Ultrastructural Studies on *Caenorhabditis briggsae*

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Abstract: A study of the fine structure of *Caenorhabditis briggsae* revealed several features not previously described in free-living nematodes. These were: chambered walls of the stoma, zonula adherens in the esophagus, daedaloid folds in the inner surface of the uterus and openings in the terminal web of the intestinal epithelium. Other structures observed in these studies were similar to those described from other free-living nematodes. *Key Words: Caenorhabditis briggsae*, Ultrastructure.

The ultrastructure of young, *Caenorhabditis briggsae* adults was studied preliminary to investigating physical and chemical changes associated with aging. Details of the fine structure of this nematode are given in this paper.

Materials and Methods

Caenorhabditis briggsae were cultivated axenically at 23 C in a medium containing 3 g yeast extract, 4 g soy peptone, 90 ml water and 10 ml liver extract from fresh beef liver as reported by Sayre *et al.* (10). All ultrastructural studies were performed on 5–8 day old females with the exception of second- and fourth-stage larvae which were examined for the presence of the striped layer (Fig. 5).

All specimens were fixed in glutaraldehyde and embedded in 1.5% agar by the methods of Hirumi *et al.* (2). Entry of the fixative was facilitated by excising the tail tip. Dehydration of the agar blocks and embedding in Epon 812 were as described by Yuen (12). Sections were cut with glass knives at 50–90 mμ (silver to gold interference colors), stained 1 hr with uranyl acetate and 3 min with lead oxide (6), and then examined with a JEM-7 or a JEM-T-7 electron microscope operated at 80 KV.

Observations

Stoma: In cross section the stoma resembled that in *Panagrellus silusiae* (13). Longitudinal sections showed that the walls of the stoma were chambered (Fig. 1), a feature not previously reported in rhabditoid nematodes. The posterior section of the stoma contains several teeth (Fig. 1) whose number and location was not determined.

Esophagus: The esophagus and the pseudocoelom are clearly delimited by the basement membrane (Fig. 4). There are two radial muscle cells in each sector and a marginal muscle cell originating at each tip of the triradiate lumen. Each muscle cell is bordered by a well-defined membrane. The muscle cell arrangement essentially is the same as in *P. silusiae* (13). In the anterior part of the procorpus, the dorsal esophageal gland containing abundant mitochondria, clusters of ribosomes and groups of microtubules lies between the radial muscle cells (Fig. 4, 6). Nuclei were not observed. At

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the level of the stoma, contiguous to the lumen, the muscle cell membranes are fused, forming clearly-defined tight juctions. In the metacorpus the tight junctions are absent, but near the lumen, zonula adherens with dense intracellular plaques mark specialized areas of contact between the cell membranes (Fig. 3). Half-desmosomes occurred at the point where the marginal muscle attaches to the plasma membrane (Fig. 3), as reported for *Nippostrongylus brasiliensis* (4), and at other points of muscle attachment (Fig. 6). In the anterior procorpus the tips of the triradiate lumen are heavily sclerotized (Fig. 2), whereas in the metacorpus the tips of the lumen are at acute angles as in *P. silusiae* (13).

HYPODERMIS: The lateral hypodermal chords possess a complex system of membranes, mitochondria and well-defined laminated structures similar to those reported by Yuen (13) for *P. silusiae* (Fig. 4, 7, 8). Clusters of mitochondria surrounded by electron-dense material occur in each of the chords (Fig. 4, 7). In longitudinal section, the laminated structures appear to terminate approximately at the level of the midsection of the procorpus. As in *P. silusiae* (13),

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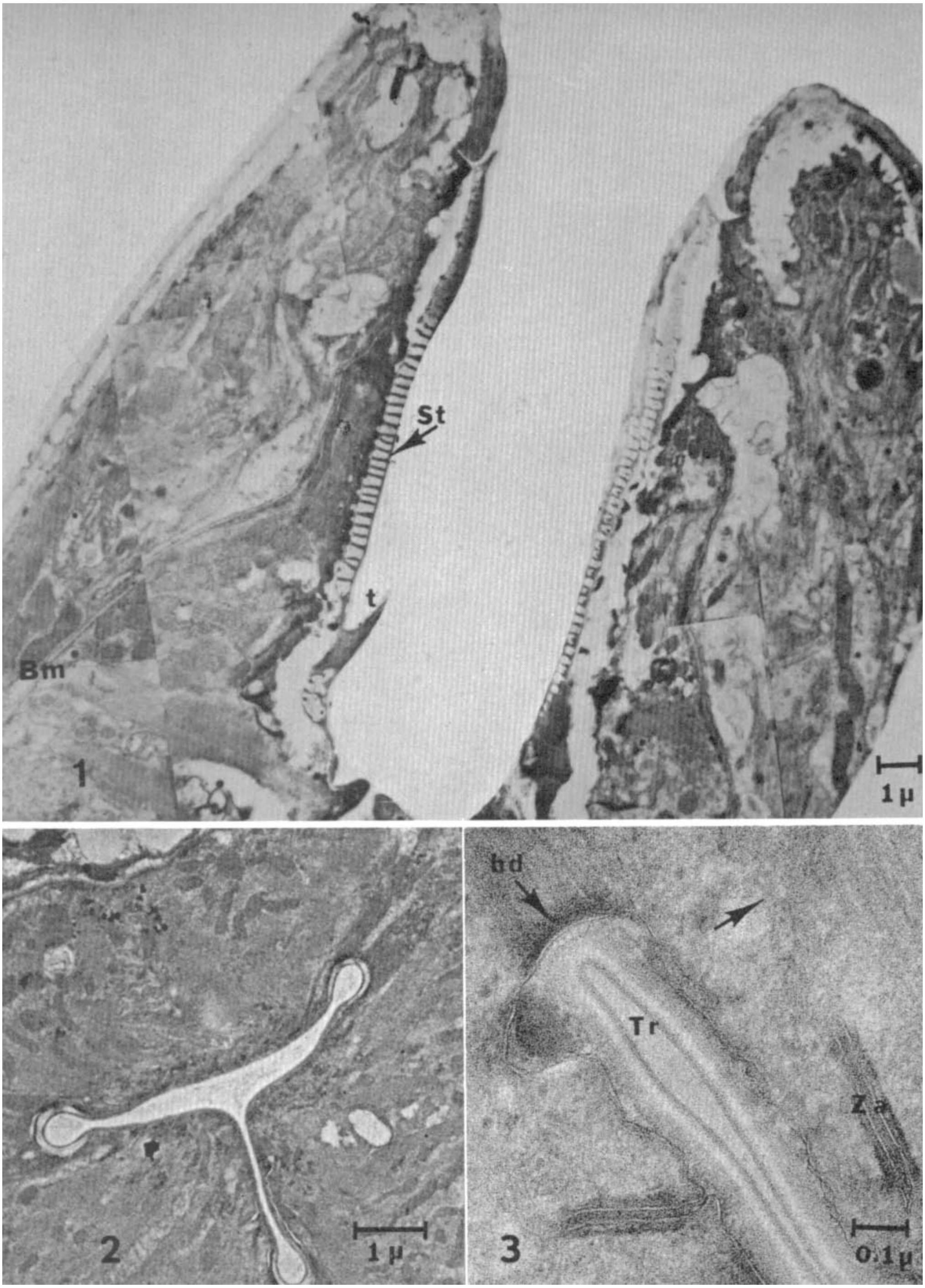
FIG. 1–3. *Caenorhabditis briggsae* fine structure. **Fig. 1.** Longitudinal section showing the chambered walls of the stoma (St). Other structures labelled are a tooth (t) and the basement membrane of the esophagus (Bm). **Fig. 2.** Cross section through the procorpus showing the heavy sclerotization of the tips of the triradiate lumen. **Fig. 3.** A zonula adherens (Za) formed by the membranes of esophageal muscle cells. A half-desmosome (hd) at the point of insertion of the marginal muscle onto the plasma membrane. The continuation of the cell membranes of the two cells is indicated by an arrow. One branch of the triradiate lumen (Tr) is shown.

FIG. 4–5. *Caenorhabditis briggsae* fine structure. **Fig. 4.** Cross section at the level of the anterior end of the procorpus. Esophageal structures labelled are the basement membrane (Bm), marginal muscle cells (Ma), radial muscle cells (Ra), the anterior portion of the dorsal gland (Dg) just posterior to the gland orifice containing many mitochondria, the junctional complexes (JC) formed where the cells meet at the walls of the stoma and the membranes delineating several of these cells (arrows). The eight somatic muscle cells (s) can be distinguished and the mitochondria (M), myofilaments (My), supporting structures (z) and membranes (arrows) of the two cells of one quadrant of the body are indicated. Other structures shown are the dorsal (dc), ventral (vc) and lateral (lc) chords, and the well-defined laminated structures (la) of the lateral, hypodermal region. **Fig. 5.** Cross section of a molting larva posterior to the esophago-intestinal junction showing the striped layer (Sl). Also shown is an area of a somatic muscle cell which contains only thick myofilaments (Tm), a band which contains both thick and thin myofilaments (TTm) and a band composed of thin filaments (Thm).

FIG. 6–9. *Caenorhabditis briggsae* fine structure. **Fig. 6.** Cross section at the level of the anterior end of the procorpus showing well-defined somatic muscle cells. The membrane delineating the cell to the left is indicated by arrows. Within the cell a supporting structure (z), the non-contractile part of the cell containing mitochondria (M), and the myofilaments (My) of the contractile portion of the cell are indicated. Other structures which are labelled are the hypodermis (h), basement membrane of the esophagus (Bm), half-desmosome (hd) marking the attachment of the radial muscles to the basement membrane, the dorsal esophageal gland (dg) between two radial muscle cells (Ra) which contains numerous, packed mitochondria (M) and microtubules (Mt). **Fig. 7.** Cross section at the level of the anterior end of the procorpus showing the well-defined laminated structures (La) found in the lateral hypodermal chords. The mitochondria (M) and a part of a somatic muscle cell (S) are also shown. **Fig. 8.** As Fig. 7 but showing the well-defined laminated structures (La) surrounding a mitochondrion (M). **Fig. 9.** Cross section slightly posterior to the esophago-intestinal junction. In this area the intestine possesses the triradiate form of the esophageal lumen, though the bacillary layer has replaced the sclerotized inner layer of the esophagus. The lateral hypodermal chords (arrows) are prominently outlined and the lateral fields (L) are conspicuous.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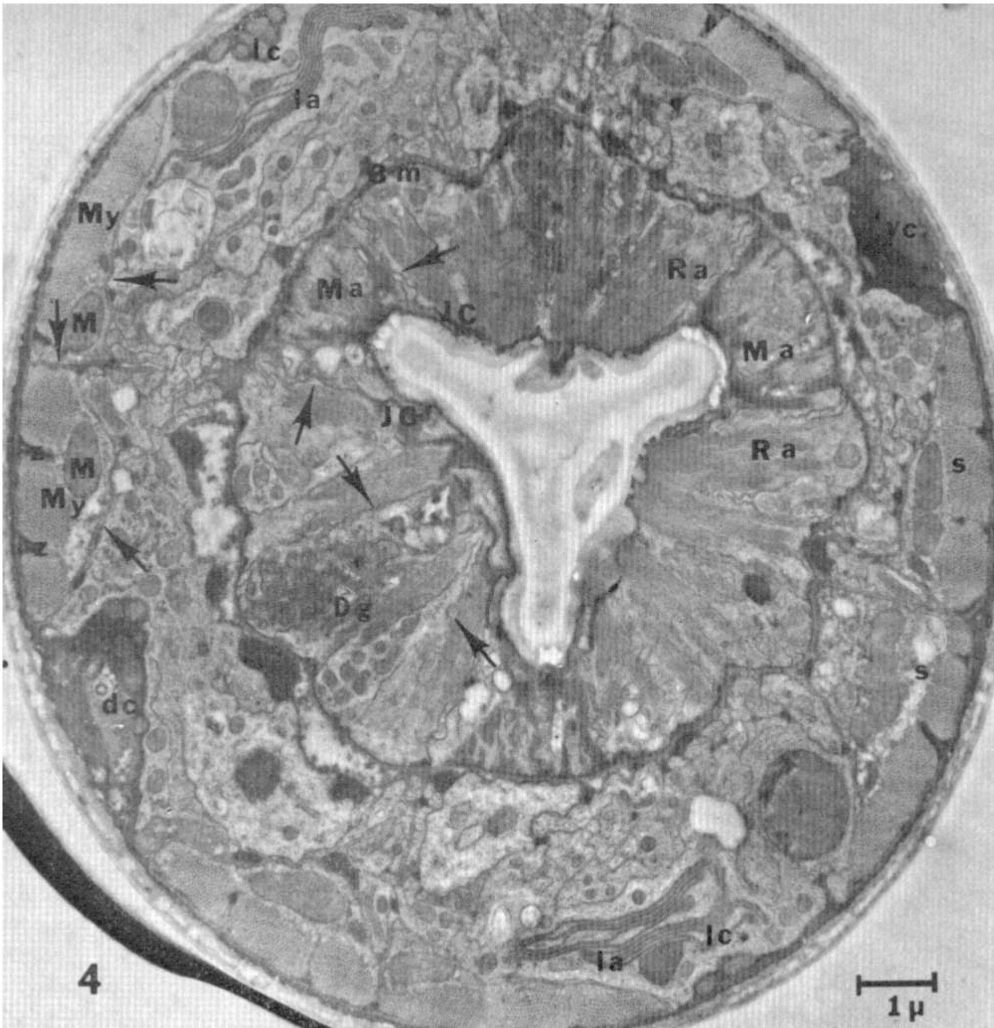
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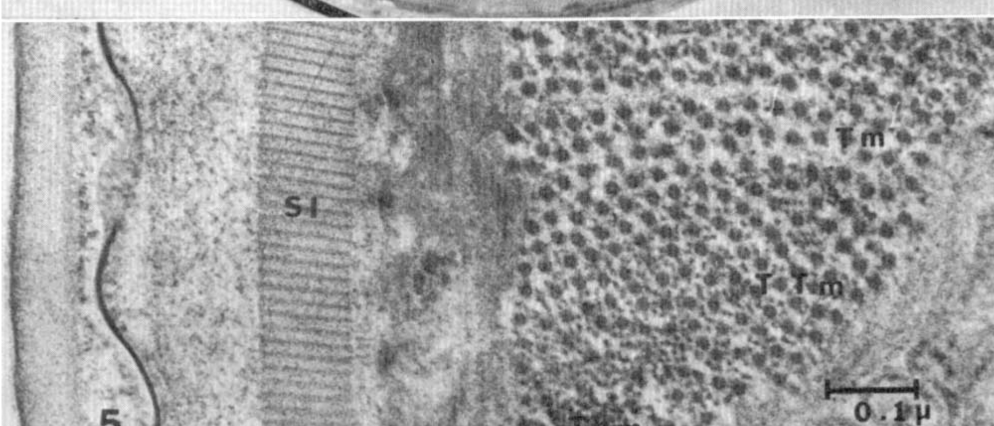
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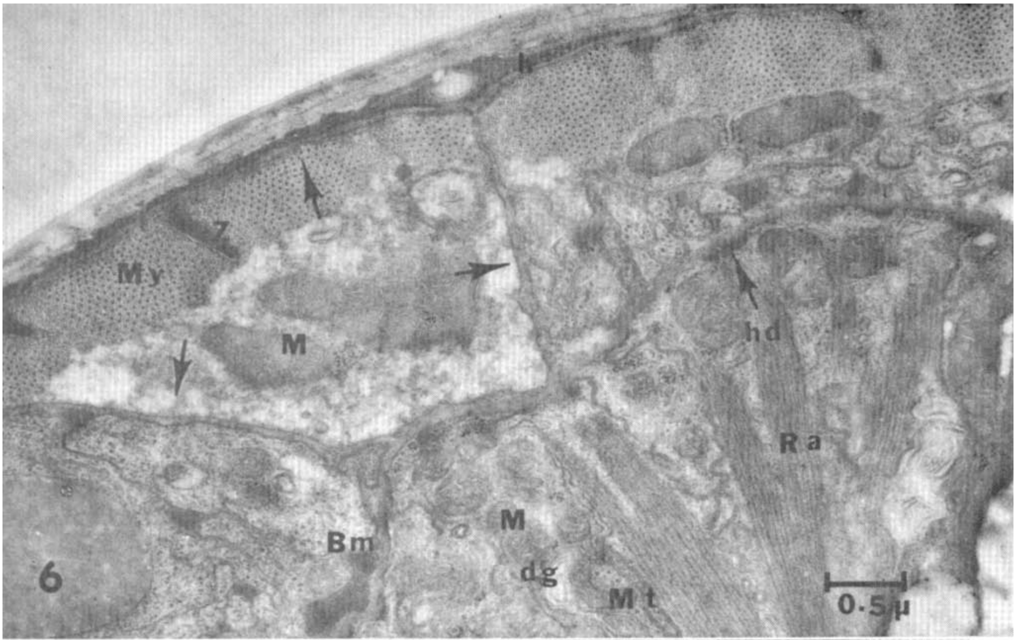

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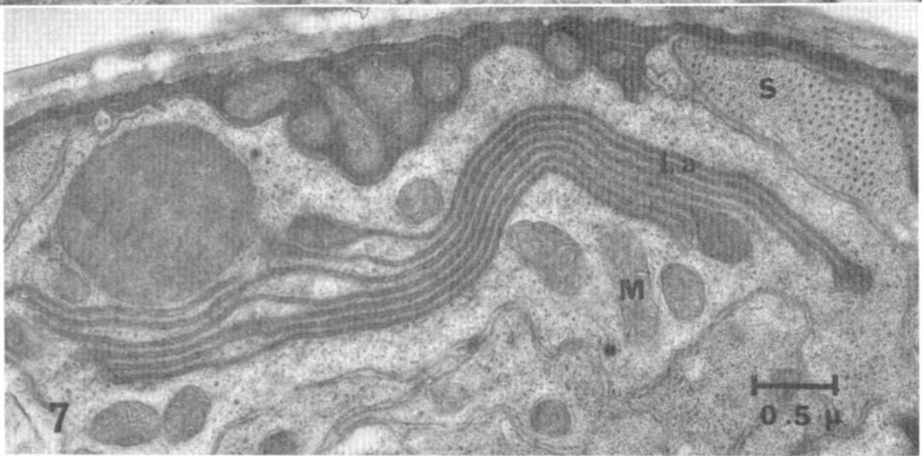

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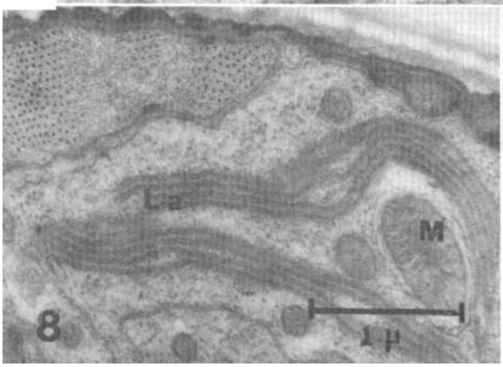

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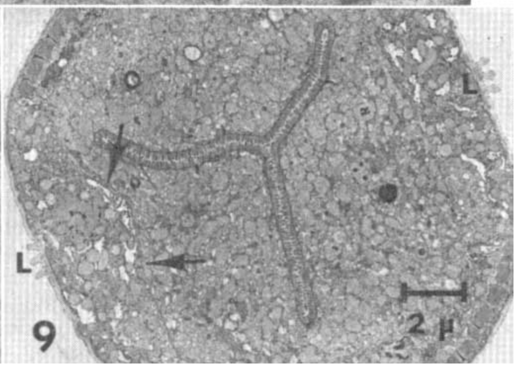

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the lateral chords posterior to the procorpus do not contain the laminated structures or membranes.

SOMATIC MUSCLES: The somatic musculature is divided into four longitudinal muscle fields, each of which consists of two platymyarian muscle cells (Fig. 4). The contractile zone contains the five bands described by Rosenbluth (7) in *Ascaris lumbricoides*, that is, two outer bands that contain thin myofilaments and two inner bands that contain both thick and thin filaments between which lies a band that contains only thick myofilaments (Fig. 5). Narrow bands of an electron-dense material between muscle fiber bundles appear to be supporting structures similar to the Z-bars of vertebrate muscle tissues (Fig. 4, 6, 9, 10). Within the non-contractile region of the cell, the nucleus (Fig. 10), ribosomes, and mitochondria (Fig. 6) were observed. At the level of the stoma each cell is surrounded by electron-dense material which clearly delimits the cell from the surrounding tissues (Fig. 6). This material is not found near the esophago-intestinal junction (Fig. 9) or at midbody.

INTESTINE: Slightly posterior to the esophago-intestinal junction, the microvillous lumen of the functional intestine is triradiate (Fig. 9) but within a short distance the intestine becomes ribbon-shaped.

The intestinal epithelium contains abundant ribosomes and mitochondria. The membranes of the epithelial cells are fused near the lumen, forming a complex similar to the tight junction (Fig. 14, 15). The structure of the junction (Fig. 15) appears similar to the terminal bar in *N. brasiliensis* (3) and the desmosome-like structure in *Heterodera rostochiensis* (11), however desmosomes were not associated with the junction.

The microvilli emanate from a continuous, thin membrane lining the lumen of the intestine. Their structure resembled that in the rat (5) and the parasitic nematode *N. brasiliensis* (3), with a core of finely packed filaments extending from the apex of each microvillus into the cytoplasm of the epithelium where they terminate as rootlets (Fig. 15). These rootlets appear to end in a clearly-defined terminal web, thicker than the microvillous border and about 16 mμ from it. The terminal web, which was also observed in *N. brasiliensis* (3), is discontinuous, indicating the surfaces have openings which allow the cytoplasm of the epithelial cells to contact the microvillous border and the rootlets of the microvilli (Fig. 14, 15).

UTERUS: The uterus epithelium contains ribosomes and mitochondria. The junctional complex of the epithelial cells nearest the lumen consists of the fusion of the cell membranes, thus forming a tight junction. Desmosomes are absent. The inner surfaces of the uterus epithelium are lined by interconnected folds, thus lending to this area a

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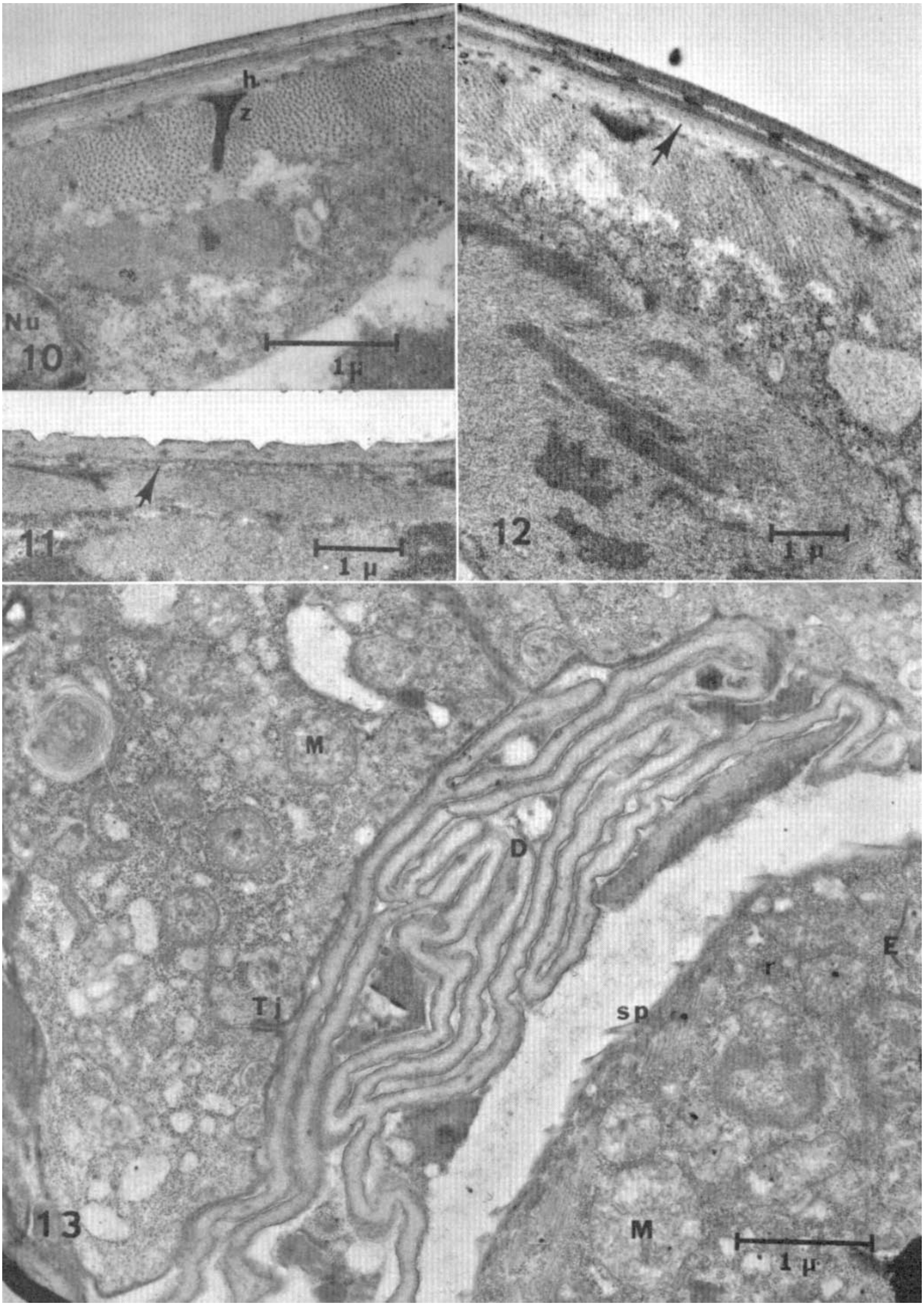
FIG. 10–13. *Caenorhabditis briggsae* fine structure. **Fig. 10.** Cross section at midbody showing the cell nucleus (Nu) within the non-contractile part of a somatic muscle cell. Also shown is a supporting structure (z) which attaches the muscle to the hypodermis (h). **Fig. 11.** Longitudinal section through the cuticle at midbody showing electron-dense zones (arrow) as small, rounded to oval areas. **Fig. 12.** Tangential section through the cuticle at midbody showing electron-dense zones (arrow) as flattened bands. **Fig. 13.** Cross section through the uterus. (E) Egg in an early stage of development showing the finely spined surface of the shell (sp), numerous mitochondria (M) and ribosomes (r). A tight junction (Tj) marks the fusion of two uterus epithelium cell membranes. (D) The daedaloid structure which lines the inner surface of the uterus.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daedaloid appearance (Fig. 13). This daedaloid structure was observed both in sections of the uterus containing eggs in the early stage of development and in those which contained well-formed larvae.

The uterus contains eggs with developing embryos, oocytes and occasionally larvae that hatched within the female. Light microscope observations of living females were carried out to confirm that some eggs hatch within the uterus. The development of the embryo within the egg was not followed, but it was observed that the outer covering of the egg bears spine-like protuberances (Fig. 13).

CUTICLE: Electron-dense zones were observed in some areas of the middle layer of the cuticle. These zones appear small and oval to rounded in longitudinal section (Fig. 11) whereas in tangential or cross section they appear as flattened bands (Fig. 12). The electron-dense zones are grouped in some areas of the cuticle and absent in other sections. It was also noted that in cross section (Fig. 10) or in a tangential cut (Fig. 12) the middle layer of the cuticle appears less dense and is thus clearly differentiated from the other layers, whereas in longitudinal section the light zone was not seen (Fig. 11).

There are three lateral ridges. Serial cross sections taken at five levels through the anterior 40% of the body showed that the ridges were absent at the level of the stoma. Slightly posterior to the stoma the ridges formed triangular protuberances 0.4μ in height. Posterior to the esophago-intestinal junction the lines appeared as bluntly or irregularly-rounded projections 0.6μ in height (Fig. 9).

In one molting larva a striped layer was observed (Fig. 5). This layer did not occur in adults or fourth-stage larvae, but it was not determined in which of the earlier stages the layer is found.

DISCUSSION

The ultrastructure of the anterior end of *C. briggsae* resembles that of *P. silusiae* (13), however *C. briggsae* contains several structures not previously described or observed in rhabditoid nematodes. First is the chambered walls of the stoma. Second is the description of the tight junctions of the membranes surrounding the esophageal muscle cells in the anterior region of the procorpus. The tight junctions can be seen in photographs of the same region of *P. silusiae* but were not described (13). Jamuar (3) observed similarly-placed tight junctions in *Nippostrongylus brasiliensis*, but called these rodlike extensions of the lumen. Lee (4) found the same structures in the third-stage larvae of *N. brasiliensis* and correctly interpreted their relation to the muscle cell membranes describing them as tight junctions. The occurrence of the zonula adherens in the nematode esophagus has not been previously reported.

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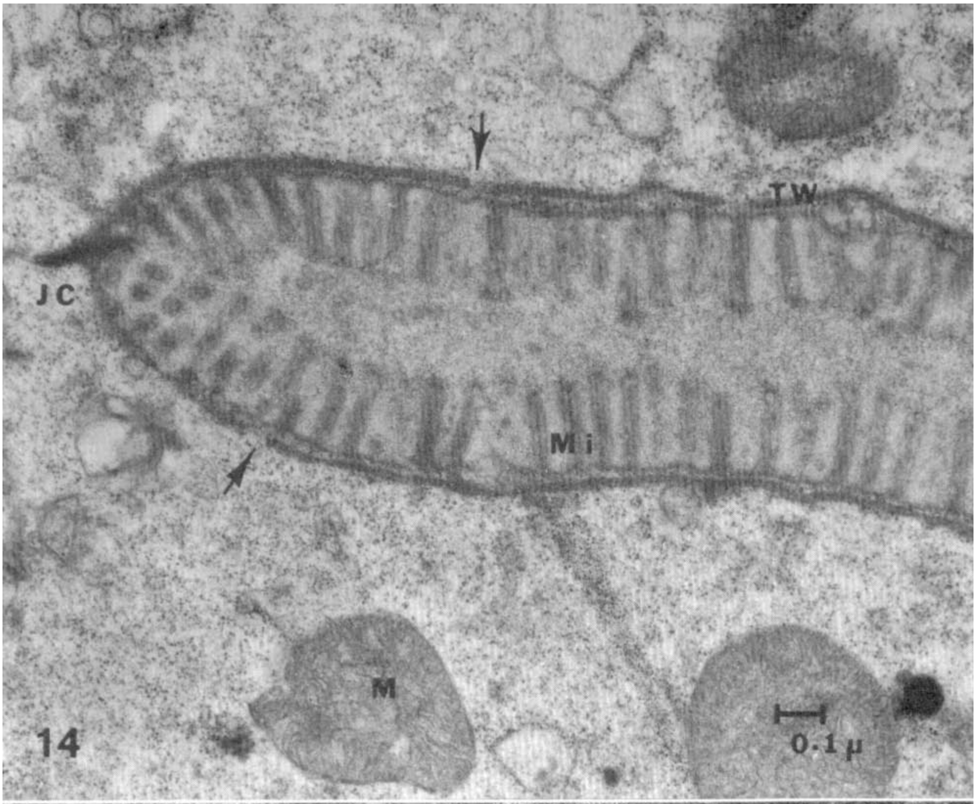
FIG. 14–15. *Caenorhabditis briggsae* fine strucure. **Fig. 14.** Cross section through the intestine showing a junctional complex (JC) between two epithelial cells, similar to but not the same as a tight junction. (M) Mitochondria of the epithelial cells. Other structures of the intestine are the microvillous border (Mi) and the terminal web (TW). Openings in the terminal web are indicated by arrows. **Fig. 15.** Cross section through the intestine. The junctional complex (JC) is seen as a grey layer sandwiched between two darker layers. A cross section through a microvillus (Mi) clearly shows the core and a longitudinal section through a microvillus shows the rootlet (R) extending to the terminal web (TW). The arrow indicates an opening in the terminal web. The cytoplasm of the epithelial cell is shown in contact with the microvillous border.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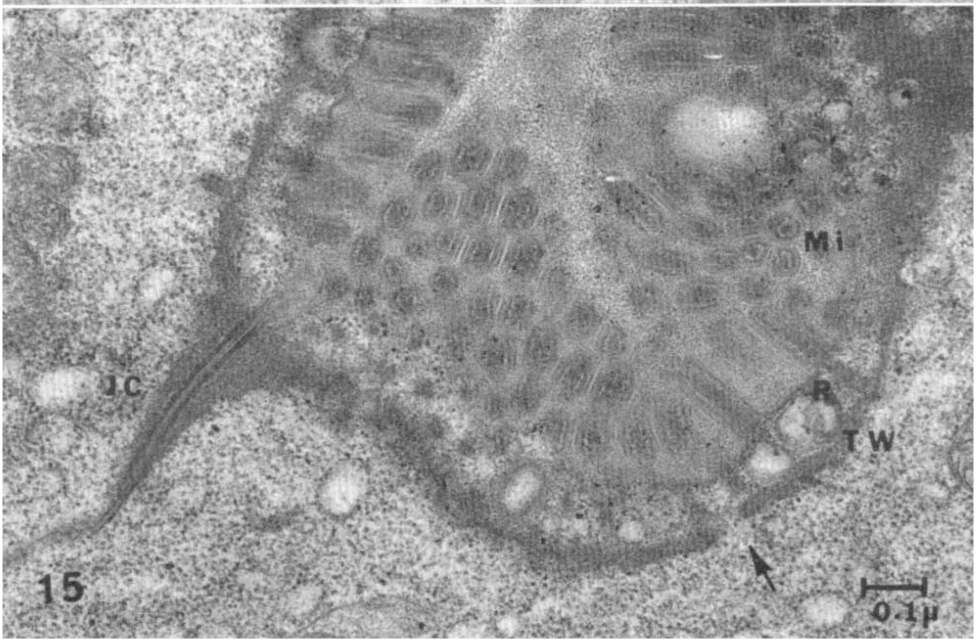
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The laminated structures in the lateral chords of *C. briggsae* at the level of the anterior end of the esophagus are similar to those reported for *P. silusiae* (13). Comparing longitudinal sections of *C. briggsae* through these structures, those through the amphids in *P. silusiae* (13), and *Haemonchus contortus* (8), we believe these laminated structures form the lower section of the amphidial pouch.

The triradiate form of the anterior part of the intestine was previously described from histological sections of *Periplectus labiosus* (9), but the present paper contains the first photograph of this structure. The morphology of the intestine and its epithelium is similar to that described for the animal parasite *N. brasiliensis* (3). Comparison of the junctional complex of *N. brasiliensis* (Fig. 7 in Citation 3) with that of *C. briggsae* (Fig. 15), indicates that they are alike. As in *N. brasiliensis* the filaments of the microvilli extend as rootlets and appear to merge with the terminal web. The openings in the terminal web were not previously described.

It is suggested that the daedaloid folds of the inner surface of the uterus are connected with the glandular activity. Coomans (1) postulated that the distal region of the uterus in *Xiphinema* contained gland cells which function in forming the egg shell. Probably the daedaloid structure forms part of the shell deposition structures in *C. briggsae*.

Wisse and Daems (11) observed electron-dense balls in the third cuticular layer of second stage *H. rostochiensis* larvae. Similar electron-dense areas are found in the cuticle of *C. briggsae*. In the present study the layers of the cuticle and the electron-dense areas each appeared quite different when viewed in a longitudinal section as compared to a cross section.

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