shaft changes its cylindrical shape to a six-lobed structure with each lobe containing a single duct (Figs. 5-E, 6-A,B). At the junction of the shaft and basal knobs these ducts gradually curve outward and become perdendicular to the long axis of the stylet (Figs. 5-F, 6-A,B,C). First, the three alternate ducts that face the corresponding gaps between the alternate two lobes of the basal knobs, i.e., the two subdorsal and one ventral ducts, open to the outer edge of the shaft and connect directly with the cells and tissues surrounding the stylet (Figs. 5-F, 6-C,D). The other three alternate ducts opposing the three knobs (one dorsal and two subventral) connect to the surrounding tissues in the same manner, but these openings are slightly below or posteriad to those of the other three ducts. At this level the ducts in the shaft have increased their diameter to 0.3 $\sim$ 0.4 μm. Therefore, ribosomes and membrane structures can be clearly observed within the channels (Figs. 5-F, 6-D).

The shaft merges with the basal knobs after the ducts have ended. Slight differences in both texture and electron density between the shaft and the basal knobs can be detected, but there is no clear-cut difference to indicate that one portion of the stylet is ending while the other begins. The stylet protractor muscles are separated into three bundles with each attaching at the base of one stylet knob (Fig. 6-A,B). The muscle fibers wrap on to the outer edge of each knob, run parallel with the stylet, and connect to the head framework of the nematode at the anterior end (Fig. 4).

Esophageal glands: The anterior part of the dorsal esophageal gland of *C. curvatum* occupies a center area of the esophagus (Figs. 7-C, 9). As the stylet lumen emerges from the stylet knobs and becomes the esophageal lumen it curves to accommodate the gland tissue. Near the very anterior end of the gland the lumen and the gland connect (Figs. 7, 8-A,C). Several funnel-shaped canals constitute the dorsal esophageal gland orifice. From there these bronchiole-like canals, which branch out into the gland, are the only connection between the lumen of the esophagus and the contents of the gland. Two types of granules are observed in the reservoir of dorsal esophageal gland process (Figs. 7, 8-A,C). One type is electron-dense, whereas the other is electron-lucent.

The two subventral esophageal glands open to the esophageal lumen at the posterior end of the metacorpus valve (Fig. 7, 8-B, C, 9-B). Similar to the orifice in the dorsal esophageal gland are several canals leading from the esophageal lumen into each of the subventral glands. Only one type of glandular granule is observed within the subventral esophageal glands (Figs. 7, 8--B,C, 9-B), and thus they can be easily distinguished from the dorsal esophageal gland process (Fig. 7). Both dorsal and subventral esophageal gland processes surrounding the esophageal lumen extend posteriad through the isthmus (Fig. 9-B,C).

The esophageal lumen within the isthmus is triradiate in shape and thus probably serves as a valve. The three glandular processes occupy the spaces between the triradiate arms of the valve. Interspaced between the glands are nerve processes (Fig. 9-B,C) which apparently connect to those in the metacorpus and extend anteriorly to the nerve tissues situated between the basal knobs of the stylet. No direct connection between these nerve tissues and the nerve ring outside of the isthmus (Fig. 9-B,C) can be seen at this level. Innervation by nerve process to esophagus must be originated from ganglia located either anterial to the procorpus or posterior to the isthmus.

Many nuclei have been observed in the lobes of esophageal glands (basal bulbs). In the dorsal esophageal glandular lobe can be seen the same glandular granules observed in the anterior process (Fig. 7, 9-D).

DISCUSSION

Studies of the feeding apparatus of tylenchid nematodes by Andrassy (1), de Grisse (4), and Goodey (5) have firmly established that the stomatostylet is composed of three parts. Our electron-microscope studies have shown, however, that the composition, morphology, and arrangements of these stylet parts are quite complex. The tooth cone is penetrated by a cylinder of shaft extension and is separated into an inner and outer ring.

The similar light-transmission densities of the tooth cone and shaft have made them almost impossible to distinguish from each other under the ordinary light microscope when they are intact. Using 0.5% sodium hypochlorite solution we were able to separate the tooth cone from the shaft and the knobs. This made possible an examination of these intricate and delicate structures with a light microscope, which has further confirmed our EM observation.

During the molting of *C. curvatum* the tooth portion of the stylet is shed along with the stomatal and body cuticle, while the shaft and knobs become temporarily invisible. EM observation during molting shows

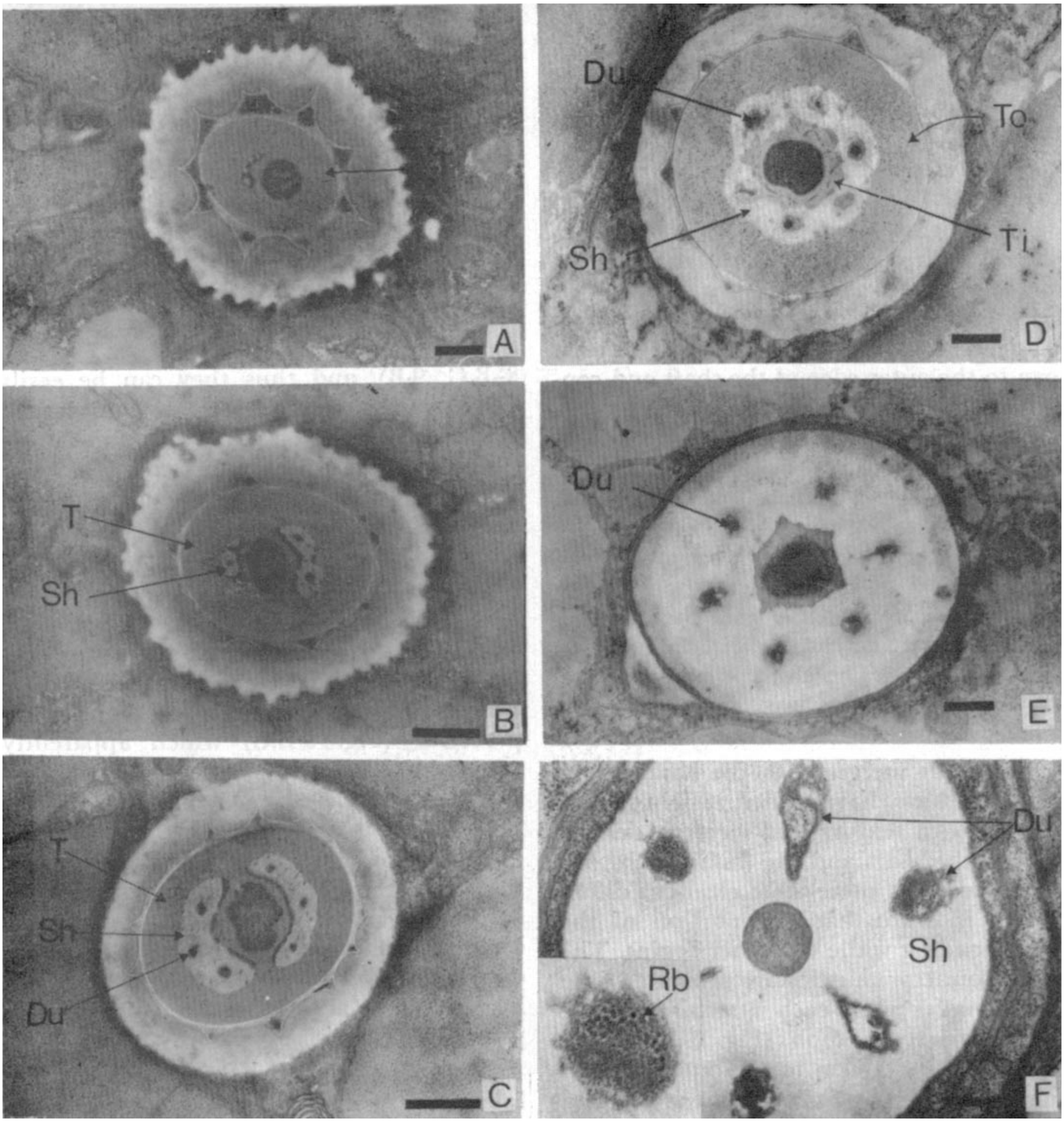

FIG. 5

that the shaft and knobs are still present, although, for some unexplained reason, these structures cannot be stained to bring out the contrast. In addition, the cells and tissues surrounding the stylet also become difficult to stain, and the cell organelles (ER, mitochondria, etc.) also become indistinct (9). This phenomenon suggests that the shaft and knobs, like the surrounding cells and tissues, are probably a part of the living system whereas the tooth cone is merely a modified cuticularized structure. The tooth cone is chemically different from shaft and knobs in that, like the body cuticle, it dissolved readily in 0.5% sodium hypochlorite, whereas the shaft and knobs remained intact.

The six ducts observed in the shaft of *C. curvatum* are distinct structures equivalent to the pores described by de Grisse (4) and Seshadri (8). No mitochondria, microtubules, or other cellular organelles have

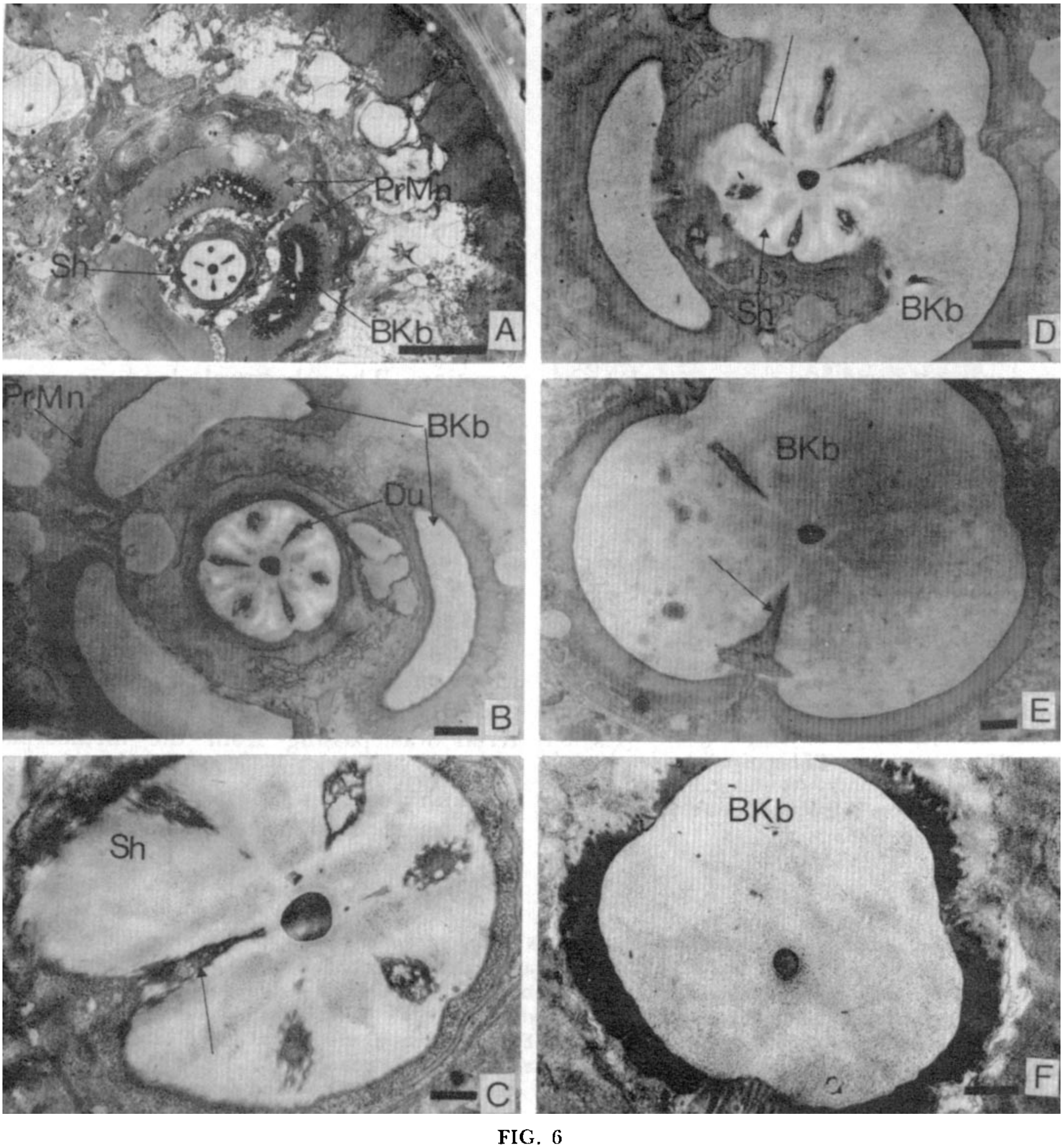

FIG. 6

FIGS. 5-6. Several cross-sections from anterior tip to the basal knobs of the stylet of *Criconemoides curvatum*. T, tooth cone; Sh, shaft; Du, duct of shaft; To, outer tooth cone; Ti, inner tooth cone; Rb, ribosomes; B Kb, basal knobs; PrMn, protractor muscles. Bars in Fig. 5 represent 0.5 μm; bar in Fig. 6-A represents 4 μm; bar in Fig. 6-B represents 1μm; bars in Fig. 6-C-F represent 0.5 μm.

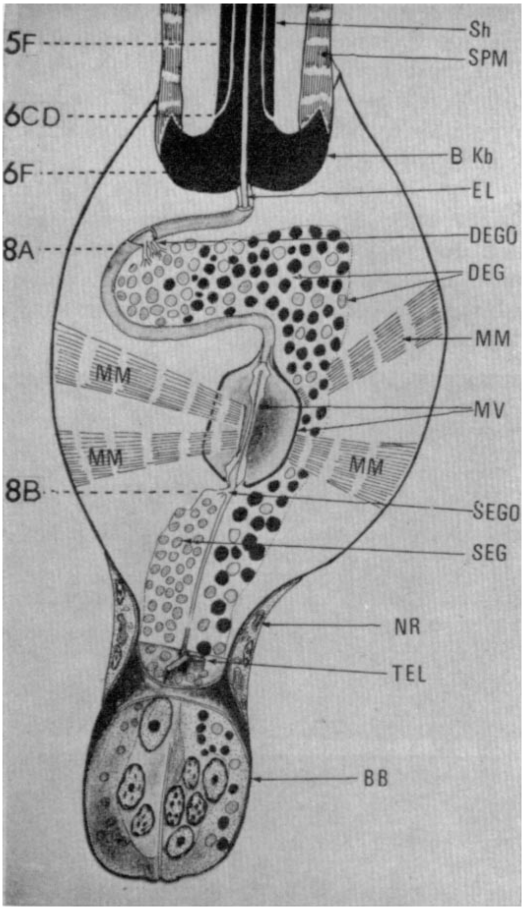

FIG. 7. A schematic drawing of the esophagus of *Criconemoides curvatum*. Sh, shaft; SPM, stylet protractor muscle; B Kb, basal knobs; EL, esophageal lumen; DEGO, dorsal esophageal gland orifice; DEG, dorsal esophageal glandular granules; MM, median bulb (metacorpus) muscles; MV, metacorpal valve; SEGO, subventral esophageal gland orifice; SEC, subventral esophageal glandular granules; NR, nerve ring; TEL, triradiate esophageal lumen; BB, basal bulb (esophageal gland lobe). Numbers 5F-8B on the left indicate approximate levels of Figs. 5, 6, 8.

been found in the anterior portion of the ducts, possibly because of their small diameter. However, we have observed distinct ribosome and ER-like membranes within the ducts near the base of the shaft. Since these ducts have been shown to open into the tissues outside of the stylet there must be a direct connection between the inner portion of the stylet and the living cytoplasm that surrounds the stylet. This evidence supports our previous hypothesis (3) that cells surrounding the shaft of the stylet serve not only to continuously nourish the shaft and knobs, both of which may be part of the living system, but also to regulate the formation and casting off of the tooth cone during molting.

The granules in the reservoir of the dorsal esophageal gland of *C. curvatum* may be of two different granule types. It is possible that these different structures may reflect concentration (or density) of their component material. The differences in electron density may be only the result of breakdown or active synthesis of glandular secretions. The subventral gland process of this nematode contains only one type of glandular granules, filled with uniformly small dark particles. The difference between the granules within the dorsal and subventral esophageal gland processes suggests that they have different functions. McElroy and Van Gundy (7) reported that globules from the dorsal esophageal gland in *Hemicycliophora arenaria* diminished during the injection phase of feeding. The subventral gland orifices opened only while food material was passing from the metacorpal chamber into the lumen of the isthmus.

Both dorsal and subventral gland processes open into the esophageal lumen

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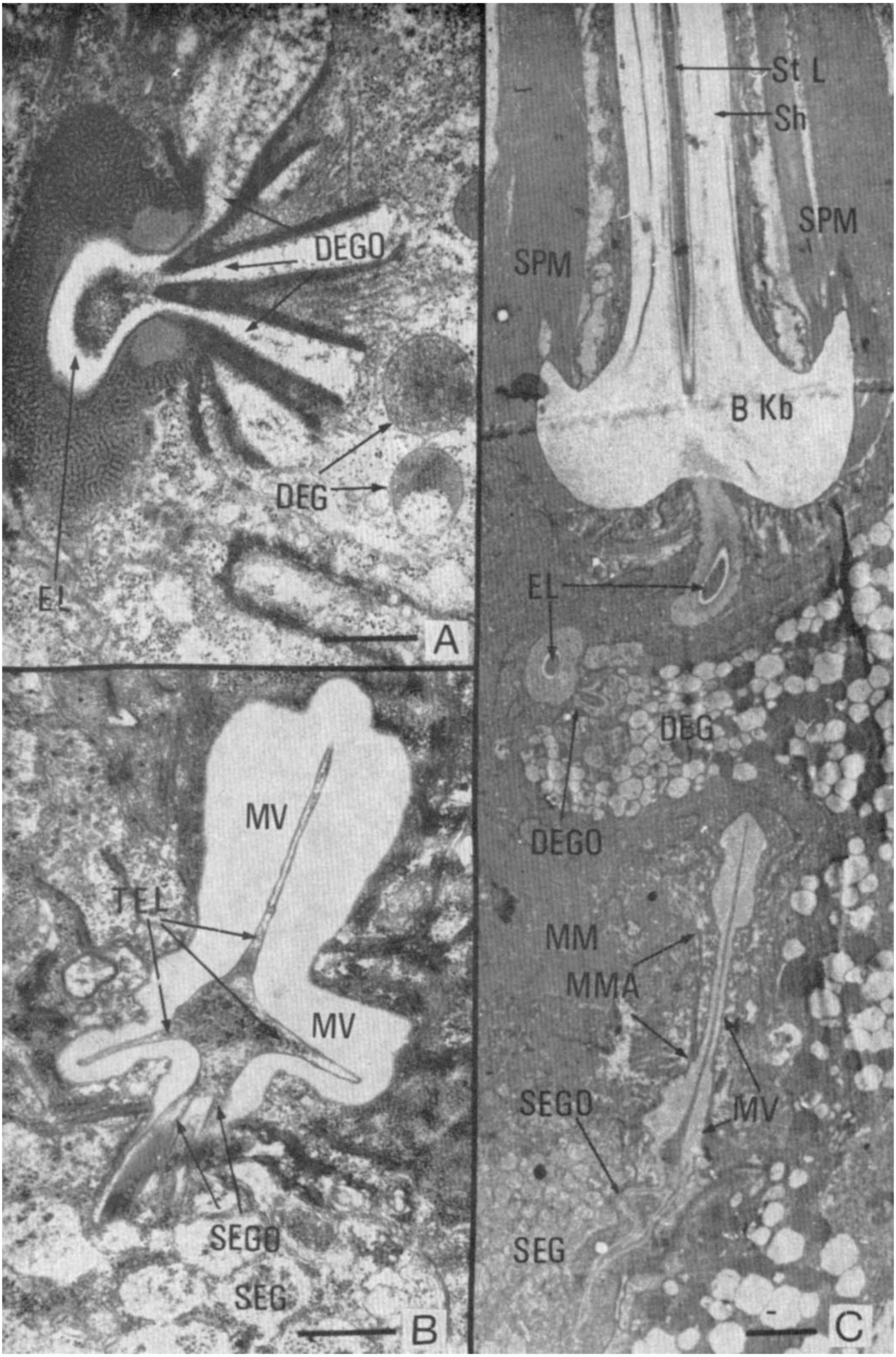
FIG. 8. Cross and longitudinal sections of esophagus of *Criconemoides curvatum*, showing orifice of both dorsal esophageal gland process and subventral esophageal gland process. St L, stylet lumen; Sh, shaft; SPM, stylet protractor muscles; B Kb, basal knobs; EL, esophageal lumen; DEGO, dorsal esophageal gland orifice; DEG, dorsal esophageal glandular granules; MM, median bulb muscles (metacorpus muscles); MMA, metacorpus muscle attachment; MV, metacorpal valve; SEGO, subventral esophageal gland orifice; SEG, subventral esophageal glandular granules; TEL, triradiate esophageal lumen.

A) Cross-section showing the connection between esophageal lumen and the dorsal esophageal gland process. Bar represents 0.3 μm.

B) Cross-section showing the connection between esophageal lumen and the subventral esophageal gland process. Bar represents 0.3 μm.

C) Longitudinal section through the stylet, procorpus, and metacorpus showing connections between esophageal lumen and both dorsal and subventral esophageal gland processes. Note the two different glandular granules in the dorsal esophageal process. Only one type of granule is in the subventral esophageal gland processes. Bar represents 1 μm.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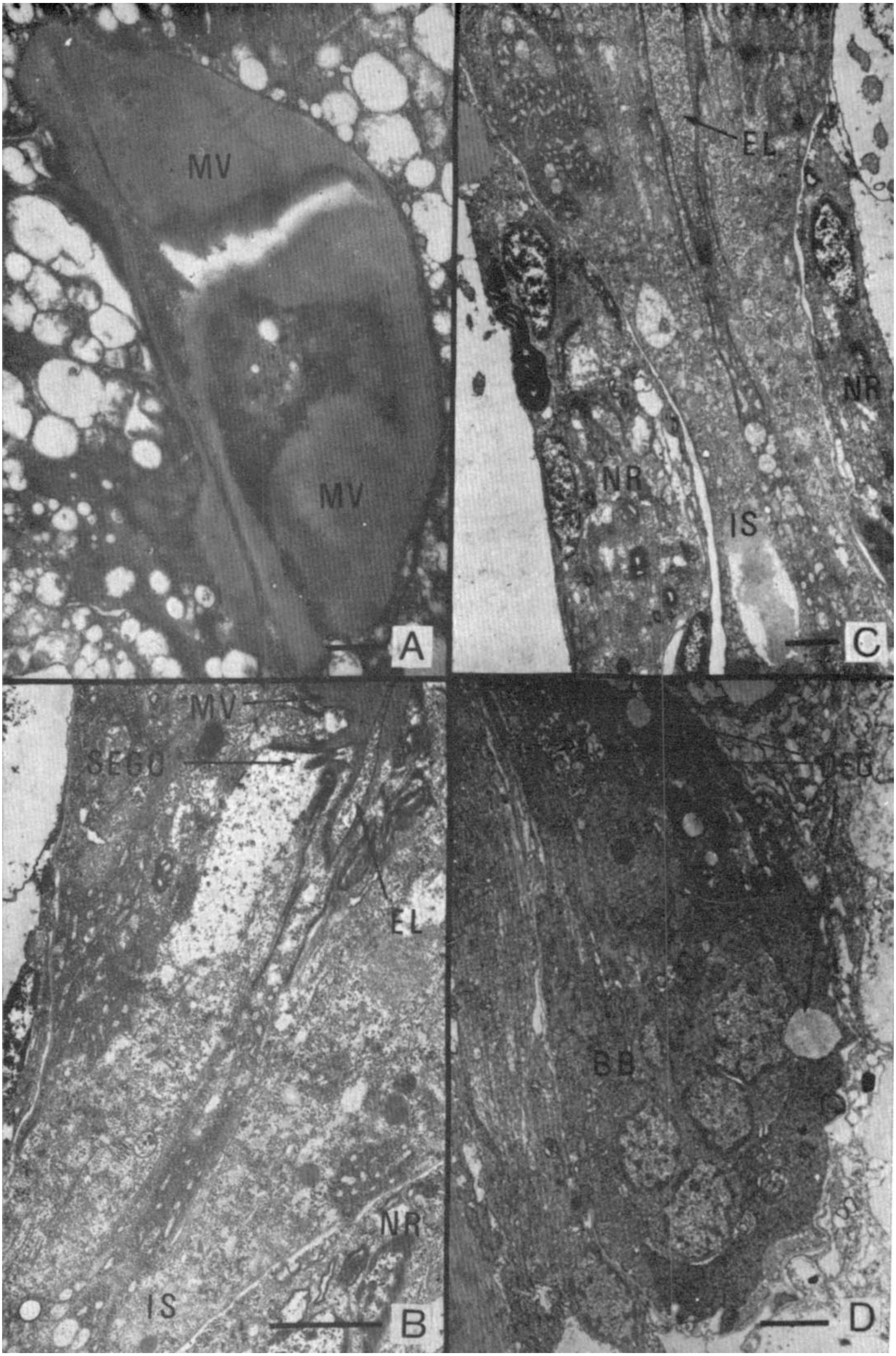
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through trachea-like branched tubes similar to those described in *Meloidogyne incognita* and *Heterodera glycines* (2). They differ from *P. penetrans,* however, which has a simple orifice in the esophagus (3). Whether such differences in esophageal orifice represent anatomical features in nematodes would be worth exploring.

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FIG. 9. Longitudinal sections through various parts of the esophagus of *Criconemoides curvatum.* MV, metacorpus valve; SEGO, subventral esophageal gland orifice; EL, esophageal lumen; IS, isthmus; NR, nerve ring; DEG, dorsal esophageal glandular granules; BB, basal bulb (esophageal gland lobe).

A) Showing cuticular structure of metacorpus valve.

B) Showing connection between esophageal lumen located at the posterior end of metacorpus and one of the subventral esophageal gland processes.

C) Showing isthmus in which the esophageal gland processes and nerve tissues pass. Surrounding the isthmus is the nerve-ring tissue.

D) Showing the same glandular granules in the dorsal esophageal gland lobe (basal bulb) as those in the anterior process of the gland. Bars represent 1.5 μm.