Morphology and Ultrastructure of the Intestine in a Plant-Parasitic Nematode, Tylenchorhynchus dubius

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Abstract: An unusual feature of the intestine in *Tylenchorhynchus dubius* is the presence, within the intestinal cytoplasm, of an extensive system of fibrillar bundles consisting of thin (14 nm diam) filaments and thick (70-90 nm diam), rod-like elements arranged in closely packed arrays. The larger of the fibrillar bundles, for which the term "intestinal fasciculi" is proposed, are evident in whole mounts and apparently correspond to the lateral or sinuous canals described in some other tylenchids. The nature and function of fasciculi are not known, but some possibilities are considered. Fasciculi were found in at least seven other species of *Tylenchorhynchus*. The intestinal cytoplasm also contains the usual subcellular organelles and large amounts of reserve materials in the form of particulate glycogen and three types of globules. The surface of the cells bordering the lumen is elaborated into numerous microvilli which have central filaments and often bear regular external projections. Although terminal bars delimit the apical margins between cells, the frequent lack of complete lateral boundaries and extensive length of the fasciculi indicate that the intestinal epithelium is a multinucleate mosaic or syncytium.

Although there are numerous descriptions of the ultrastructure of the intestine in nematodes (see 5, 9, 12 for references), only a few have dealt with the intestine of plant parasitic nematodes (21, 23, 39, 41). This paper describes the morphology and ultrastructure of the anterior intestine of the ectoparasitic phytophagous nematode *Tylenchorhynchus dubius* (Bütschli, 1873) Filipjev, 1936. The intestine of some plant parasitic nematodes, including *T. dubius*, contain structures that have been described previously as lateral canals or sinuous canals (33, 35, 36). The fine structure of these "canals" in *T. dubius* is described, and their possible nature and function are discussed.

MATERIALS AND METHODS

T. dubius, originally collected in The Netherlands, were kept on Kentucky bluegrass in a greenhouse. Active, mature specimens were selected from samples obtained with a Baermann funnel and prepared for electron microscopy as described in an earlier paper (7). Fine structural observations reported apply only to the anteriormost 50 μm of the intestine. Results from light microscopy were based on studies of whole mounts in glycerine, heat-relaxed specimens in water mounts and semi-thin sections (0.5-1 μm). The semi-thin sections were cut from material fixed and embedded as for electron microscopy, placed on gelatin-coated slides, stained with alkaline methylene blue (1% methylene blue in 1% borax) and mounted in immersion oil.

RESULTS

Light microscopy: In *T. dubius*, which has a ditylenchoid-type esophagus, the junction between the well-developed basal bulb of the esophagus and the intestine is clearly defined. Between the glandular portion of the basal bulb and the anterior portion of the intestine there is a small specialized region, the esophago-intestinal valve (Fig. 1). Within the esophago-intestinal valve, which is composed of a number of small cells, the cuticular lining of the esophagus terminates a short distance anterior to the commencement of the microvillar border lining the lumen of the intestine. From examination of semi-thin sections, it is evident that the intestinal lumen is small and somewhat variable in shape. Immediately posterior to the esophago-intestinal valve it is normally triradiate in cross-section but further posteriorly it becomes irregularly flattened dorso-ventrally (Fig. 2). In all specimens examined histologically the lumen was closed, although in living specimens it is often partially dilated anteriorly.

The cytoplasm of the epithelial cells forming the wall of the intestine contain

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numerous globules. The anteriormost 10-15 μm of the intestine contains relatively fewer globules than does the remainder. The difference is most evident in young or starved specimens. Normally in older specimens, the cells of this anteriormost region also become filled with globules. On the basis of their staining affinities with alkaline methylene blue, three kinds of globules designated as Types 1, 2 and 3 can be distinguished (Fig. 2). Type 1 is relatively small- to medium-sized (1-2 μm diam) and stains intensely dark blue. Type 2 is usually large to very large (3-7 μm diam), frequently larger than the cell nuclei, and stains dark greenish-blue. Types 1 and 2 are both probably proteinaceous, although Type 1 might be a 'pigment' granule. Type 3, although quite variable, is usually medium to large (1.5-3 μm diam) and stains pale bluish-green. In thin, lightly stained sections they often appear gray. Globules similar to Type 3 are present also in cells in the lateral chords and extracellularly in the pseudocoel. Extracellular globules (lipids?) associated with the intestine also have been reported in other tylenchids (30).

It is possible also to distinguish inclusions corresponding to the fibrillar bundles described in the next section. In semi-thin sections, the larger of these bundles (fasciculi) are evident as light blue regions in the cytoplasm (Fig. 2 and 3). When a large fasciculus is sectioned close to its long axis, as in Fig. 3, faintly appearing, fine longitudinal striae sometimes can be discerned. Examination of whole mounts of numerous specimens from our stock culture showed that fasciculi were very difficult to see in living nematodes, but were conspicuous in the same specimens after heat relaxation. The fasciculi remained clearly visible after fixation in 5% formalin and through processing to glycerine (Fig. 4) in the intestines of 67 of 70 specimens prepared as permanent glycerine mounts. Examination of similarly preserved specimens of *T. dubius* in the Canadian National Collection (Nematoda), which had been collected in association with different host plants from localities throughout Europe and North America, showed that fasciculi could be seen in the intestine of 50 of the 55 specimens examined.

Histologically, the remainder of the cytoplasm, which is largely restricted to the interstices between globules, is heterogeneous, but individual components (e.g. glycogen) or organelles could not be distinguished or

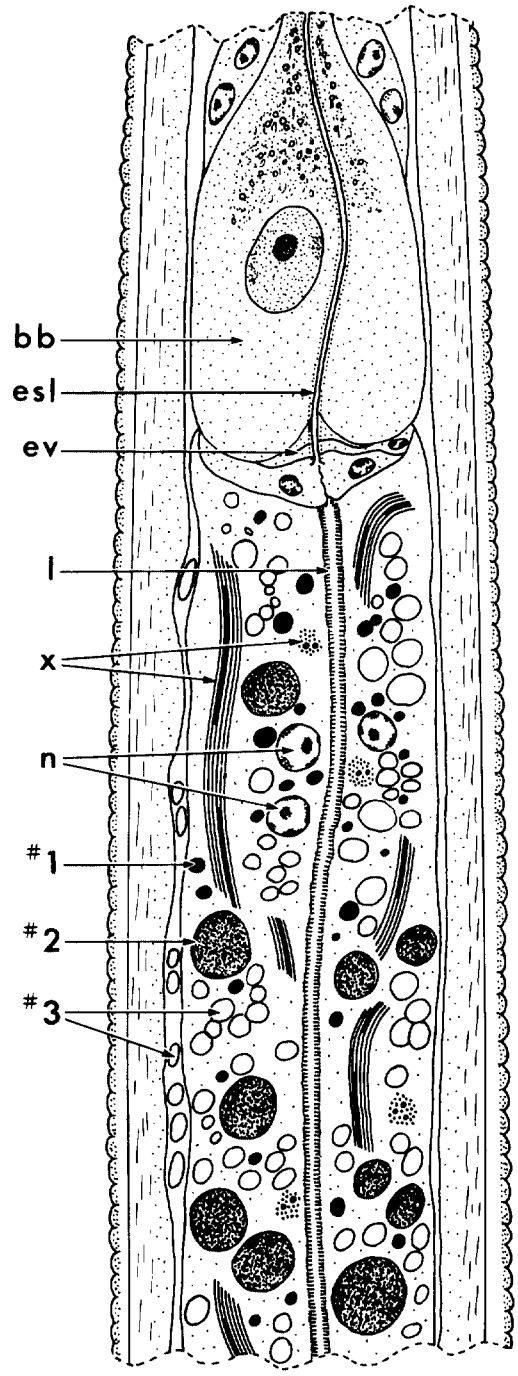


FIG. 1. Diagrammatic representation of a longitudinal section through the anterior part of the intestine and its junction with the esophagus. bb = Basal bulb of esophagus; esl = lumen of esophagus; ev = esophago-intestinal valve; l = lumen of intestine; x = fasciculi; n = nuclei of intestine; 1, 2, 3 = globules, Types 1, 2 and 3.

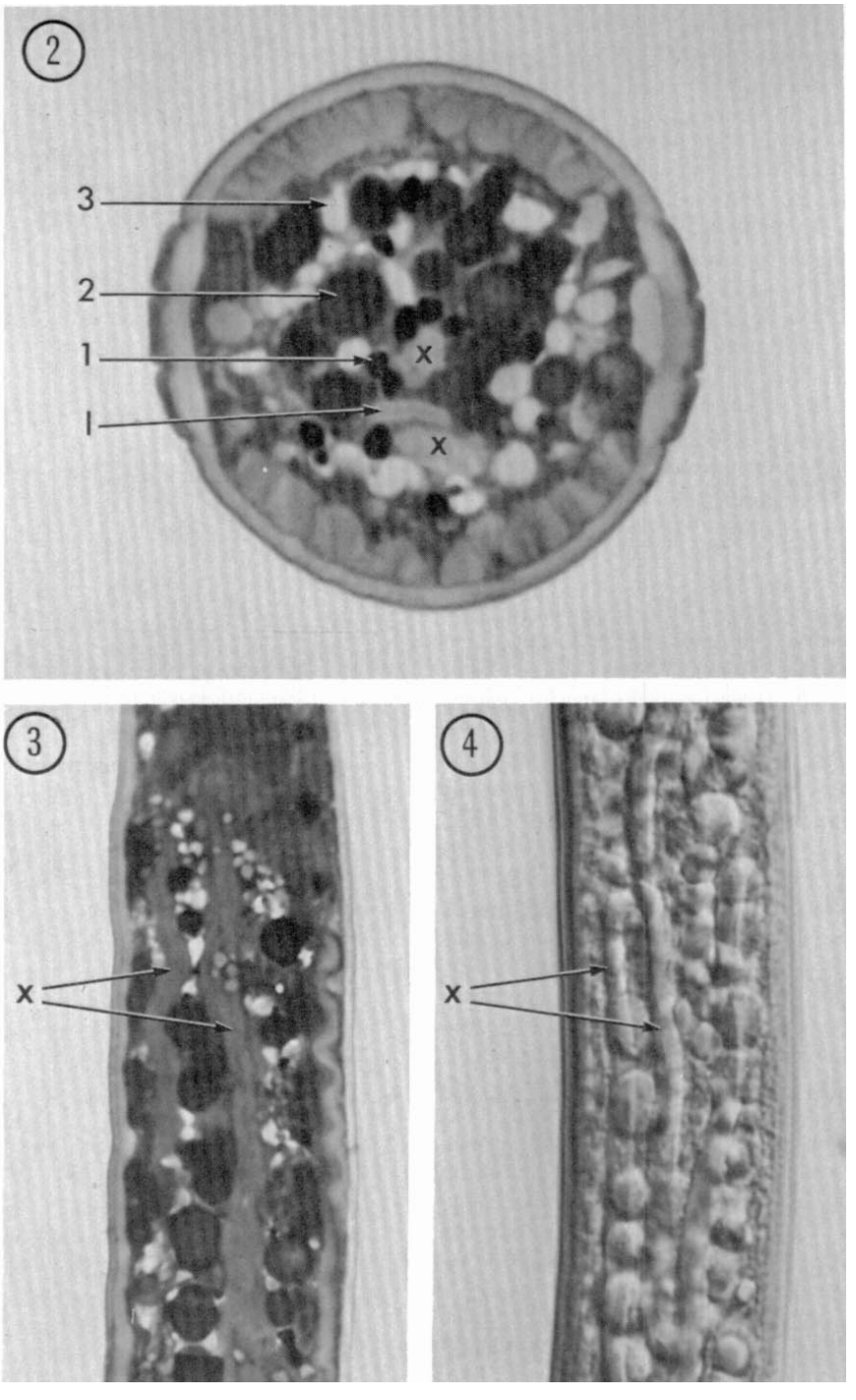


FIG. 2-4. **2.** Cross-section through anterior intestine about 30 μm posterior to the junction of intestine and esophagus. At this level the lumen (l) is flattened dorsoventrally. The three types of globules (1, 2 and 3) can be distinguished. Semi-thin section stained with methylene blue and photographed using a green (Kodak No. 58) filter (× 2600). **3.** Longitudinal section showing extensive longitudinal profiles of fasciculi (x) in anterior intestine (× 1200). **4.** Photomicrograph showing fasciculi (x) as they appear in glycerine whole mounts. Differential interference contrast (× 1200).

identified with any certainty with the light microscope. The intestinal nuclei are usually round and have a prominent nucleolus.

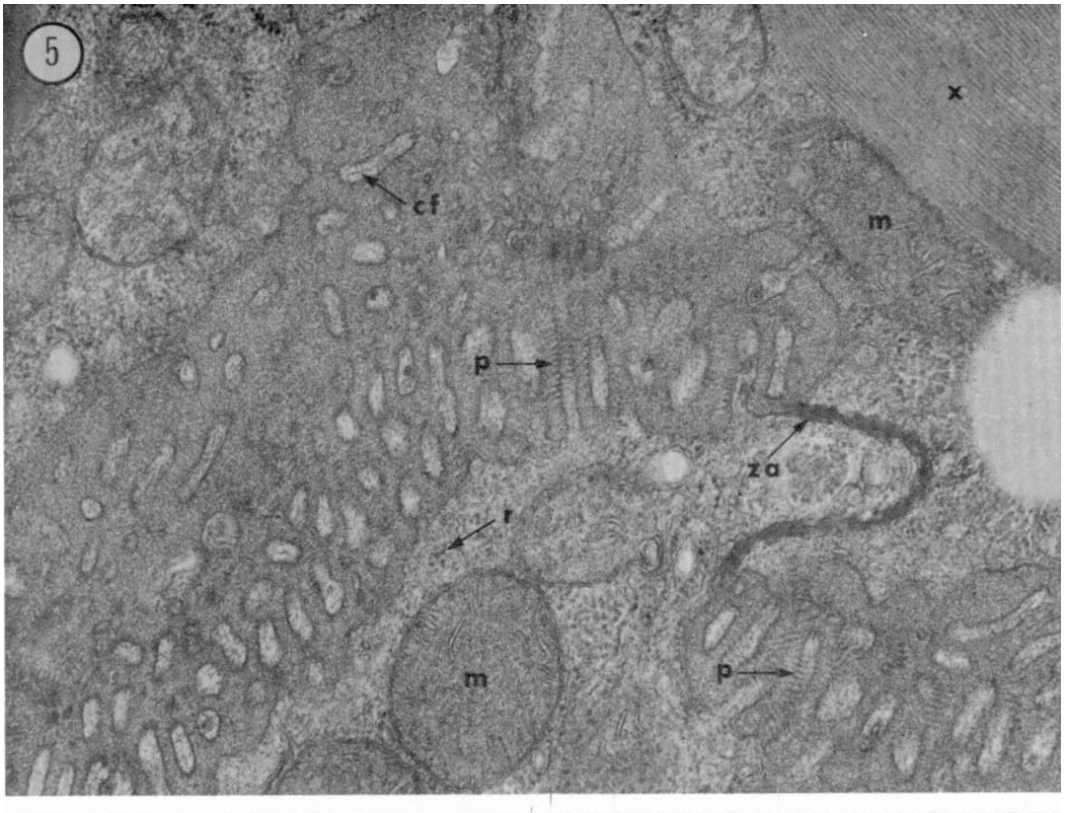
Electron microscopy: The microvillar border on the apical (luminal) surface of the intestinal cells is composed of small microvilli about 60 nm diam and 500-700 nm long. The microvilli have central filaments which extend a short distance into the submicrovillar cytoplasm, but there is no evidence of a submicrovillar layer or terminal web (Fig. 5-7). A conspicuous feature of many of the microvilli is a regular radiating array of small projections on the outer surface. These projections may be present over the whole surface of a microvillus, over only a part of the surface or they apparently may be absent. The lumen, including the spaces between the microvilli, is filled with a granular material which sometimes contains a few intermixed membranous vesicles.

The cytoplasm of the intestinal cells contains a moderate number of mitochondria which are concentrated in the apical portion of the cells close to the microvillar border (Fig. 5). Ribosomes are scattered throughout the cytoplasm, but only occasionally are they associated with membranes. Whorls of smooth membrane (or perhaps phospholipid) are present, often spatially associated with accumulations of glycogen (Fig. 6). The glycogen is usually in the form of large (alpha) particles, 150-300 nm diam, made up of smaller (beta) particles (16).

The three types of storage globules evident with the light microscope are also readily distinguishable with the electron microscope. All three types are composed of homogeneous material lacking any discernible regular substructure. Type 1 is moderately to very electron-dense. Often the center of these globules is empty in sections, but this is likely an artifact. Type 2 is less electron-dense, and always remains intact in sections. Type 3 is either electron-lucent or only slightly electron-dense. They are frequently irregular in outline and are often linked together, forming rather extensive conglomerates.

An unusual and hitherto undescribed fine structural feature of the intestine of *T. dubius* is the presence of two-component fibrillar arrays or bundles within the intestinal cytoplasm. The larger of the fibrillar bundles, for which we propose the term "intestinal fasciculi", are evident at the light microscope level in both suitably prepared sections and whole mounts. Fasciculi probably correspond to the lateral canals or sinuous canals described in the intestine of several other tylenchids (33, 35, 36). Fasciculi are composed of filaments and rod-like elements arranged in linear paracrystalline arrays (Fig. 6, 8 and 10). They were present in all 14 specimens examined at the ultrastructure level, varying in size from 0.3 to 2 μm diam or more. Fasciculi oriented in different directions are frequently present close together in sections (Fig. 6); others appear to join or diverge, and it is possible that the bundles may, at least in part, form an anastomosing network. Both the filaments and rod-like elements lie parallel to each other and to the longitudinal axis of the fasciculi. The filaments, about 14 nm diam, are closely and evenly spaced in a fairly regular hexagonal arrangement (Fig. 8 and 9). The individual filaments extend for considerable distances. Since no clear evidence of terminations within the bundles was observed, it is possible that they extend the entire length of the fasciculus. The substructure of the filaments evident in cross-section (Fig. 9) indicates that they might be composed of subunits about 5 nm diam. The rod-like elements, about 70-90 nm diam, are considerably less numerous than the filaments and apparently are distributed randomly within the bundles. The length of the individual rods also was not determined. However, some at least do not extend the full length of the bundle but terminate within it.

The cell nuclei contain a moderate amount of condensed chromatin, which is mostly clumped at the periphery, and a large granular nucleolus (Fig. 7). The lateral junctions between cells are characterized by junctional complexes (terminal bars) at the apical margin adjacent to the intestinal lumen (Fig. 5). Each junctional complex, which is of the zonula adhaerens type (18), is made up of an intercellular space of uniform width (about 18 nm) filled with a moderately dense material, the adjoining cell membranes and conspicuous bands of dense material in the subjacent cytoplasmic matrix. Although the lateral junctions between cells are very evident in the apical part of the cells because of the presence of zonulae adhaerentes, they are much less evident in the mid and basal portions, and only in a few instances could the lateral boundaries be followed along their entirety. Whenever two nuclei were observed close together in the same section, the intervening cytoplasm was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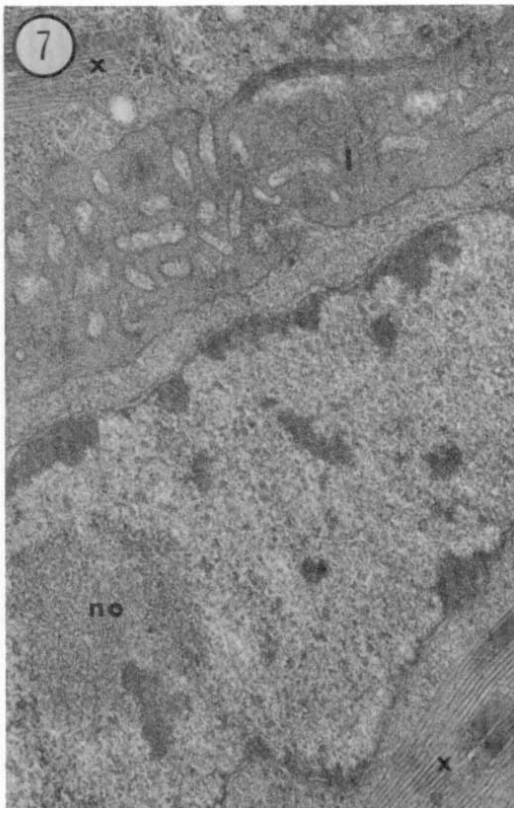
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examined carefully for evidence of lateral cell boundaries. None was observed, although where nuclei are close together, it could be expected that the lateral cell membranes, if present, would be more or less normal to the plane of the section and therefore likely to be evident. On the basal surface of the cells there is a very thin basal lamina that separates the intestinal epithelium from the pseudocoel.

DISCUSSION

The subcellular organization of the intestinal cells of *T. dubius* is in most respects similar to that described in other nematodes. Characteristically, the intestinal microvilli of nematodes have filaments in the central core and an external surface coat (glycocalyx?) often organized into projections or filaments. In the intestine of most free-living and animal parasitic forms, the core filaments extend into a submicrovillar layer or terminal web (e.g., 2, 11, 12, 17), although in some it is only poorly developed (10) or apparently absent (9, 26, 40). In the larvae of animal parasitic nematodes, however, a terminal web is absent (6, 12, 27), although one may be present in the adult stage of the same species (12, 24). In *T. dubius*, a terminal web is not present nor has one been described in the intestine of any other plant parasitic nematode.

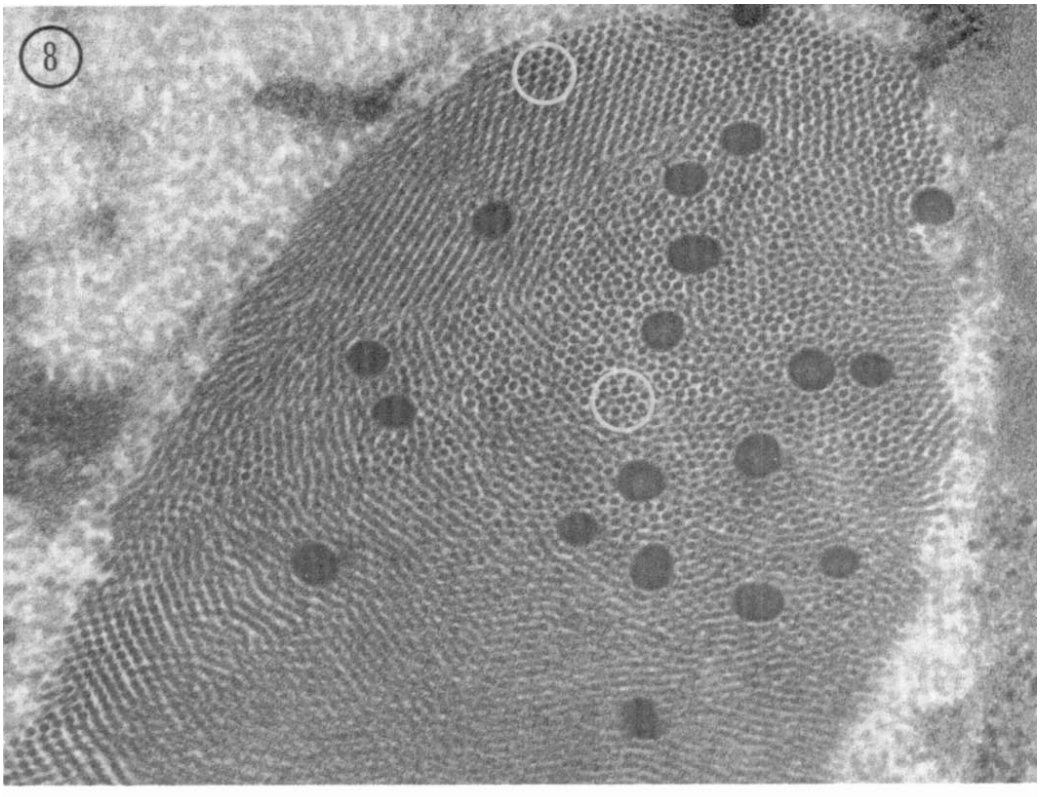
The unusual feature of the *T. dubius* intestine is the presence of extensive, two-component, fibrillar bundles or fasciculi. We do not know of any comparable structures in animal or plant tissues that would explain the function or nature of these structures. Possibilities that we have considered are that the fasciculi are paracrystalline storage inclusions; aggregations of virus particles or inclusions associated with a virus infection; contractile elements or strengthening elements.

Most large paracrystalline storage inclusions, thought to be proteinaceous, tend to be spherical in form and are more compactly structured than the fasciculi (25, 29). Because of the considerable length of individual fasciculi and the presence of a discrete second component, it seems unlikely that they are storage inclusions.

The possibility that the fasciculi are aggregations of virus particles or inclusion bodies associated with a virus infection seems unlikely. Relatively few filamentous viruses are known and these are mostly plant viruses (37), some with arthropod vectors (34). None of the described filamentous viruses or virus-like particles are as large as the 70-90 nm diam rod-like component of the fasciculi, but some do correspond in general appearance and diameter to the 14 nm diam filaments (3, 13). In view of the existence of filamentous viruses in plants and invertebrate vectors, one cannot exclude the possibility that the fasciculi are aggregations of filamentous virus particles or inclusions associated with a virus infection. However, since fasciculi were observed in nearly all specimens of *T. dubius* examined, the relationship would not seem to be parasitic but rather that the fasciculi form a part of the normal endowment of the intestinal cells.

The distribution of the fasciculi for considerable distances throughout the intestine, perhaps forming an interconnected network, would be most consistent with a contractile or strenghthening (supportive) role. The possibility that there might be contractile elements in the intestinal epithelium of nematodes has been advanced by several authors. Wisse and Daems (39) reported the presence of bundles of filaments in the intestine of second-stage larvae of *Heterodera rostochiensis* and suggested that the bundles, which consisted of one type of filament only, might be contractile. Peristaltic-like movements of the intestine are known to occur in nematodes. In some larger nematodes there are somato-intestinal muscles extending from the body wall to the gut (5, 8). In others such as

FIG. 5-7. **5.** Electron micrograph showing apical portions of several intestinal cells. Mitochondria (m), ribosomes (r) and part of a fasciculus (x) are present within the cytoplasm. The microvilli bordering the lumen have a filamentous central core (cf) and often radiating projections (p) on the outer surface. A conspicuous terminal bar (za) joins the apical margin of two adjacent cells (× 37,000). **6.** Portion of an intestinal cell containing parts of two fasciculi (x) which although oriented in slightly different directions come very close together. Profiles such as this suggest that the fasciculi might form an anastomosing network. In one of the fasciculi two of the thick, rod-like elements appear to terminate abruptly (arrows). s = Smooth membrane whorl; * = glycogen; l = lumen of intestine (× 30,000). **7.** Nucleus of intestinal cell with large granular nucleolus (no) and condensed chromatin which is located mainly at the periphery. x = Fasciculi; l = lumen of intestine (× 31,000).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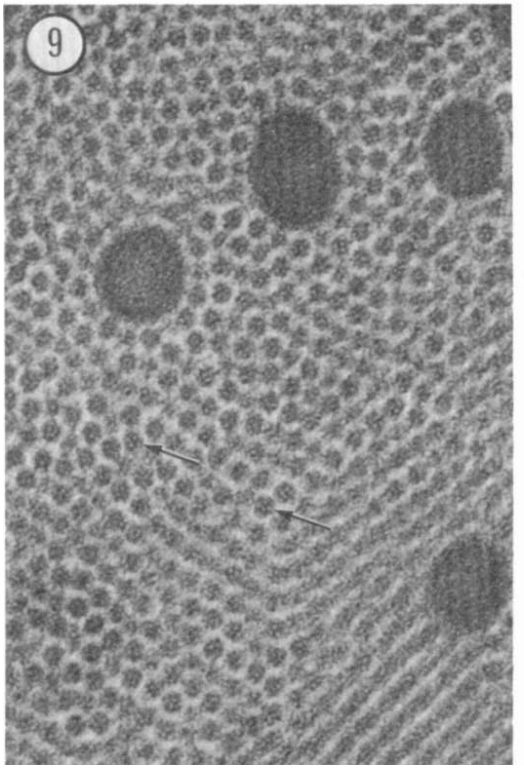
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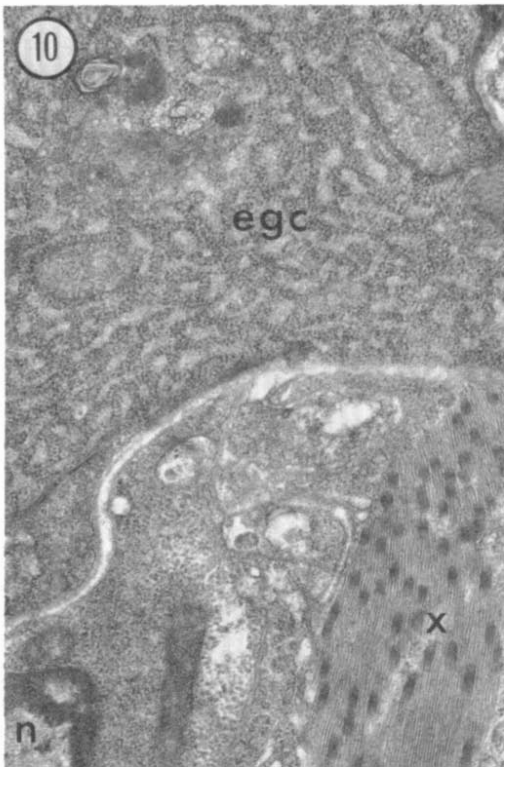
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the oxyurid, *Aspiculuris tetraptera*, the outer surface of the intestine is covered with a network of muscle fibers (28). Intestinal muscles have not been observed in any plant parasitic nematode; however, movements of the intestine, especially the anterior part, have been reported in several species (1, 20, 31). The role of such movements in terms of intestinal function is not clear (15).

The possibility that the fasciculi are a kind of contractile apparatus would be most plausible if the dimensions of the filaments and rods approximate those of other structures with known contractile capabilities. In striated muscle the thin (actin) myofilaments are 5-7 nm diam and the thick (myosin) myofilaments vary from 16-20 nm diam (19). In the obliquely striated muscles of nematodes these dimensions are slightly larger; 8 nm and 22 nm, respectively (22). However, some muscles specialized for strong prolonged tonus, such as the paramyosin muscles present in some molluscs, annelids and echinoderms, have thick filaments 33-90 nm diam (4, 42). These various dimensions compare with 14 nm for the filaments and 70-90 nm for the rods in the fasciculi of *T. dubius*. Thus, in diameter, the filaments considerably exceed the diameter of actin myofilaments although they are of a similar size to myosin myofilaments. The thick, rod-like elements considerably exceed the diameter of myosin myofilaments but are similar in size to paramyosin filaments. However, unlike paramyosin filaments which are somewhat variable in cross-sectional size and profile, the thick elements in the fasciculi are quite uniform and regular. The ultrastructural organization of smooth muscle is still not fully understood, and although both thin (6.5 nm) and thick (10-20 nm) filaments have been reported, ordered arrays are uncommon (32). Thus, it appears that the two-component fasciculi in *T. dubius* intestinal cells are not related to any known two-component contractile system, although this does not exclude the possibility that the fasciculi are an unusual kind of contractile system. There is evidence (38) that 5-nm-diam microfilaments found in many cells are contractile, and it is perhaps significant that the substructure evident in transverse sections of the filament component of the fasciculi is also about 5 nm diam.

If the intestine lacks an intrinsic contractile apparatus, any movements must then be brought about indirectly. Even if this is the case, the intestine, in some nematodes at least, is nevertheless subjected to repeated distortions (1, 14, 20, 31) and it is possible that strengthening elements have been evolved to enable the intestine to better withstand the stresses involved. The fasciculi might conceivably form a network of such strengthening elements.

If the role of the fasciculi is either contractile or supportive, it would be most effective if the system was functionally continuous between cells. This could be accomplished either by specialized intercellular attachments (which were not observed) between fasciculi in adjacent cells or by the fasciculi extending freely between cells. Although complete lateral boundaries between adjacent cells were observed in a few instances, usually they appeared to be incomplete with only the apical portion (terminal bar) in evidence. In the animal parasitic nematodes *Ancylostoma caninum* (2) and *Strongyloides myopotomi* (12), parts of the intestine are also reported to be syncytial with terminal bars, but in *Cyathostoma lari* (11) the intestine is syncytial with no terminal bars. At the light microscope level, individual cells are seldom observed in adult Tylenchoidea. In *Ditylenchus dipsaci*, the intestinal cells are apparently quadrinucleate (8). We think that the intestine of *T. dubius* is functionally a syncytium or multinucleate mosaic. The presence of terminal bars indicates that cytokinesis accompanies nuclear division during formation of the adult intestine and that subsequently the unspecialized parts of the lateral cell junctions disappear, allowing the fasciculi to ramify throughout large portions of the intestine unimpeded by cell boundaries.

FIG. 8-10. **8.** Cross-section through a fasciculus. The small (14 nm) filaments are arranged in a basic hexagonal pattern (circles) which, however, tends to be disrupted in the vicinity of the thick, rod-like elements. The filaments also are arranged in groups or domains which differ slightly in orientation (× 84,000). **9.** Part of Fig. 8 at higher magnification showing evidence of substructure in filaments (× 180,000). **10.** Part of intestine near junction with esophagus. egc = Esophageal gland cell; n = nucleus of a cell associated with esophago-intestinal valve; x = fasciculus in intestinal cell (× 18,000).

Fasciculi (lateral or sinuous canals) have been described previously in some other tylenchids, including *Macrotrophurus arbusticola, Belonolaimus gracilis, Helicotylenchus canalis* and *Tylenchorhynchus canalis* (33, 35, 36). These few reports suggest that fasciculi are unique to a few species only and, consequently, might be of specific taxonomic value. We have observed, however, that fasciculi occur in several other species of the genus *Tylenchorhynchus*, including *T. bursifer, T. claytoni, T. maximus, T. martini, T. nudus, T. capitatus* and *T. parvus* in addition to *T. dubius*. The diagnostic significance of fasciculi for species within this genus, at least, is doubtful.

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