Phil. Trans. R. Soc. Lond. B **306**, 1–18 (1984)
Printed in Great Britain

THE OCCURRENCE OF SUBMICROVILLAR ENDOTUBE (MODIFIED TERMINAL WEB) AND ASSOCIATED CYTOSKELETAL STRUCTURES IN THE INTESTINAL EPITHELIA OF NEMATODES

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(*Communicated by E. N. Willmer, F.R.S. – Received* 18 *April* 1983)

[Plates 1–9]

CONTENTS

	PAGE
Introduction	2
Materials and methods	3
Method of preparation	3
Light microscopy	3
Electron microscopy	4
Classification	4
Results	4
Order Strongylida	4
Haemonchus contortus	4
Ostertagia spp.	5
Nematodirus spp.	5
Trichostrongylus colubriformis	5
Nippostrongylus brasiliensis	5
Dictyocaulus viviparus	6
Muellerius capillaris	6
Ancylostoma caninum	6
Syngamus trachea	7
Trichonema sp.	7
Strongylus edentatus	8
Oesophagostomum columbianum	9
Metastrongylus sp.	9
Order Rhabditida	10
Strongyloides papillosus	10
Pelodera strongyloides	10
Order Ascarida	10
Syphacia obvelata	10
Toxocara canis	10

Vol. 306. B 1125 [Published 10 May 1984

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Ascaris suum 11
Heterakis gallinae 11
Order Spirurida 11
Litomosoides carinii 11
Order Trichinellida 11
Trichuris ovis 11
Discussion 11
References 17
List of abbreviations used in figures 18

The intestines of 22 genera of nematodes from five different orders were examined for the presence of an endotube, the submicrovillar entity previously described for *Haemonchus contortus*, a member of the order Strongylida. The endotube can be obtained by blunt dissection as a complex with the microvilli essentially free of the rest of the cytoplasm.

Representatives of all three suborders and eight families of the order Strongylida possessed an endotube but of the representatives of the four other parasitic orders and the one free-living group examined only one genus, *Strongyloides* (Rhabditida) possessed this structure. The thickness of the endotube ranged from about 80 nm in *Metastrongylus* up to 6 μm in *Strongylus*. In all samples the filamentous cores of the microvilli, whether formed into an axial bundle (as usual) or dispersed in a net (as in *Dictyocaulus*), which extended 0.1–0.5 μm below the base of the microvilli terminated in the luminal surface of the endotube. The basal side of the endotube was usually associated with a layer of microfibrils. The depth and distribution of the microfibrillar layer determined the extent to which the endotube–brush-border complexes could be dissected free from other cytoplasmic components.

There was electron microscopic evidence for an endotube-like entity not associated with the microvilli in the intestine of *Syphacia* (Ascarida). A survey of published electron micrographs of nematode intestines indicated that the true submicrovillar endotube occurred only in members of the order Strongylida and the genus *Strongyloides* (Rhabditida) in which the structure here described as an endotube has previously been described as terminal web.

Introduction

The syncytial intestine of adult *Haemonchus contortus* contains a submicrovillar structure seen in stained, thin sections as an electron dense, homogeneous layer some 0.2 μm thick containing fenestrations about 0.2 μm in diameter (Munn 1977). The filamentous axial cores of the microvilli connect to the layer which, together with the microvilli, can be separated from the rest of the intestinal epithelium by blunt dissection to yield a tubular complex. The tube extends the whole length of the intestine. Because of its tubular form and because it is intracellular, the submicrovillar entity has been named an endotube (Munn 1981). The intestine of another strongylid, *Ancylostoma caninum*, although partially cellular also contains an endotube with which is associated an extensive microfibrillar system (Munn & Greenwood 1983). The intestines of a range of other nematodes have now been examined to see whether or not they possess

endotubes. The ability to isolate the endotube in tubular form (as a complex with the microvilli) has been taken as the principal criterion for its existence in a particular nematode. A survey of published electron micrographs has been carried out to look for evidence for the occurrence of the endotube in nematodes not presently available for direct examination.

Materials and methods

Method of preparation

All dissections were carried out using a Zeiss dissecting microscope with lateral illumination from light guides against a matt black background. The nematodes were placed in phosphate-buffered saline solution (pH 7.2) containing 8 g l^{-1} NaCl, 0.2 g l^{-1} KCl, 1.15 g l^{-1} $Na_2HPO_4 . 2H_2O$, 0.2 g l^{-1} KH_2PO_4 in a glass Petri dish (10 cm in diameter). The nematodes were handled and held at the anterior tip with no. 4 watchmaker's tweezers. For the smaller nematodes an incision was made in the cuticle near to the posterior end with a pointed scalpel blade (no. 11, Swann-Morton) or the posterior tip of the nematode was cut off. The intestine was then extruded by pulling the worm past the back of the scalpel blade held so that there was only a narrow gap between it and the surface of the Petri dish. Usually this procedure also extruded the reproductive organs and in some cases the body wall muscles. For larger nematodes, those with very tough cuticle and those in which the organs were very tightly packed within the cuticle it was necessary to make several incisions round the cuticle and remove it in sections so as to release the intestine without risk of damage.

The intestines were subjected to the procedure described by Munn & Greenwood (1983) for the blunt dissection of the endotube–brush-border complex. Each intestine was held at the anterior tip (preferably the pharynx which usually remained attached to the intestine) and again pulled through the narrow gap formed by holding the back of the scalpel blade close to the surface of the Petri dish. Success in this procedure depended on experience in controlling the gap between the blade and the dish, the tension applied to the intestine and the type of nematode. For two of the nematodes (*Syngamus*, *Oesophagostomum*) for which there was other evidence for the presence of an endotube this could not be freed of cytoplasm by dissection immediately the nematodes were obtained from their hosts. For these worms it was necessary to keep them at 4 °C at least overnight before the dissection was successful (see Results).

At least ten nematodes of each genus (except for *Strongylus* only five of which were available) were examined. Both males and females were used but most information was on the larger females. There was no evidence for differences in the fine structure of the intestines relative to the sex of the worms. Samples for sectioning were taken from the various regions along the length of each intestine. At least three of the samples were chosen at random for sectioning. No systematic examination for variation in structure at the electron microscope level along the length of the intestines was attempted except where phase contrast light microscopy of the whole intestine or endotube has indicated differences. (In other studies on *Haemonchus* to be reported elsewhere, the endotube and associated structures have the same appearance and organization for some 98% of the length of the intestine; small differences occur at the most anterior end.)

Light microscopy

Intestines and pieces dissected from them, in phosphate-buffered saline solution were examined by bright field transmitted light with a Wild (Heerbrug) microscope fitted with

fluotar, phase contrast objectives, a Type III transmitted light base and a Mka 5 automatic photographic system. XPI film (Ilford) was used at ASA 640.

Electron microscopy

Intact or fragmented nematodes were fixed with a solution of 20 g l^{-1} glutaraldehyde in 80 mM phosphate buffer pH 7.4 containing 0.18 M sucrose and 1.4 mM calcium chloride, for 1–2 h at room temperature and then overnight at 4 °C. The samples were washed with the phosphate buffered medium and then post-fixed for 1 h with 10 g l^{-1} osmium tetroxide solution at room temperature. The samples were dehydrated with ethanol, transferred to 1,2-epoxypropane and finally embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate.

Classification

The scheme of classification followed here is that proposed by Chitwood (1969). Other schemes differ in detail and in the status given to the larger groupings but these do not alter the general conclusions reached here about the distribution of nematodes with endotubes.

Results

Intestines could be dissected from all but two of the 22 genera of nematodes examined. Each intestine was examined whole by light microscopy and then tested to see if an endotube complex could be dissected from it. All the preparations were examined by phase-contrast light microscopy and, for nine of the genera, by electron microscopy after sectioning. The intestines (of four of the genera) and the whole nematodes or pieces thereof (of 17 of the genera) were also examined by electron microscopy.

Order Strongylida

Haemonchus contortus

The structure of the syncytial intestine and endotube–brush-border complex of *H. contortus* is described in detail elsewhere (Munn 1977; Munn 1981; Munn & Greenwood 1983) but a brief description is given here for ease of comparison with the results obtained with other nematodes. The luminal surface is clearly visible within the intact intestine (figure 1*a*, Plate 1) and in partially dissected preparations is continuous with the endotube (figure 1*b*). The isolated endotube usually appears 'clean', that is, free of particulate material (such as mitochondria) derived from the bulk of the cytoplasm. The luminal surface of the endotube is coated with microvilli which are most clearly seen at the cut ends of the endotube (figure 1*c*,*d*). The basal surface of the endotube commonly has a pitted appearance.

In electron micrographs of sections the endotube is an electron-dense layer about 0.2 μm thick, irregularly, but frequently, pierced by fenestrations 0.15–0.25 μm in diameter (figure 2). The axial cores of the microvilli are about 0.03 μm in diameter and extend about 0.15 μm into the cell to end at the luminal surface of the endotube. Microtubules are occasionally present, sometimes passing through the fenestrations in the endotube, and loose arrays of microfilaments about 11 nm in diameter occur on its basal side.

Ostertagia spp.

The intestine of *Ostertagia* has an overall diameter of about 100 μm. It is oligocytous (Chitwood & Chitwood 1974) with two cells in any circumference. The length of the cells has not been established. The surface of the lumen is clearly visible within the intact intestine; the luminal diameter varies between about 30 μm and 50 μm (figure 3*a*).

An endotube can be readily dissected but only short lengths can be obtained free of basal cytoplasm (figure 3*b*). In the electron microscope the tubular form of the endotube is clear (figure 4, plate 2). It is 0.1 μm thick, with sharply defined fenestrations 0.1–0.2 μm in diameter. The fenestrations occur less frequently than in *Haemonchus* endotube. The microvilli, the luminal surfaces of which are coated with contortin-like material (Munn 1977), are up to 1 μm long. Their axial cores extend about 0.19 μm into the cell to connect to the luminal surface of the endotube.

The endotube material extends basally to cover the cytoplasmic side of the terminal bar. This is most clearly seen in sections of the intact intestine (figure 5) where, unlike the isolated endotube preparation (figure 4) the lateral membranes are not collapsed. There is a loose network of microfibrils on the basal side of the endotube.

Nematodirus spp.

The intestine of *Nematodirus* is oligocytous (Lee & Martin 1980). It is some 50–80 μm in overall diameter (figure 6*a*,*b*). The luminal surface is visible within the intact intestine and is continuous with the endotube which may be exposed by blunt dissection (figure 6*c*). The luminal diameter varies from about 5 μm anteriorly to about 30 μm posteriorly. Intestines of this nematode and *Trichostrongylus* were examined only by phase-contrast microscopy.

Trichostrongylus colubriformis

As with *Nematodirus* the lumen was comparatively narrow (figure 7*a*). Its surface was continuous with the endotube which could be obtained free of basal cytoplasmic material only in relatively short lengths (figure 7*b*). The overall diameter of the endotube was about 10 μm and that of the intestine was 40 μm.

Nippostrongylus brasiliensis

Some faint outlines of cells could be discerned in the intact intestine dissected from *N. brasiliensis*. The luminal lining was clear and an endotube could be obtained by blunt dissection (figure 8). At its free end there was a fringe of short microvilli (figure 8 inset).

Electron micrographs of sections of the intestine showed that the endotube is normally some 0.14 μm thick (figure 9) although in places it was only 0.06 μm thick. Fenestrations about 0.16 μm wide were present. The microvilli were about 0.7 μm long and 0.1 μm wide with a filamentous core about 5 nm in diameter extending 4 nm (figure 9) to 8 nm into the cytoplasm to the luminal surface of the endotube. The endotube material extended along the terminal bar region of the lateral plasma membrane. Microfilaments were not seen on the basal side of the endotube.

Dictyocaulus viviparus

The intestines of the specimens examined were 5–6 cm long. They were multinucleate syncytia throughout their length. On dissection they were found to have a prominent basement membrane and in some places a thin, cellular layer outside this. Compared to the diameter of the lumen, 0.1–0.25 mm, the intestinal cytoplasm was often quite thin. Endotube–brush-borders could be dissected from the rest of the cytoplasm and pieces up to 2.5 cm long were obtained. The cross-sectional shape of the lumen is irregular (figure 10*a*, plate 3) and the endotube–brush-border complex obtained therefrom retained this irregular outline in an exaggerated form (figure 10*b*,*c*). It was not possible to dissect out a complete endotube free of cytoplasm because breaks occurred in already exposed pieces when we were trying to increase the exposed length. There is no doubt, however, that *Dictyocaulus* has an endotube continuous throughout the length of the intestine. By light microscopy the endotube preparations did not appear 'clean' (figure 10*b*,*c*). Electron microscopy showed that the preparations retained what appeared to be fat globules, small electron-dense globules, mitochondria, coated vesicles and some small pieces of membrane within a network of microfilaments (figure 11*a*) apparently connected to the endotube, via basally directed prolongations as seen in oblique sections of the luminal surface of the intestine (figure 11*b*). The isolated endotube (figure 11*a*) resembled that seen in sections of intact intestine but it was closely applied to the plasma membrane at the bases of the microvilli as though the network of filaments within the microvilli which extended about 0.3 μm into the cytoplasm before joining to the surface of the endotube in the intestine had shortened. The outline of the endotube was very irregular and its thickness varied from about 0.15 μm to about 0.4 μm. The microvilli were about 0.9 μm long.

Muellerius capillaris

It was not possible to dissect *Muellerius* but electron micrographs of sections of the nematode indicated the presence of a structure which could be equated with an endotube (figure 12*a*). It formed a very thin (about 16 nm), but continuous layer immediately below the luminal plasma membrane. The axial filaments of the short, infrequent microvilli were connected to the layer (figure 12*b*). It resembled the endotube of *Metastrongylus*.

Ancylostoma caninum

The very large polygonal cells at the anterior end of intestines isolated from *A. caninum* are clearly visible at low magnification. A faint polygonal pattern may be discerned in the mid-region of the intestine while posteriorly no pattern can be seen. The luminal surface is clearly visible throughout the length of the intestine.

An endotube–brush-border complex can be separated from the intestine by blunt dissection (Munn & Greenwood 1983). The outline of the cells at the anterior end is visible on the isolated endotube (figure 13*a*, plate 4), due to retention of the terminal bar (Munn & Greenwood 1983). A reticulum consisting primarily of an array of approximately parallel fibrils with less prominent cross connections is present at the basal surface of the endotube (figure 13*d*).

The microvilli are about 6 μm long (figure 13*b*,*c*). As shown by electron microscopy they have a prominent axial core about 0.05 μm in diameter which extends 0.12–0.16 μm into the cell to end in the endotube which forms an electron-dense, amorphous layer about 0.5 μm thick. This is perforated irregularly by fenestrations about 0.1 μm in diameter. The isolated

endotube–brush-border complex has a layer of loosely intertwined microfilaments about 10 nm diameter at the basal surface which retains numbers of mitochondria and occasional small, electron-dense globules.

In the region of the terminal bar the endotube material is extended so that it encloses the cytoplasmic surface of the plasma membrane. The endotube does not appear to join directly to the plasma membrane but to a particulate layer coating its surface (Munn & Greenwood 1983).

Syngamus trachea

Although light and electron microscopical examination of sections of the intestine of *Syngamus* indicated the presence of a substantial endotube (figures 15*a*, *b* and 16, plate 5 and see Borgers *et al.* 1975) it was not possible to isolate this entity from the nematodes immediately after they were obtained from the host. Blunt dissection of intestines from dead worms kept overnight at 4 °C in phosphate-buffered saline exposed short lengths of endotube, but it was necessary to keep the worms at 4 °C for six days before it was possible to obtain complete endotubes from the intestines. Figure 15*e* shows a phase-contrast light micrograph of the cut end of such an endotube. Although microvilli are clearly visible in sections of intact intestine (figure 15*a*, *b*) and at the cut end of the short lengths of endotube obtained from freshly dead nematodes, they are difficult to detect in the aged preparations (figure 15*c*, *d*, *e*). Similarly, a network associated with the basal surface of the endotube visible in endotube preparations from freshly dead worms (figure 15*f*) is not readily seen in the preparations from aged worms. This network may be equated with the long strands of material on the basal side of the endotube seen in electron micrographs (figure 16) or may be basally directed prolongations of the endotube itself. In general, the microvilli of the isolated endotube–brush-border complex are considerably collapsed (figure 17) compared to those of the intact intestine (figure 16) with loss of the 0.045 μm diameter axial cores which in the intestine project about 0.4 μm into the cytoplasm to join with the luminal face of the endotube. These degenerative changes resemble some of the changes induced experimentally in *Haemonchus* endotube–brush-border complexes by incubation with proteases (Munn (1981) and unpublished observations) and it is inferred that the ageing process which aids the release of the endotube involves proteolytic cleavage of bonds between the endotube-linked fibrils and other cytoskeletal elements in the intestinal cytoplasm. The overall thickness of the endotube in the intact intestine is about 5 μm; its basal surface is very irregular and it is much perforated with interconnecting fenestrations (figure 16). In sections of the aged preparation the endotube appears collapsed (figure 17) with occlusion of the fenestrations and compaction of the very fine fibrillar components when compared to the endotube *in situ*.

Trichonema sp.

The intestine of *Trichonema* is some 6 mm long in the male and 9–10 mm in the female. It is cellular with some 46 cells. These are narrow and semi-annular at the anterior end of the intestine but become broad and polygonal in shape towards the posterior. Two rows of nuclei are visible with the dissecting microscope at the anterior. The surface of the lumen visible within the intestine is continuous with the exposed endotube (figure 18*a*).

An endotube is present throughout the length of the intestine. It can be isolated completely free of cell components other than the microvilli. These are not visible in the intact preparation

with the light microscope (figure 18*a*), but can be seen, as a dark band (figure 18*b*) in transverse sections. Electron microscopy of sectional microvilli reveals them to be 0.8–1 μm long, about 0.09 μm in diameter and at 0.13 μm centre-to-centre, closely packed (figure 19). Each microvillus has a prominent axial core some 0.5 μm in overall diameter which extends about 0.2 μm into the cell and terminates in the proximal surface of the endotube.

The endotube is between 1.5 and 2.4 μm thick. In any one place its surfaces are more or less parallel and fairly smooth. They are interrupted only by the fenestrations. These are of variable diameter in the range from about 0.05 to 0.35 μm. The fenestrations are branched and irregular in their direction so that only rarely is a connection between the basal and the distal surface of the endotube seen in a thin section. Openings of the fenestrations at the proximal surface of the endotube are far more common than at the distal surface and it is inferred that the branched forms seen (figure 19) represent connections between single openings on the one side with multiple openings on the other side. The endotube appears to be composed of densely packed fine fibrils and there are microfibrils (some 13 nm in diameter) extending into the cytoplasm from the distal surface (figure 20).

Strongylus edentatus

The intestine is some 2.5–3.5 cm long and composed of very large, polygonal multinucleate cells. It was possible to obtain an endotube from the posterior end of the intestine. More anteriorly the tubular form did not survive blunt dissection and the intestine broke into fragments with prolongations with an irridescent blue colour presumed by analogy with the similar colour of the *Haemonchus* endotube–brush-border complex to be an interference colour owing to the presence of the long microvilli visible by phase contrast microscopy. The long microvilli were also visible on the endotube obtained from the posterior end of the intestine (figure 21*a*, plate 6); it was noted that many were becoming detached and some were ballooning out. When seen free of cytoplasmic organelles the surface had a dotted appearance with, in places, a reticulum of fibrils (figure 21*b*). In electron micrographs the endotube has an overall thickness of 4–6 μm. It is perforated by slightly twisting fenestrations about 0.3–0.5 μm in diameter (figure 22). The edges of the fenestrations are marked by an electron-dense layer some 16 nm thick but the endotube apparently is composed of closely packed, fine fibrils which run in a direction approximately perpendicular to the axial cores of the microvilli (figure 23*b*). These axial cores are about 64 nm in overall diameter and extend about 0.5 μm below the luminal plasma membrane to end in the luminal surface of the endotube. In the endotube

Description of plate 5

Figure 16. Electron micrograph of thin section of part of intestine of *Syngamus trachea*. Scale bar, 1 μm.

Figure 17. Electron micrograph of this section of endotube preparation from *S. trachea*. Pronounced degenerative changes have occurred in the microvilli, the endotube and the underlying arrays of microfilaments; compare with figure 16. Scale bar, 0.5 μm.

Figure 18. (*a*) Phase contrast light micrograph of a partially dissected intestine from *Trichonema*. The endotube is seen to be continuous with the surface of the lumen (arrowed) of the intestine. (*b*) Light micrograph of section of an endotube preparation. Scale bars, (*a*) 50 μm; (*b*) 20 μm.

Figure 19. Electron micrograph of part of an endotube preparation from a thin section adjacent to the section shown in figure 18*b*. Scale bar, 0.5 μm.

Figure 20. Electron micrograph of the basal side of the endotube from *Trichonema* to show its densely packed fine fibrils and associated microfibrils. Scale bar, 0.25 μm.

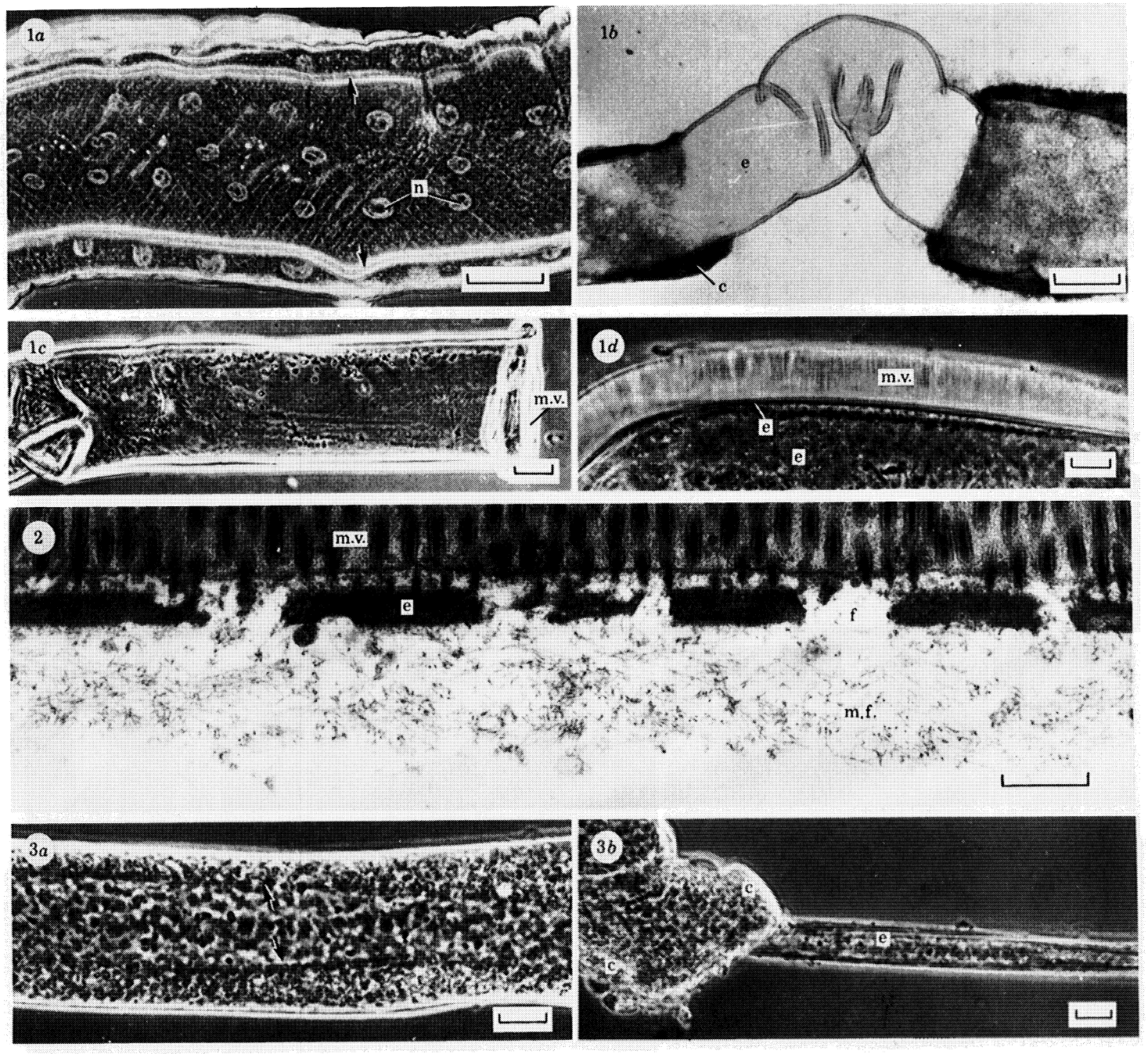


FIGURE 1. Light micrographs of (*a*) part of an intact intestine from *Heamonchus contortus*. Rows of nuclei (n) and the surface of the lumen (arrows) are clearly visible. The cross-hatching effect is due to organization of the microvilli into blocks of aligned rows. (*b*) A partially dissected intestine of *H. contortus* showing that the endotube (e) is continuous with the luminal surface visible within the intact portions of the intestine. (*c*) A piece of endotube from *H. contortus*. Microvilli are most clearly visible at the cut end of the endotube which, characteristically, has everted for a short distance. (*d*) The microvilli exposed at the cut end of the endotube at higher magnification. Scale bars, (*a*, *b*), 50 μm; (*c*) 10 μm; (*d*) 10 μm.

FIGURE 2. Electron micrograph of a piece of an endotube preparation from *H. contortus* to show its apearance in thin section. The endotube (e) is perforated with fenestrations (f); on its basal surface there is a layer of microfibrils. Scale bar 1 μm.

FIGURE 3. Phase contrast light micrographs of (*a*) the intact intestine of *Ostertagia circumcincta*. The surface of the lumen is visible (arrows); (*b*) partially dissected intestine to show the endotube protruding from the end of the basal cytoplasm. Scale bars 10 μm.

(*Facing p.* 8)

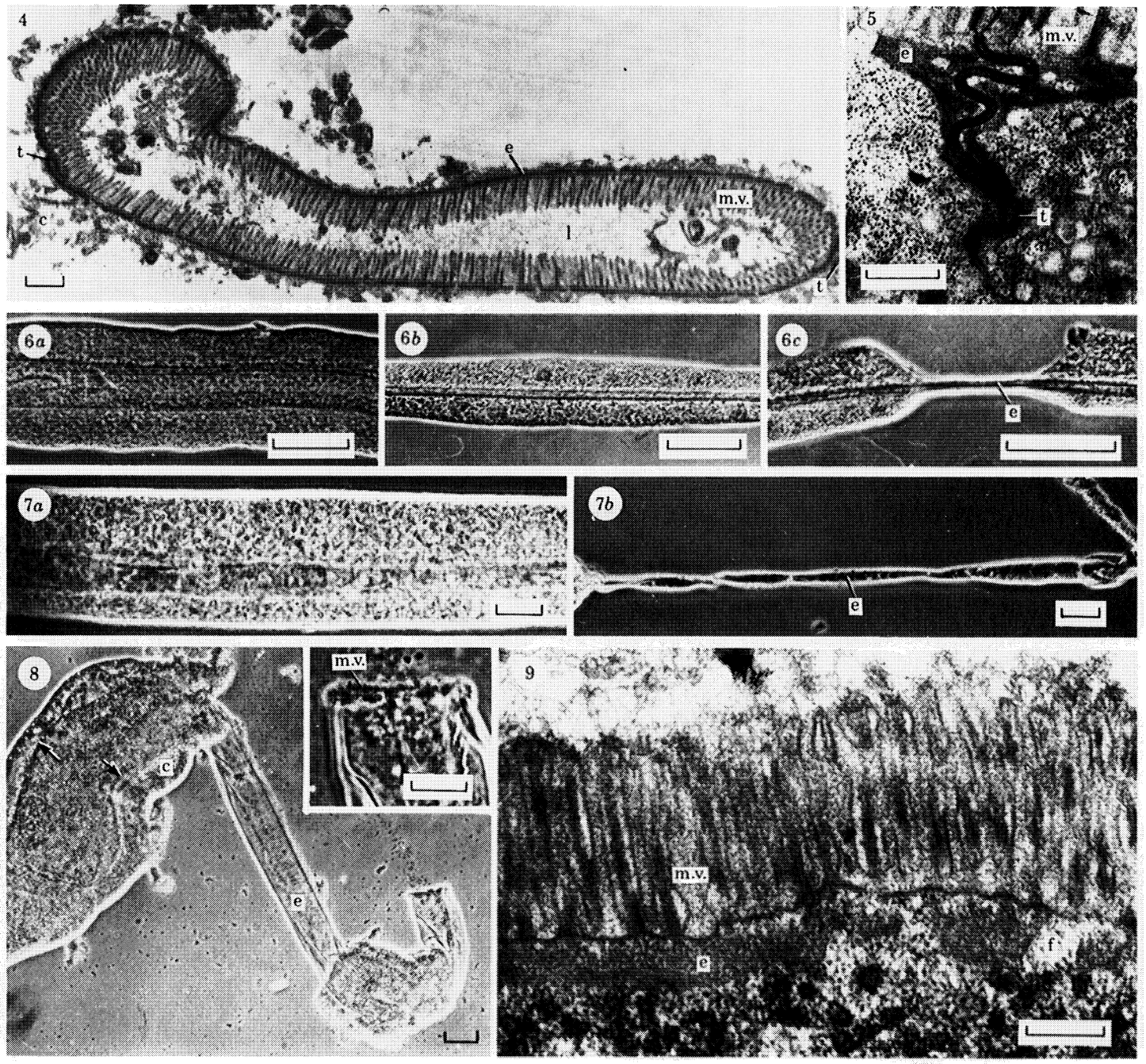


FIGURE 4. Electron micrograph of a cross-section of an endotube–brush-border complex obtained from *Ostertagia*. Two terminal bars (t) and small amounts of basal cytoplasm are retained. Scale bar, 1 μm.

FIGURE 5. Electron micrograph of part of a transverse section of intestine of *Ostertagia* to show the extension of the endotube (e) along the terminal bar (t). Scale bar, 0.5 μm.

FIGURE 6. Phase contrast light micrographs of (*a*) posterior, (*b*) anterior regions of intact intestine from *Nematodirus*. The luminal surface is clearly visible and, (*c*), is continuous with the endotube exposed by blunt dissection. Scale bars, 5 μm.

FIGURE 7. Phase contrast light micrographs of (*a*) intact intestine of *Trichostrongylus* and (*b*) a piece of endotube dissected from it. Scale bars, (*a*), 10 μm; (*b*), 20 μm.

FIGURE 8. Phase contrast light micrograph of partially dissected intestine from *Nippostrongylus brasiliensis*. The surface of the lumen is visible within the intact portion of the intestine (arrows) and is continuous with a short length of exposed endotube. A fringe of microvilli can just be discerned at the free end of the endotube (shown at higher magnification in the inset). Scale bar, 20 μm (inset, 10 μm).

FIGURE 9. Electron micrograph of a section of part of the intestine of *N. brasiliensis*. Scale bar, 0.25 μm.

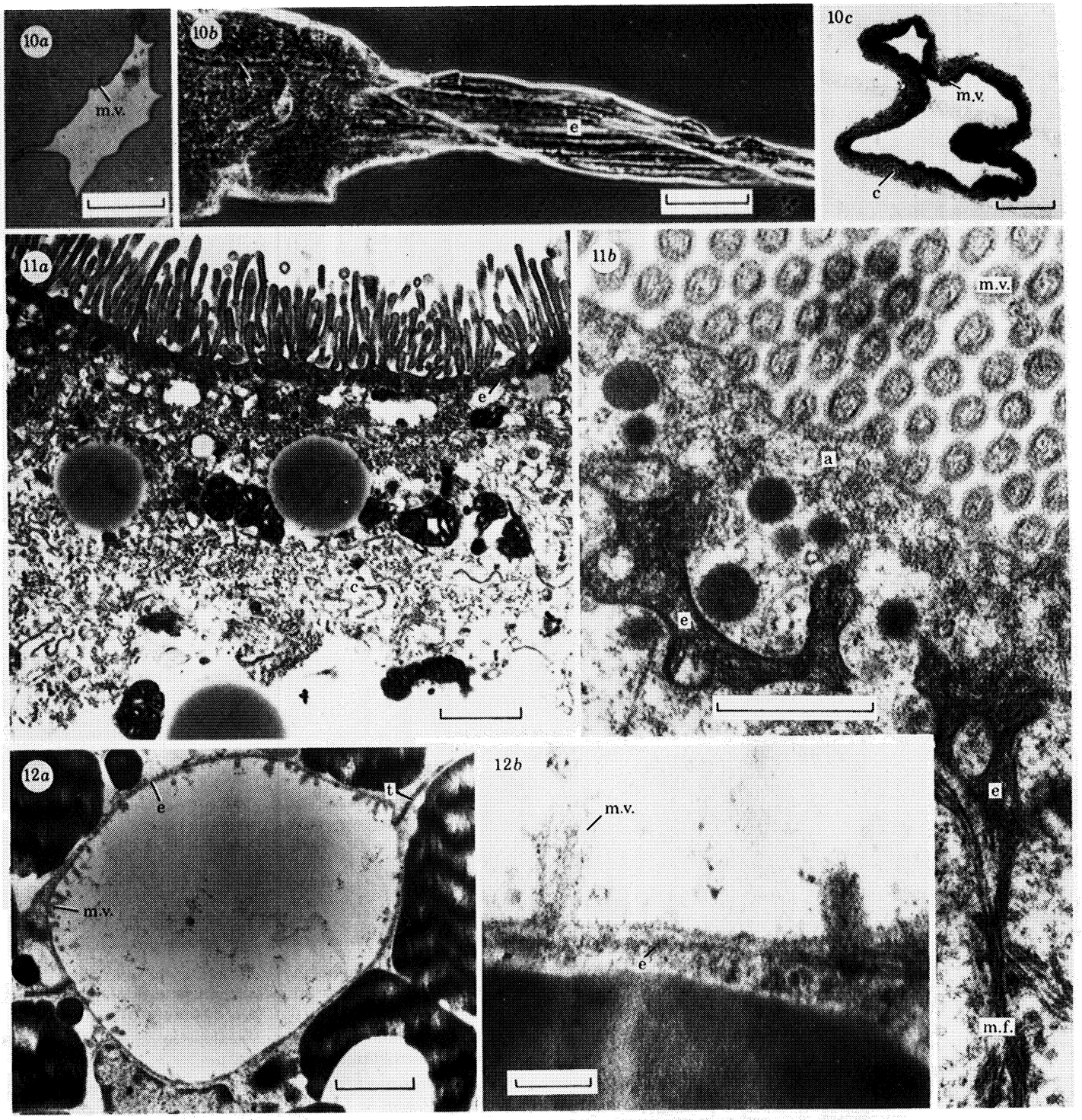


FIGURE 10. Light micrographs of (*a*) cross-section of part of the intestine, (*b*) partially dissected intestine showing the protruding endotube to be continuous with the lumen surface (arrow) and (*c*) cross-section of the endotube–brush-border complex with associated layer of basal cytoplasm, from *Dictyocaulus viviparus*. Scale bars, (*a*, *b*), 50 μm; (*c*) 10 μm.

FIGURE 11. Electron micrographs of parts of endotube preparations from *Dictyocaulus viviparus*. (*a*) This is from a thin section adjacent to the 0.5 μm thick section shown in figure 10*c*. (*b*) This is to show the basal prolongations of the endotube with which microfilaments are associated. Scale bars, (*a*) 1 μm; (*b*) 0.5 μm.

FIGURE 12. Electron micrographs of parts of this transverse sections through the intestine of *Muellerius*. (*a*) The oval shaped lumen is lined with short, sparse microvilli the axial cores of which join to the thin continuous layer immediately below the plasma membrane as shown at higher magnification in (*b*). The adjacent cytoplasm is packed with large, heavily staining granules. Scale bars, (*a*) 1 μm; (*b*) 0.1 μm.

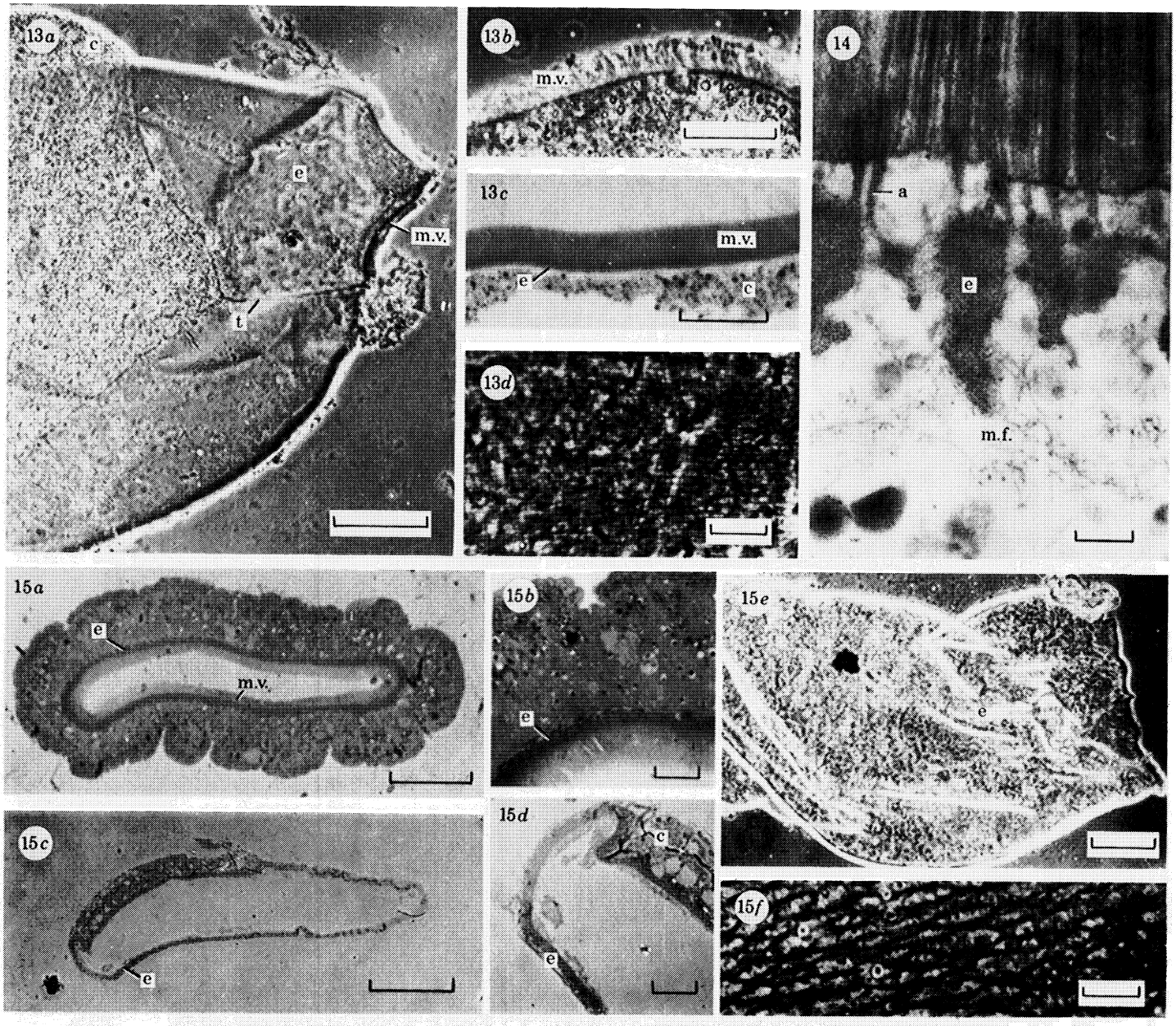
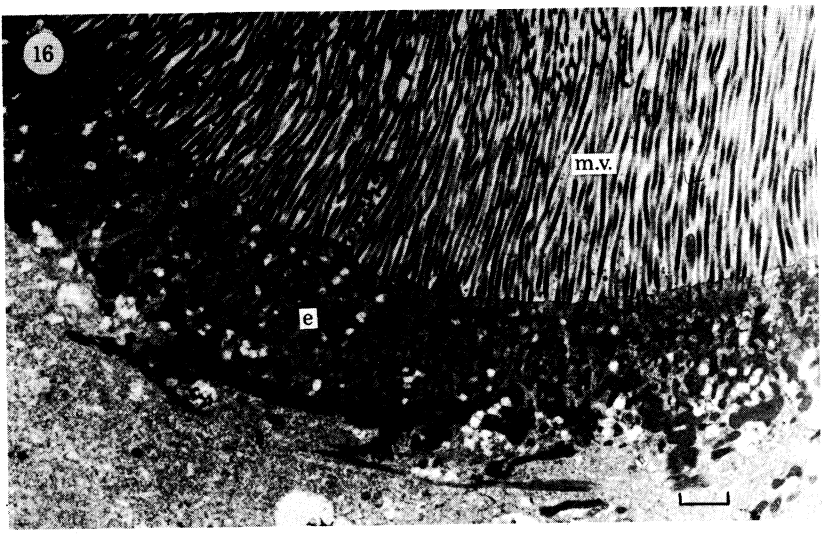
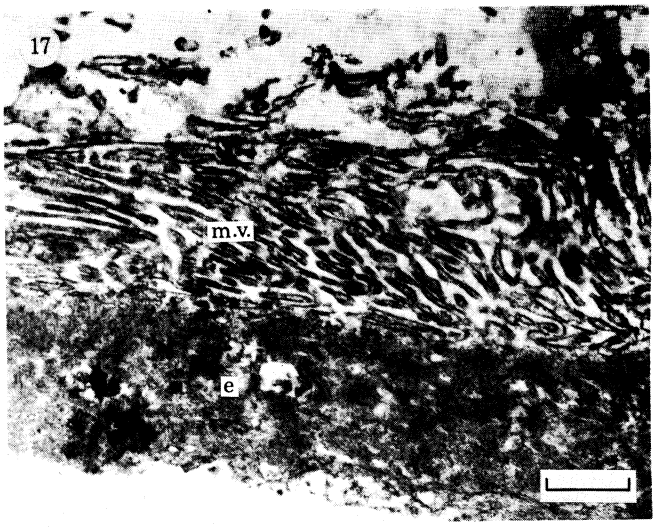


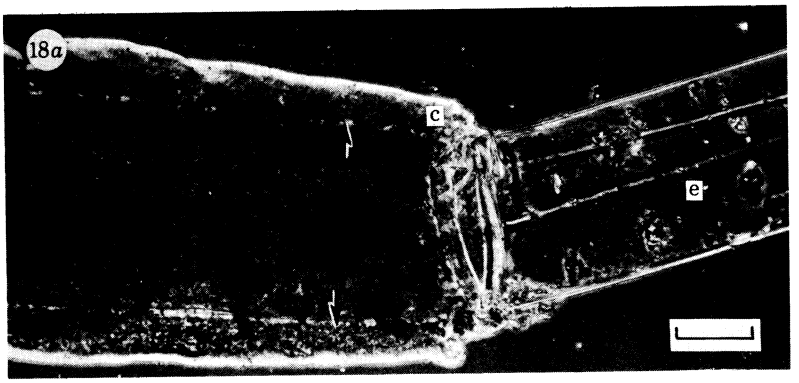
FIGURE 13. Light micrographs of (*a*) partially dissected intestine, (*b*), (*c*) a fringe of microvilli and (*d*) the reticulum on the basal side of an endotube from *Ancylostoma caninum*. (*a*), (*b*), (*d*). These are phase contrast micrographs; (*c*) is a micrograph of a section of an endotube preparation. Scale bars, (*a*) 50 μm; (*b*,*c*) 20 μm; (*d*) 10 μm.

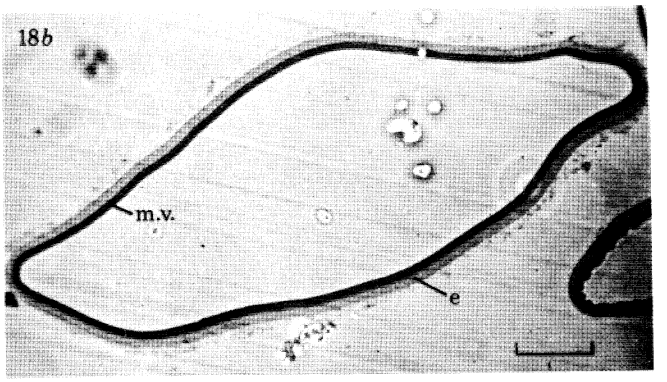
FIGURE 14. Electron micrograph of a thin section of an endotube preparation from *Ancylostoma* showing connections to the axial cores of the microvilli and, on the basal side, the microfibrils. Scale bar, 0.2 μm.

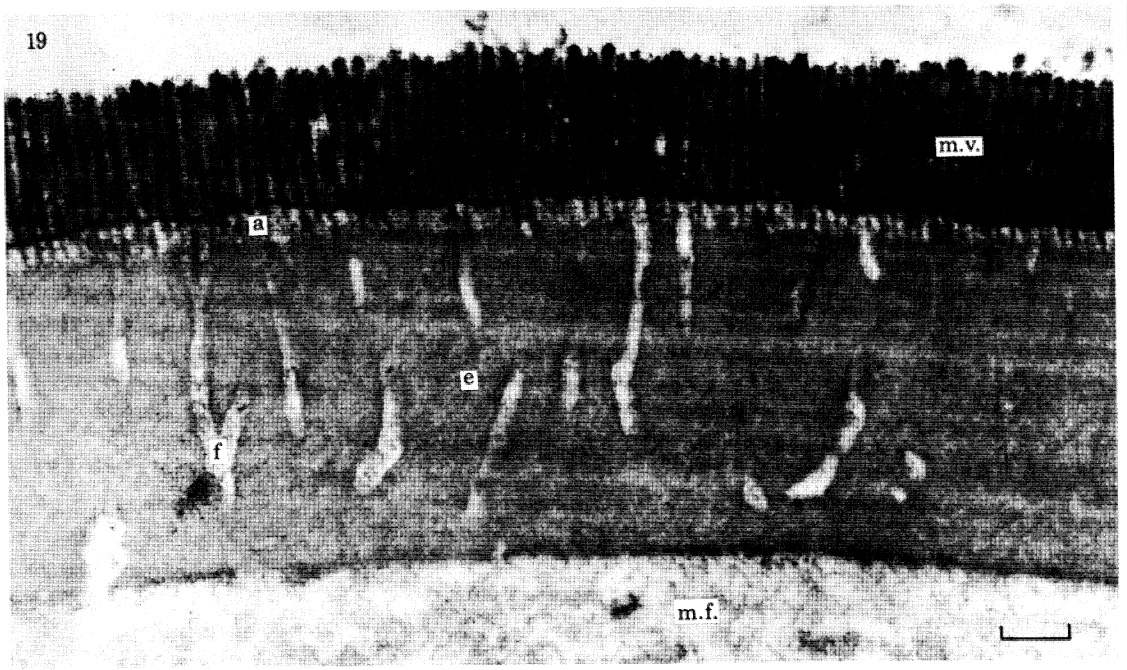
FIGURE 15. Light micrographs of (*a*), (*b*) a transverse section of the intestine of *Syngamus trachea*, (*c*), (*d*) a transverse section of an endotube preparation obtained from an intestine kept at 4 °C for 6 d. (*e*) Phase contrast light micrograph of an endotube preparation from an intestine aged as in (*c*) and (*d*), and (*f*) the reticulum on the basal surface of a piece of endotube from fresh intestine. Scale bars, (*a*,*c*), 50 μm; (*b*,*d*,*f*), 10 μm; (*e*) 100 μm.

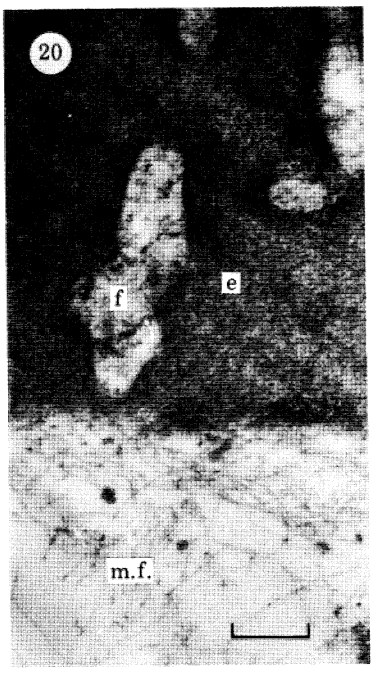












FIGURES 16–20. For description see p. 8.

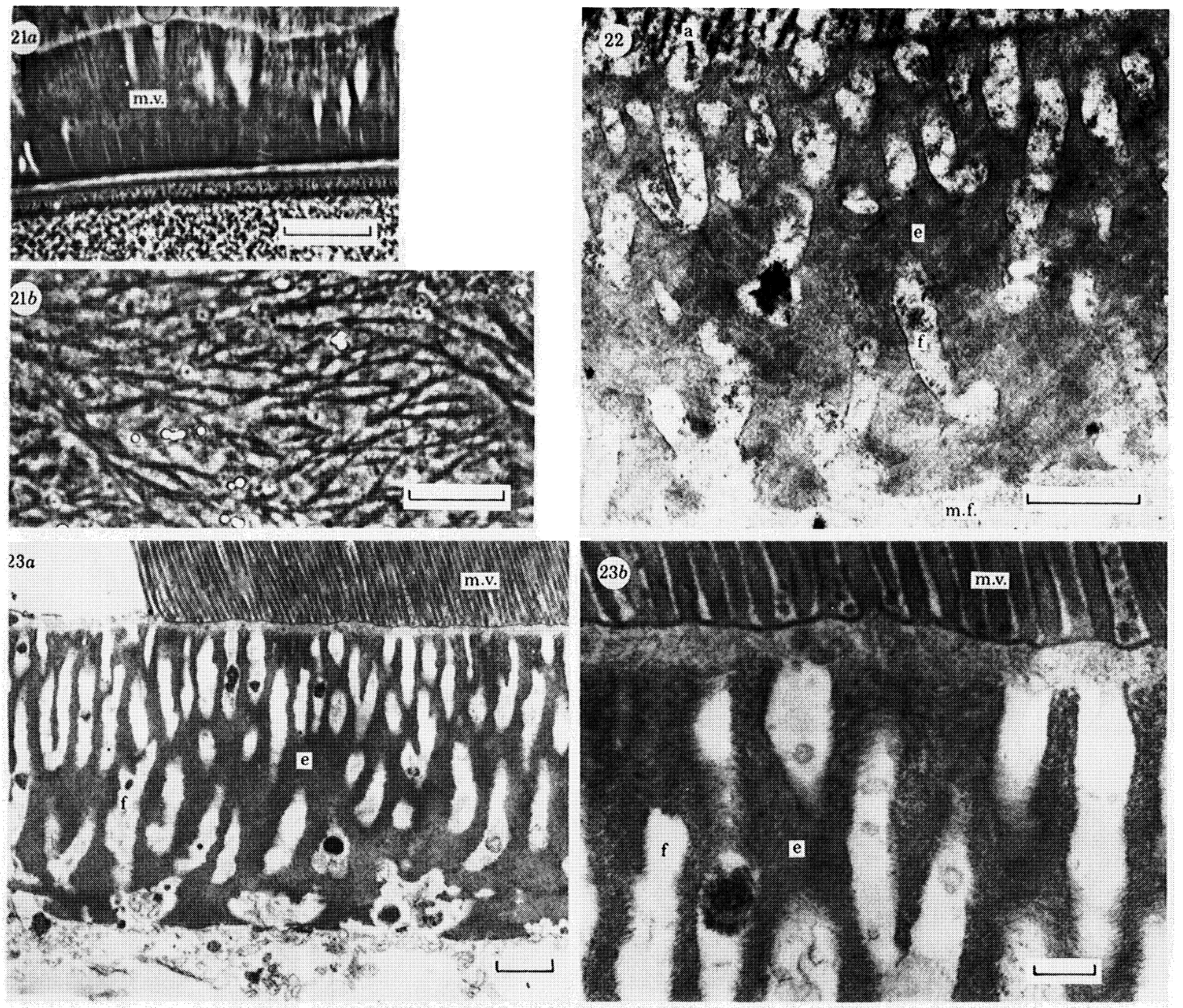
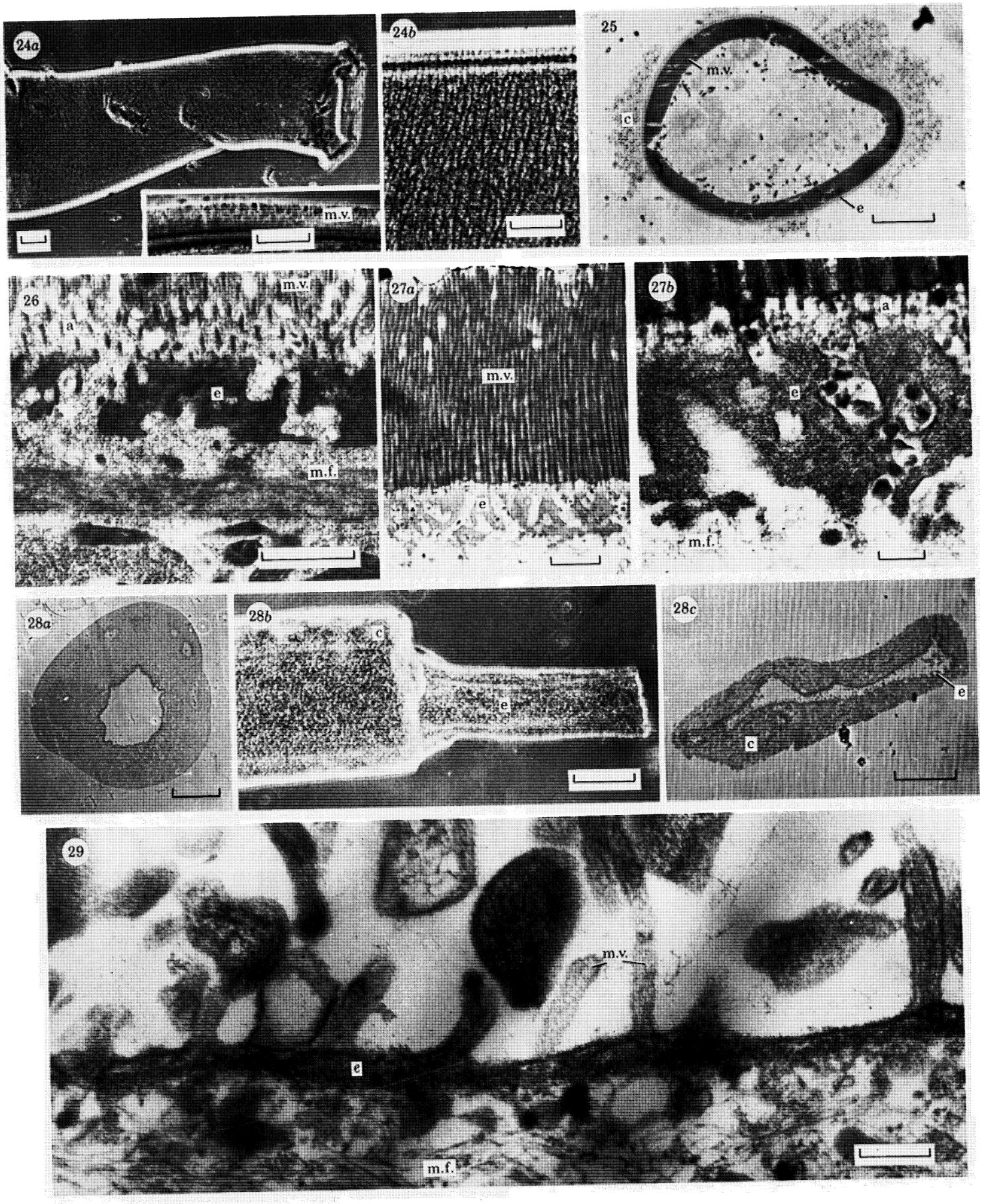


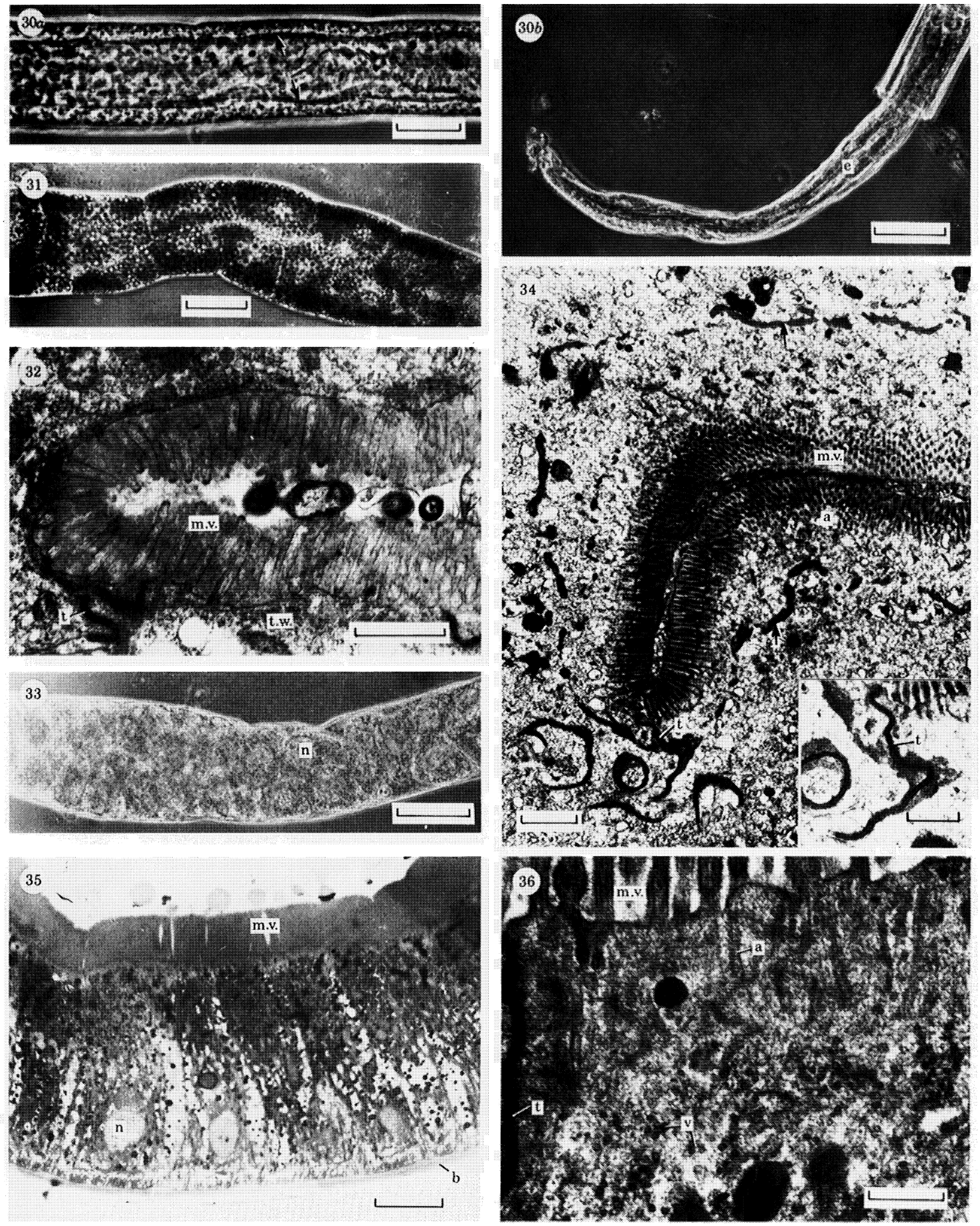
FIGURE 21. Phase contrast light micrographs of parts of endotube preparations from the intestine of *Strongylus endentatus* showing (*a*), the long microvilli, numbers of which are ballooned, and (*b*), the reticulated appearance of the basal surface. Scale bars, (*a*) 20 μm; (*b*) 10 μm.

FIGURE 22. Electron micrograph of a thin, slightly oblique section of an endotube in *S. edentatus* intestine. Scale bar, 1 μm.

FIGURE 23. Electron micrographs of an endotube preparation from *S. edentatus* showing (*a*) partial loss of microvilli, and (*b*) loss of axial cores from remaining microvilli, and fibrous nature of the endotube. Scale bars, (*a*) 1 μm; (*b*) 0.25 μm.



FIGURES 24–29. For description see over.



FIGURES 30–36. For description see opposite.

Description of plate 7

Figure 24. Phase contrast light micrographs of endotube preparations from *Oesophagostomum columbianum*. The endotube from the anterior part of the intestine retains a prominent reticulum (*c*) shown at higher magnification in (*b*). A fringe of microvilli is present at the cut end of the tube (inset). Scale bars, 20 μm.

Figure 25. Light micrograph of a transverse section through an endotube–brush-border preparation from *O. columbianum*. Scale bar, 20 μm.

Figure 26. Electron micrograph of the endotube and associated structures in a section of the intestine of *O. columbianum*. Scale bar, 1 μm.

Figure 27. Electron micrograph of part of a thin section adjacent to that shown in figure 25. (*a*) Shows part of the endotube–brush-border complex at low magnification and (*b*) shows the endotube, axial core filaments and basal side microfibrils in detail. Scale bars, (*a*) 1 μm; (*b*) 0.2 μm.

Figure 28. Light micrographs of (*a*) a transverse section through the intestine, (*b*) a partially dissected intestine and (*c*) a transverse section through an endotube preparation from *Metastrongylus*. Scale bars, (*a*) 50 μm; (*b*), 100 μm; (*c*) 20 μm.

Figure 29. Electron micrograph of this section showing the endotube, short sparse microvilli and, basally, an irregular array of microfibrils in *Metastrongylus*. The lumen of the intestine contains numerous bacteria-like profiles. Scale bar, 0.2 μm.

Description of plate 8

Figure 30. Phase contrast light micrographs of parts of intestines from *Strongyloides papillosus*. (*a*) The surface of the lumen (arrows) is clearly visible in the intact intestine and (*b*) is continuous with the endotube. Scale bars, (*a*) 20 μm; (*b*), 50 μm.

Figure 31. Phase contrast light micrograph of the intestine of *Pelodera strongyloides*. Scale bar, 20 μm.

Figure 32. Electron micrograph of a thin section through part of the intestine of *P. strongyloides*. Scale bar, 1 μm.

Figure 33. Phase contrast light micrograph of part of an intestine from *Syphacia obvelata*. The intestine is multicellular; the surface os the lumen is not visible. Scale bar, 50 μm.

Figure 34. Electron micrograph of a thin transverse section through the apical regions of two intestinal cells from *S. obvelata*. The lumen is occluded. Material (arrowed) resembling an endotube is present. It is associated with the terminal bar (t), shown at higher magnification in *inset*, but comparatively remote from the base of the microvilli. Scale bar, 1 μm.

Figure 35. Light micrograph of part of a transverse section of the intestine of *Toxocara canis*. Scale bar, 20 μm.

Figure 36. Electron micrograph of parts of the apical region of intestinal cells from *T. canis*. Scale bar, 0.5 μm.

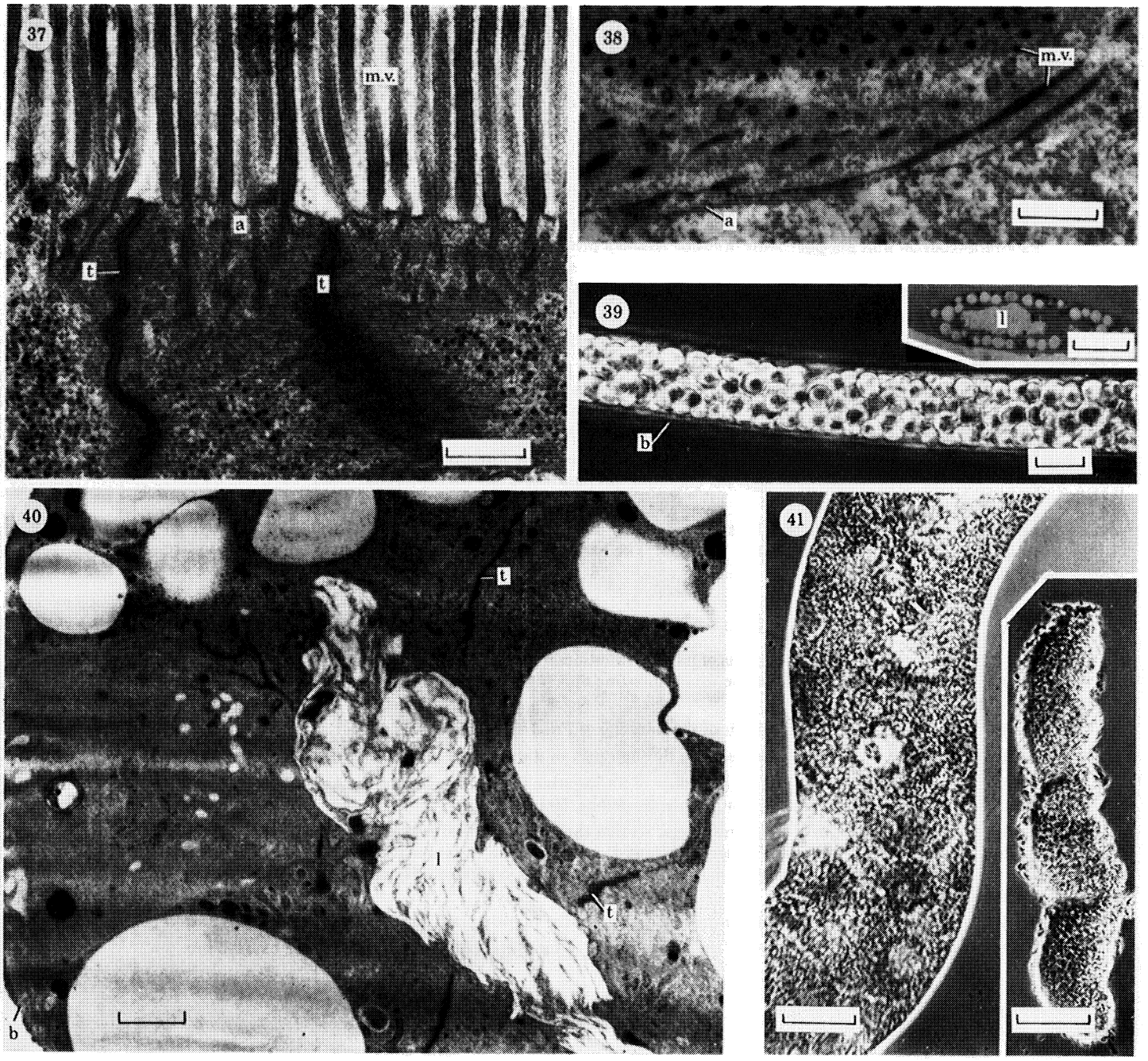


FIGURE 37. Electron micrograph of parts of the apical region of intestinal cells from *Ascaris suum* showing one terminal bar in vertical section and one in oblique section. Scale bar, 0.5 μm.

FIGURE 38. Low power electron micrograph of the luminal surface of the intestine of *Heterakis gallinae*. Scale bar, 1 μm.

FIGURE 39. Light micrographs of intestine from *Litomosoides carinii*. (*a*) Intact intestine by phase-contrast; *inset* section. Scale bars, 20 μm.

FIGURE 40. Electron micrograph of a section through part of the intestine of *L. carinii*. Scale bar, 1 μm.

FIGURE 41. Phase contrast light micrographs of the intestine of *Trichuris ovis* and (inset) three cells obtained therefrom. Scale bar, 100 μm (inset 50 μm).

preparations the cores of the microvilli are no longer present and this probably accounts for the fact that many of the microvilli are lost (figure 23*a*).

Some fibrils and large electron-dense particles are retained within the fenestrations of the isolated endotube (figure 23). The particles may be aggregates of much smaller particles seen within the fenestrations of the endotube in the intestine (figure 22).

Oesophagostomum columbianum

Although apparently cellular at the most anterior end the intestine of *Oesophagostomum* had an endotube throughout its entire length. It was not possible to obtain much clean endotube from fresh intestine but it could be isolated from worms left overnight at 4 °C in phosphate-buffered saline solution. Even then the endotube could not readily be obtained clean from the anterior portion of the intestine (figure 25, plate 7) and in this region the endotube had a reticulum on the basal surface with prominent transverse components and less noticeable longitudinal components (figure 24*b*). Unlike those in *Ancylostoma* the apical lateral plasma membranes (terminal bars) at the anterior end of the intestine were not visible on the endotube. Between a third and a half of the endotube from the posterior end could be obtained easily, virtually free from cytoplasm. This region showed little or no sign of a reticulum. In electron micrographs material that might be equated with the reticulum was seen as bundles (about 0.5 μm thick) of thin microfibrils associated with the basal surface of the endotube (figure 26) but these were not seen in electron micrographs of the isolated endotube. There were thicker microfibrils (about 12 nm diameter) between the bundles and the endotubes and, to a lesser extent, on the cytoplasmic side of the bundles. The overall thickness of the endotube is about 1 μm but it has a very irregular outline, particularly on the basal side (figures 26 and 27). The fenestrations are irregular in shape and numerous. The endotube appears to be composed of very fine fibrils oriented predominantly perpendicular to the microvilli cores. The microvilli are about 4.5 μm long and 0.1 μm wide. They have an axial core about 42 nm wide which extends about 0.25 μm below the microvilli and ends on the luminal side of the endotube.

At the basal surface of the endotube from the posterior part of the intestine there is an irregular array of filaments each some 6 nm in width (figure 27*b*).

Metastrongylus sp.

The structures associated with the surface of the irregularly shaped lumen of *Metastrongylus* do not appear very substantial in cross section compared to the overall thickness of the intestine (figure 28*a*). Nonetheless, it was possible to obtain short lengths of endotube by dissection as shown in the phase contrast micrograph (figure 28*b*). The endotube is continuous with the luminal surface visible within the intestine. The longest pieces of endotube which could be obtained were only about three times the length of the piece shown in figure 28*b*. In all cases they retained a significant amount of other cytoplasmic material. Microvilli could not be seen by phase contrast microscopy at the free end, but were visible in sections (figure 28*c*) and, as shown by electron microscopy (figure 29), they were rather sparse, quite short, and irregularly occurring structures. The endotube was only 75 nm thick and closely applied to the luminal plasma membrane. There was a loose network of microfibrils immediately below the endotube extending deep into the cytoplasm. Some of the microfibrils appeared to be associated with the basal face of the endotube (figure 29).

Order Rhabditida

Strongyloides papillosus

The overall diameter of the intestine of *S. papillosus* is about 80 μm. The lumen is comparatively wide, diameter 45–50 μm and its surface, which is clearly visible within the intestine (figure 30*a*) is continuous with the endotube (figure 30*b*, plate 8) which can be obtained by blunt dissection.

Pelodera strongyloides

Two free-living nematodes, *Pelodera* and *Panagrellus*, have been examined but only *Pelodera* was sufficiently large to permit dissection of the intestine by the procedures described here. The intestine is composed of large, polygonal cells; the lumen is not visible within the intact intestine by light microscopy (figure 31). No evidence for the presence of an endotube could be obtained by dissection but electron microscopy showed the presence of a substantial terminal web (figure 32).

Order Ascarida

Syphacia obvelata

The intestine of *Syphacia* appeared cellular; the luminal surface was not visible within the intestine (figure 33). It was not possible to dissect out an endotube but the small size of the intestine was a major difficulty. An electron-dense layer with some similarities to an endotube is present in electron micrographs of sections of *Syphacia* intestine (figure 34). This layer has large discontinuities and is, for the most part 1–3 μm from the basal extensions of the axial cores of the microvilli. It does however adjoin and spread to coat the cytoplasmic surface of the terminal bar.

Toxocara canis

The intestine of *Toxocara* is multicellular with some 20–24 cells around a circumference. The cells are about 50 μm tall and the microvilli some 10 μm or more long (figure 35). An endotube could not be obtained by blunt dissection.

In electron micrographs (figure 36) the axial core filaments of the microvilli extend at least 0.35 μm into the cytoplasm into a layer of microvesicles, some 50 nm in diameter, similar in appearance to those in *Ascaris* (see figure 37, plate 9). Longitudinal bundles of filaments similar to the core filaments of microvilli, but not in the same hollow cylindrical arrangement are seen much deeper in the cell between the longitudinally arrayed mitochondria but there is no evidence that the two sets of filaments are connected. While there is a hint of the presence of finely fibrous material in the region of the terminal bars and near the bases of the microvilli there is no real sign of a terminal web and certainly no sign of an endotube.

Ascaris suum

The cellular intestine of this nematode has been extensively described. We have confirmed that there is no evidence for the existence of an endotube either by dissection or appearance in the electron microscope. The microvillar core filaments are arranged to form a hollow cylinder (see also Sheffield (1964)). Like those of *Toxocara*, they retain this arrangement within the cytoplasm of the cell. The terminal web system is not very substantial except immediately adjacent to the terminal bars (figure 37).

Heterakis gallinae

The intestine of *H. gallinae* is cellular with a narrow, irregularly shaped lumen. Microvilli are sparse. Typically they project at an acute angle and the basal extensions of their axial core filaments lie close to the plasma membrane (figure 38). Although some of the filaments seem to fuse there is no evidence of a terminal web.

Order Spirurida

Litomosoides carinii

The intestine of *Litomosoides* is composed of what appear to be a rather loosely associated group of cells with flattened surfaces between adjacent cells and rounded surfaces on the side next to the basement membrane. No lumen was visible by light microscopy of the intact intestine (figure 39*a*) although one is visible in sections (figure 39*b*). It was not possible to obtain any evidence for an endotube by dissection nor by electron microscopy, nor was there any sign of a true brush border (figure 40). (Some cells appeared to have wispy prolongations and the lumen was filled with similar material.)

Order Trichinellida (*Subclass Adenophorea*)

Trichuris ovis

The intestine of *T. ovis* is composed of roughly polygonal cells within a tubular basement membrane. The lumen could not be seen. The intestine readily broke up into strings of cells at the luminal surface of which were a few short microvilli (figure 41). There was no evidence for the presence of an endotube, by dissection or by electron microscopy.

Discussion

The criterion for the identification of an endotube used here is that it is possible to obtain a tubular entity (with associated brush border membrane) by blunt dissection of the intestine. By this criterion an endotube is present throughout the length of the intestine of eleven of the thirteen strongylid genera examined here. Of the other two genera, from *Strongylus* it was possible to obtain endotube as such from only a comparatively short region at the posterior of the intestine although fragments from all other parts of the intestine showed very small lengths of endotube-like extensions at their edges and contained endotube as judged by its appearance in the electron microscope (figure 22). It was not practicable to dissect the intestine from *Muellerius*. Examination of sections of the intestines from all 13 of these genera showed the presence of material compatible with that of an endotube. Published electron micrographs of sectioned intestines of strongylids similarly support the contention that all members of this order possess endotubes. By analogy with the entity present at the base of microvilli in mammalian enterocytes, the relevant structures in the nematodes have been designated 'terminal web'. Precise identification of the endotube with the terminal web of mammalian cells must await the demonstration of the presence of analogous proteins in the two systems. It may prove that the two are not strictly analogous but because of the widespread use of the term 'terminal web' to describe structures in nematode intestines it is proposed that the endotube be considered as a modified terminal web. The structure designated as the terminal web of *Haemonchus placei* by Smith & Harness (1972) is closely similar in appearance to the endotube of *H. contortus* and

the appearance of the 'terminal web', particularly at its junction with the terminal bar in *Trichostrongylus colubriformis* (see plate 5*c* in Smith & Harness (1972)) is compatible with its being an endotube, as demonstrated here (figure 7).

Inspection of electron micrographs of sections of intestine of *Cyathostoma lari* (Colam 1971*c*) shows that it has many structural features analogous with those of the other strongylids described here. Notably, the terminal web is very similar in appearance to the endotube of *Syngamus* (figures 16 and 17 and see Borgers *et al.* (1975)). The microvilli of *C. lari* intestine are 5–25 μm in length and their axial cores extend 0.2–0.3 μm into the apical cytoplasm and then join to the terminal web. The intestine is syncytial (Colam 1971*c*) and it seems extremely likely that the terminal web in this nematode forms an endotube.

As noted elsewhere (Munn 1977; Munn & Greenwood 1983) there is close similarity in the appearance of the terminal web of *Ancylostoma caninum* (Miller 1967) and that of *H. contortus*. Miller (1967) pointed out that microfibrillar elements associated with the terminal web were confined to the side away from the microvilli. These microfibrils are seen here (figure 14) in sections of the isolated endotube-complex and may well contribute to the reticulum seen by phase contrast light microscopy (figure 13*d*). An alternative explanation of the reticulum seen on the basal side of the endotube of *A. caninum* and also the endotubes of *Syngamus* (figure 15*f*), *Strongylus* (figure 21*b*) and *Oesphagostomum* (figure 24*a*, *b*) is that it is a planar view of the basally directed prolongations of the endotube seen in electron micrographs of transverse (vertical) sections of the endotubes (figure 14, 16, 23*a* and 27). There is insufficient evidence to decide between these alternatives at the present time.

The parasitic member of the Rhabditida examined here, *Strongyloides papillosus* has an endotube (figure 30) and the structure designated as terminal web in electron micrographs of *S. myopotomi* (Colley 1970) is very similar in appearance to an endotube. On the other hand the intestines of two species of *Rhabdias* parasitic in frogs studied by Colam (1971*a*) had no distinct terminal web.

None of the Ascarida tested for the presence of an endotube possessed one. The structure seen in sections of *Syphacia* (figure 34) may be related to an endotube but its comparative remoteness from the cytoplasmic extensions of the microvillar axial core fibrils, even though these seem remarkably long, suggests that this entity should not be classified as an endotube. It is noteworthy that the luminal surface is not visible by phase contrast microscopy within the intact intestine. Micrographs of the intestine of other members of the order presented here or published by other authors (see table 1) contain no evidence that they possess and endotube. Indeed, for several there is little sign of any kind of terminal web system. For example, there is no evidence in published electron micrographs of *Aspicularis tetraptera* (Ascarida, Oxyurina) intestine (Lee & Anya 1968; Comley 1980) that this contains any significant amounts of material organized into a terminal web. The intestine of *Cosmocerca* (Ascaridina) is cellular and in the posterior region, has microvilli up to 25 μm in length. These have prominent axial filaments which extend about 0.5 μm into the apical cytoplasm; the terminal web, however, is finely granular and rather inconspicuous (Colam 1971*b*).

Neither representative of the other parasitic groups examined, *Litomosoides carinii*, from the order Spirurida and *Trichuris ovis*, from the subclass Adenophorea, order Trichinellida, yielded evidence for the presence of an endotube, or any significant terminal web material. Previously published electron micrographs of *Litomosoides*, using material fixed with osmium tetroxide, do not show much detail of the intestinal cells (Kagei 1963). A more useful study of the intestinal

epithelium of *Dirofilaria immitis* (Lee & Miller 1969) revealed that the microvilli lacked electron-dense central cores and that although the area immediately beneath the apical plasma membrane was free of organelles, no terminal web was apparent. The intestinal cells of *Brugia malayi* also have very little or no terminal web (Vincent *et al.* 1975).

The structure of the intestine of the free-living marine nematode *Pontonema vulgaris* (subclass Adenophorea) has been described by Jennings & Colam (1970). The intestine is cellular with numerous microvilli about 1 μm in length with axial cores which extend into the submicrovillar cytoplasm. This region of the cell is free of ribosomes and membranes but has no sign of a terminal web.

The one free-living nematode examined here, the rhabditid *Pelodera strongyloides* gave no evidence for the presence of an endotube although the terminal web appeared quite substantial (figure 32). Accounts of the structure of the intestine of several representatives of the order Tylenchida have been published. The intestine of the plant parasite *Tylenchorhynchus dubius* appears to be extensively syncytial; a few complete lateral cell boundaries are present but the majority have only the apical portion remaining. The microvilli are short with axial filaments extending only a short distance into the cytoplasm, but there is no distinct submicrovillar layer or terminal web (Byers & Anderson 1973). The intestinal cells of another tylenchid, *Anguina calamagrostis* described by Wu (1968), apparently lack any significant amounts of organized terminal web.

There is a prominent fibrillar terminal web in the anterior intestine of the passively feeding tylenchid *Hexatylus viviparus* (Shepherd & Clark 1976). In the mid intestine the microvilli are longer but the terminal web is more tenuous. The terminal web in the anterior and mid intestine of *Aphelenchoides blastophthorus* is similar to that in *H. viviparus* (Shephard *et al.* 1980). In neither does the terminal web resemble the endotube described here. The cells in the intestine of *Pratylenchus penetrans* have no terminal web and the microvilli lack axial core filaments (Kisiel *et al.* 1974).

Observations on the intestinal structure of parasitic nematodes relating to the presence or absence of an endotube are summarized in table 1. The occurrence of the endotube seems, on the present evidence, to be confined to members of the orders Strongylida and Rhabditida with all genera of the former group that have been dissected showing evidence for the presence of an endotube. Members of both these orders have intestines that are either syncytia or composed of only a few cells (Chitwood & Chitwood 1974), but it is not all nematodes with this kind of organization that have endotubes; as noted above, *T. dubius*, the intestine of which is extensively syncytial, lacks a terminal web (Byers & Anderson 1973).

The terminal web of *Muellerius* may prove to be an endotube but there is no direct evidence for this. From its appearance in the electron microscope it is no more substantial than the terminal web in *Pelodera*. On the other hand, the corresponding entity in *Nippostrongylus* which is equally lacking in substance (figure 8) yields an endotube by dissection (figure 9). The other correlate with the presence of an endotube, the visibility of the lumen surface within the intact intestine when examined by light microscopy (table 1 and see following paragraph) cannot be used, since it was not possible to dissect the intestine from *Muellerius*.

It is noteworthy that all the intestines that were shown to possess an endotube had a lumen with a surface clearly visible when the intact intestine was examined microscopically (see figures 1*a*, 3*a*, 6, 7*a*, 8, 10*b*, 13*a*, 18*a*, 28*b* and 30). The reason for the visibility of the lumen surface is not obvious. It is not dependent on the relative diameter of the lumen nor the length of the

TABLE 1. SUMMARY OF OBSERVATIONS ON THE PRESENCE OR ABSENCE OF AN ENDOTUBE IN THE INTESTINES OF PARASITIC NEMATODES

order and suborder	family	genus	host	site	nematode intestine lumen visible	endotube indicated†	endotube observed	references
Ascarida Ascaridina	Ascaridae	*Ascaris suum*	pig	intestine (lumen)	no	no	no	1,2,3,4,5
		Cosmocerca ornata	frog	intestine (lumen)	—‡	no	—	6
		Phocanema decipiens	seal	stomach (lumen)	—	no	—	7
	Toxocaridae	*Toxocara canis*	dog	intestine (lumen)	no	no	no	1
Heterakina	Heterakidae	*Heterakis gallinae*	pheasant	caecum (lumen)	no	no	no	1
Oxyurina	Syphacidae	*Syphacia obvelata*	rat	intestine (lumen)	no	no	no§	1
	Aspicularidae	*Aspicularis tetraptera*	mouse	caecum (lumen)	—	no	—	8,9
Rhabditida Rhabditina	Rhabdiasidae	*Strongyloides papillosus*	sheep	intestine (mucosa)	yes	—	yes	1
		S. myopotomi	nutria	intestine (mucosa)	—	yes	—	10
		Rhabdias bufonis	frog	lung (bloodfeeder)	—	no	—	11
		R. sphaerocephala	frog	lung (bloodfeeder)	—	no	—	11
Strongylida Tricho-strongylina	Tricho-strongylidae	*Haemonchus contortus*	sheep	abomasum (mucosa)	yes	yes	yes	1,12,13,14
		H. placei	cattle	abomasum (mucosa)	—	yes	—	15
		Ostertagia circumcincta	sheep	abomasum (mucosa)	yes	yes	yes	1
		O. trifurcata	sheep	abomasum (mucosa)	yes	yes	yes	1
		Nematodirus spathiger	sheep	intestine (mucosa)	yes	—	yes	1
		N. battus	sheep	intestine (mucosa)	yes	yes	yes	1,16
		Trichostrongylus colubriformis	sheep	intestine (lumen)	yes	—	yes	1,15

	Heligmosomatidae	*Nippostrongylus brasiliensis*	rat	intestine (mucosa)	yes	yes	yes	1,17,18
	Dictyocaulidae	*Dictyocaulus viviparus*	calf	lung (bronchi)	yes	yes	yes	1
		Muellerius capillaris	sheep	lung	—	yes	—	1
Strongylina	Ancylostomatidae	*Ancylostoma caninum*	dog	intestine (mucosa)	yes	yes	yes	1,7,14,19,20,21
	Syngamidae	*Syngamus trachea*	pheasant	trachea (mucosa)	yes	yes	yes	1,2
	Strongylidae	*Cyathostoma lari*	seagull	orbital sinus (mucosa)	—	yes	—	22
		Trichonema sp.	horse	colon	yes	yes	yes	1
		Strongylus endentatus	horse	colon	—	yes	yes	1
	Oesophagostomidae	*Oesophagostomum columbianum*	sheep	colon (lumen)	—	yes	yes	1
Metastrongylina	Metastrongylidae	*Metastrongylus* sp.	pig	lung	yes	yes	yes	1,23
Spirurida	Onchocercidae	*Litomosoides carinii*	cotton rat	thorax	no	no	no	1,24
Filariina		*Dirofilaria immitis*	dog	heart	no	—	—	25
		Brugia malayi	primates	lymphatics	no	—	—	26
Trichinellida		*Trichuris ovis*	sheep	caecum (mucosa)	no	no	no	1
		Capillaria hepatica	mouse	liver	no	—	—	27

† From appearance in electron micrographs, ‡ no observation made, § see text.

References: 1, this paper; 2, Borgers & De Nollin 1974; 3, Borgers *et al.* 1975; 4, Kessel *et al.* 1961; 5, Sheffield 1964; 6, Colam 1971*b*; 7, Andreassen 1968; 8, Lee & Anya 1968; 9, Comley 1980; 10, Colley 1970; 11, Colam 1971*a*; 12, Munn 1977; 13, Munn 1981; 14, Munn & Greenwood 1983; 15, Smith & Harness 1972; 16, Lee & Martin 1980; 17, D. L. Lee 1969; 18, Jamuar 1966; 19, C.-C. Lee 1969; 20, Miller 1967; 21, Browne & Chowdhury 1959; 22, Colam 1971*c*; 23, Jenkins & Erasmus 1969; 24, Kagei 1963; 25, Lee & Miller 1969; 26, Vincent *et al.* 1975; 27, Wright 1963.

microvilli. A simple explanation is that the endotube holds the lumen open. This implies a skeletal role for the endotube, (see below). Freshly isolated endotube–brush-border complexes from *Haemonchus* retain their circular profile as do the preparations from other intestines with lumina of relatively small diameter. Endotube preparations from intestines with comparatively wide lumina, for example *Ancylostoma* and *Strongylus*, are much more collapsed and all collapse to some extent during fixation with glutaraldehyde.

The restriction of endotubes to nematodes with intestines composed either of very large cells or a syncytium points to a skeletal role as the primary function of the endotube. This may simply be to withstand the pressure generated within the lumen by the pharyngeal pump. It is presumed that the microfibrillar elements with which the endotube is associated are also involved in this role. Although all the endotubes had microfibrils associated with them, their quantity and extent were variable. The ease with which preparations of the endotubes could be obtained free of basal-side cytoplasm depended on the extent to which the microfibrils were retained and this in turn is attributed to the total amount of microfibrils present initially and, presumably, the strength of the binding between them and the endotube.

A partial contractile role for the endotube cannot be excluded since preliminary experiments have shown that isolated endotubes from *Haemonchus contortus* contract when exposed to ATP (E. A. Munn, unpublished observations).

In addition to the intrinsic interest in the existence of endotube–brush-border complexes there is interest in the use of single membrane preparations for studies of metabolite and ion fluxes. Of the complexes tested, that from *Haemonchus* seems to be the most useful from this point of view with that from *Dictyocaulus* as a second choice. Although the intestine of *Dictyocaulus* is much longer its luminal diameter is not significantly greater, nor does it so easily yield an endotube–brush-border preparation.

We are especially grateful to the many people who have generously helped personally or by advice with the supply of the live, or freshly dead, adult nematodes used in this study. *Ancylostoma caninum* from dog intestine were supplied by Mr D. Seymour (Pfizer Central Research Laboratories, Sandwich, Kent and Mr P. A. Kingsbury (Wellcome Research Laboratories, Berkhampstead, Hertfordshire) who also supplied the *Toxocara canis*. *Syngamus trachea* and *Heterakis gallinae* were obtained from pheasants provided by Mr R. Green (Home Farm, Babraham). *Trichonema* sp. and *Strongylus edentatus* were supplied by Mr C. Wannop (Equine Research Laboratories, Newmarket). *Nippostrongylus brasiliensis* and *Syphacia obvelata* were supplied by Dr Jean Martin and Mr M. Wainwright (Molteno Institute, University of Cambridge). *Litomosoides carinii*, from the cotton rat, were provided by Dr M. Worms (National Institute for Medical Research, Mill Hill, London). *Metastrongylus* and *Muellerius capillaris* were provided by Mr A. Duncan and Mr W. Potter (Playles Meat Ltd abattoir). *Dictyocaulus viviparus* from calves were provided by Mr R. Sandercock (Glaxo, Bury Green, Herts). *Ascaris suum* were obtained from a local abattoir. *Haemonchus contortus* were from sheep infected as described by Coadwell & Ward (1975). *Ostertagia circumcincta*, *O. trifurcata*, *Nematodirus spathiger*, *N. battus*, *Oesophagostomum columbianum*, *Trichostrongylus colubriformis*, *Strongyloides papillosus* and *Trichuris ovis* were obtained from naturally infected sheep at Babraham. Cultures of the free-living nematodes *Pelodera strongyloides* and *Panagrellus redivivus* were supplied by Dr D. J. Hooper (Rothamsted Experimental Station, Harpenden).

We also thank Dr D. W. T. Crompton (Molteno Institute, University of Cambridge) and Dr A. M. Shepherd (Rothamsted Experimental Station, Harpenden) for their advice and help.

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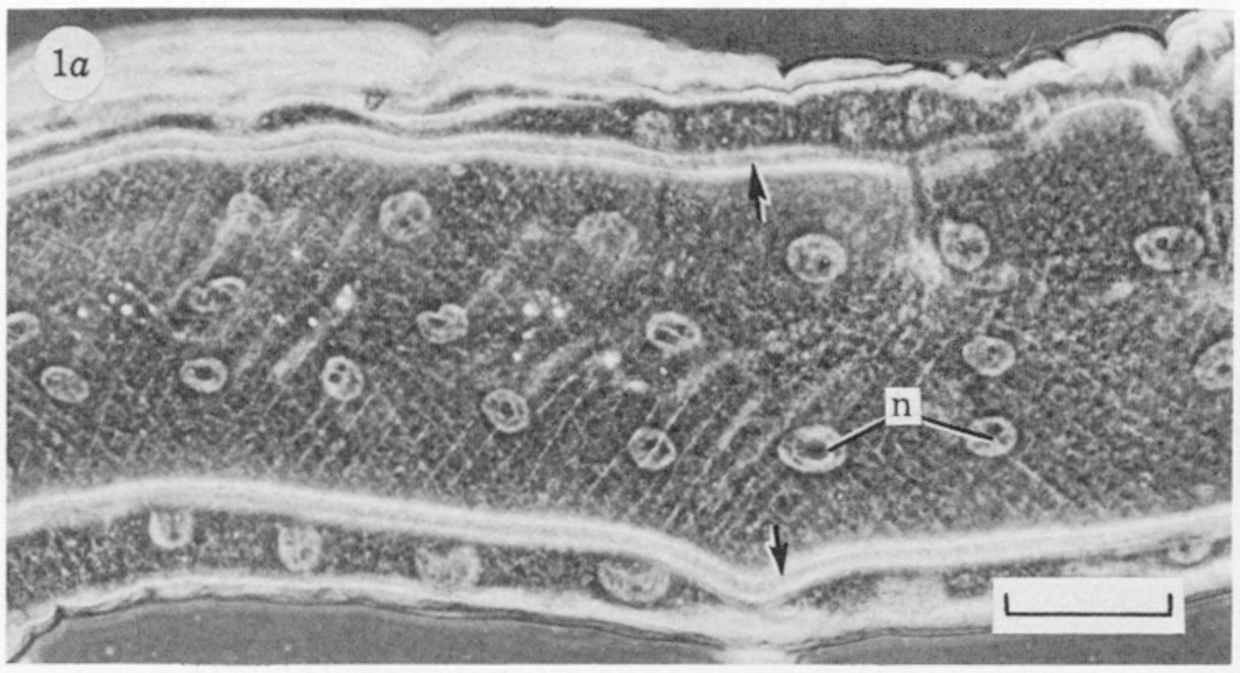
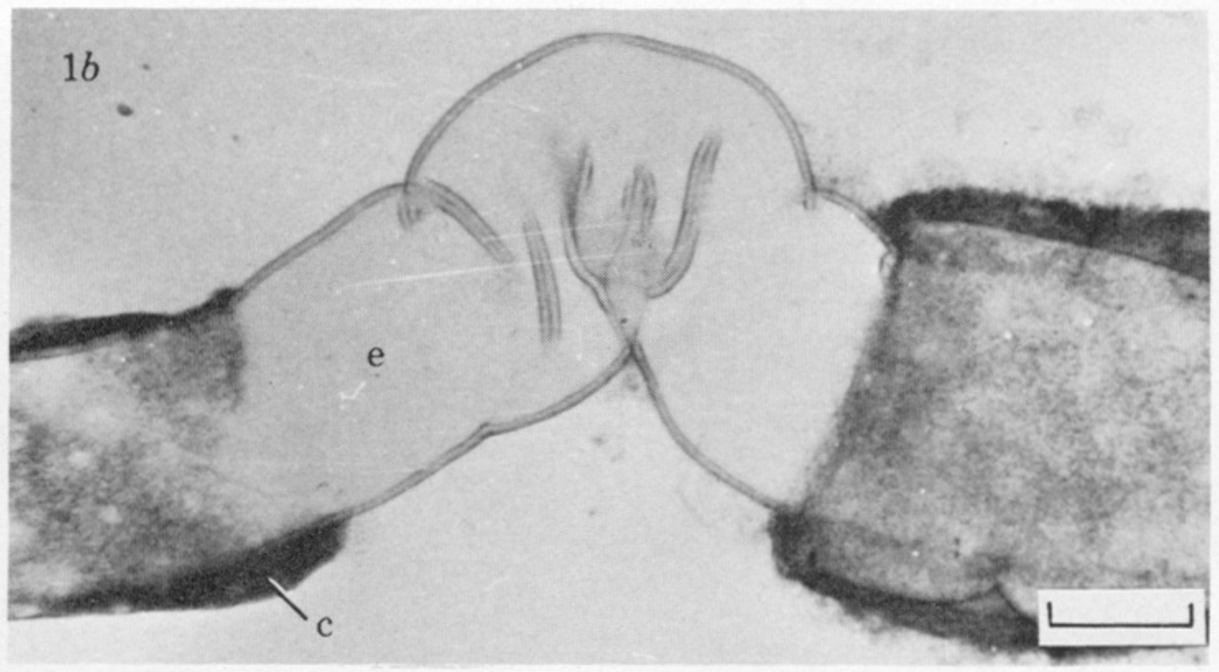
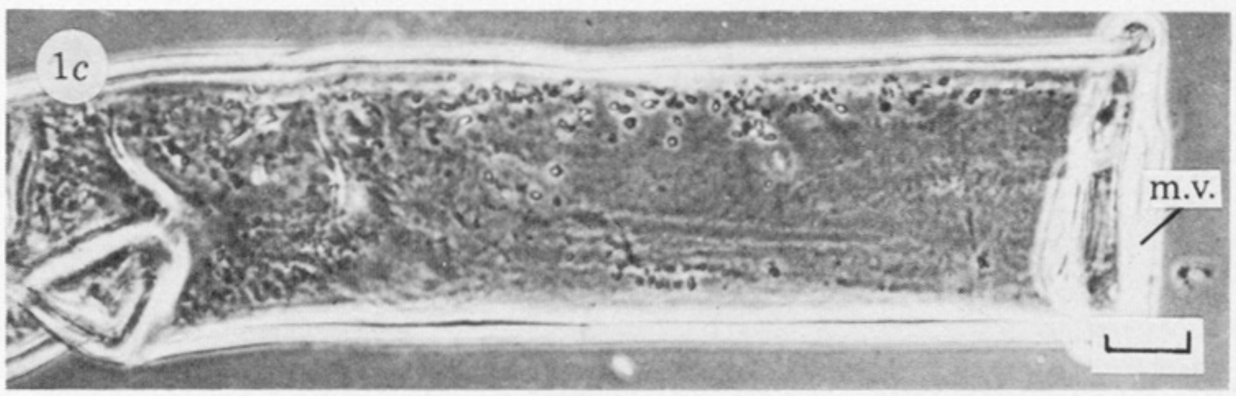
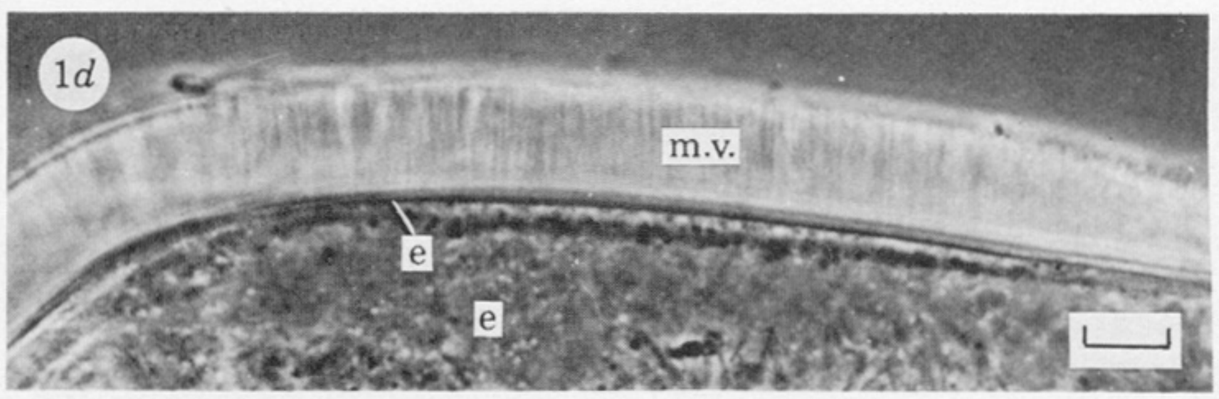
List of abbreviations used in figures

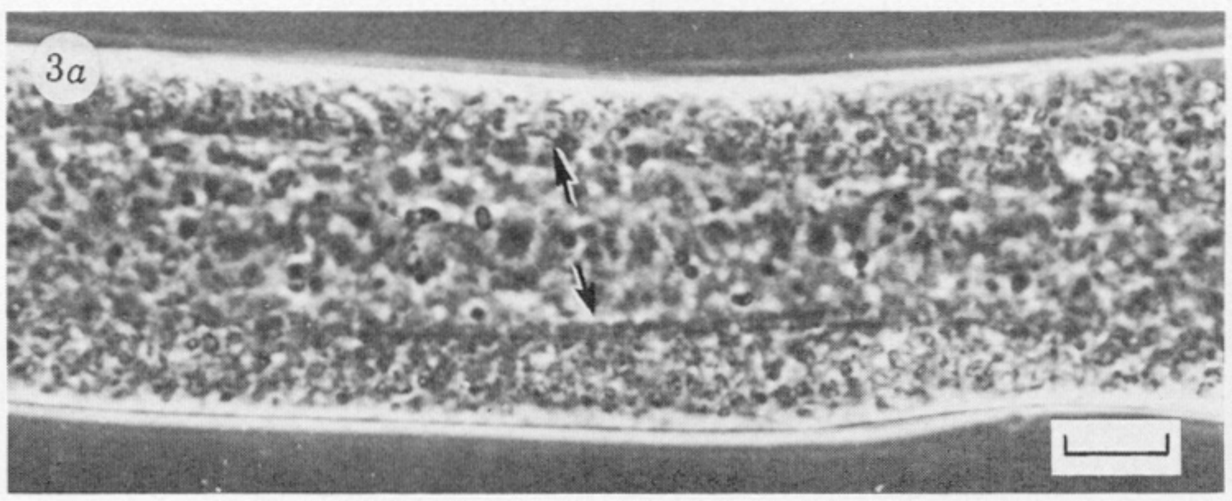
a	axial core of microvillus	m.f.	microfibril
b	basement membrane	m.v.	microvillus
c	basal cytoplasm	n	nucleus
e	endotube	t	terminal bar
f	fenestration in endotube	t.w.	terminal web
l	intestinal lumen	v	microvesicle in *Ascaris* and *Toxocara*











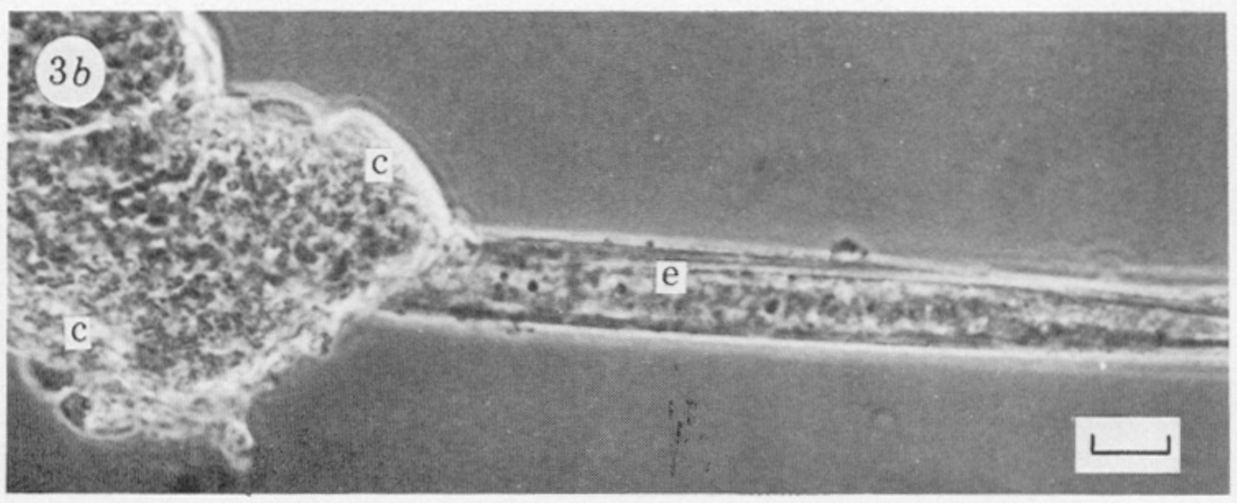
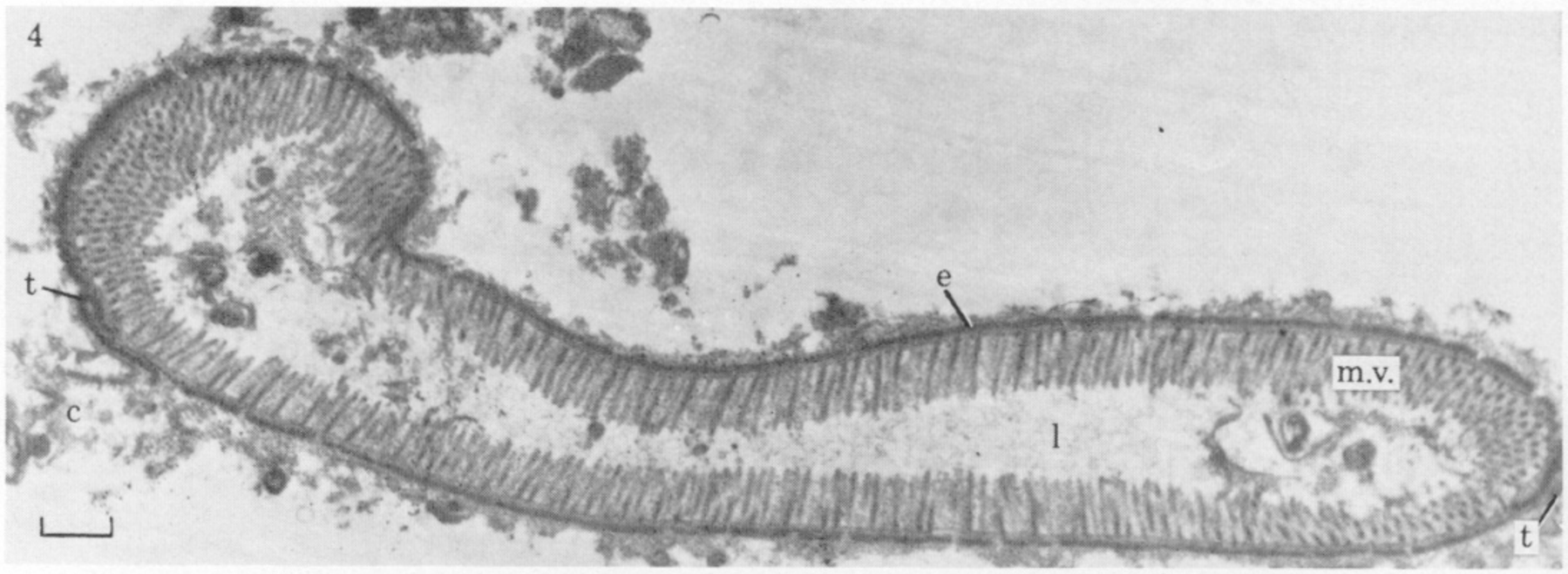
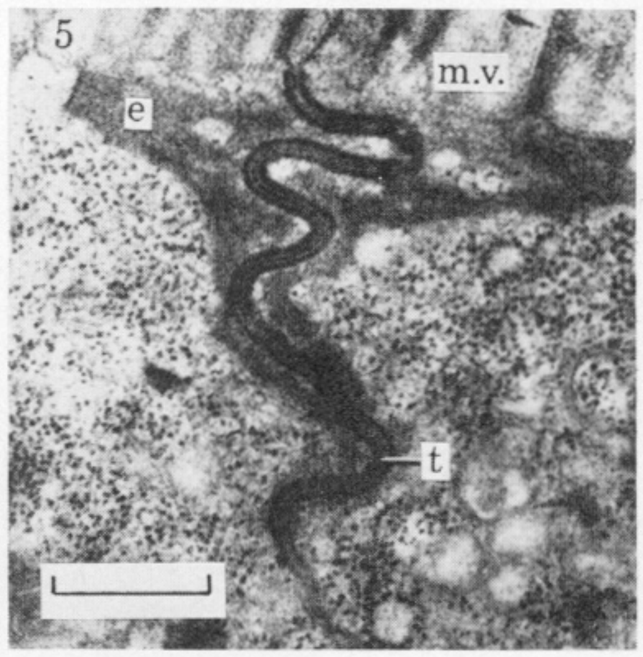


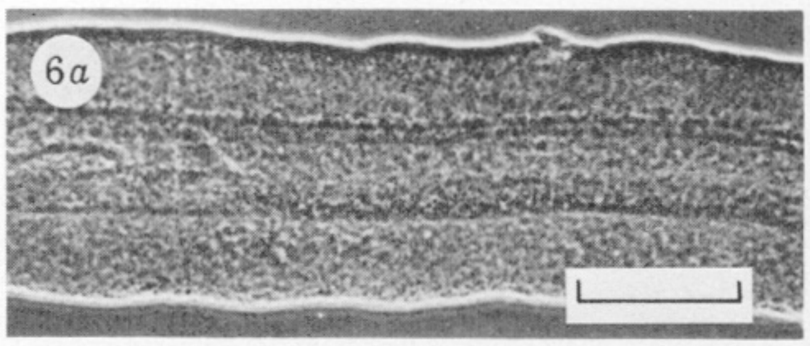
FIGURE 1. Light micrographs of (*a*) part of an intact intestine from *Heamonchus contortus*. Rows of nuclei (n) and the surface of the lumen (arrows) are clearly visible. The cross-hatching effect is due to organization of the microvilli into blocks of aligned rows. (*b*) A partially dissected intestine of *H. contortus* showing that the endotube (e) is continuous with the luminal surface visible within the intact portions of the intestine. (*c*) A piece of endotube from *H. contortus*. Microvilli are most clearly visible at the cut end of the endotube which, characteristically, has everted for a short distance. (*d*) The microvilli exposed at the cut end of the endotube at higher magnification. Scale bars, (*a*, *b*), 50 μm; (*c*) 10 μm; (*d*) 10 μm.

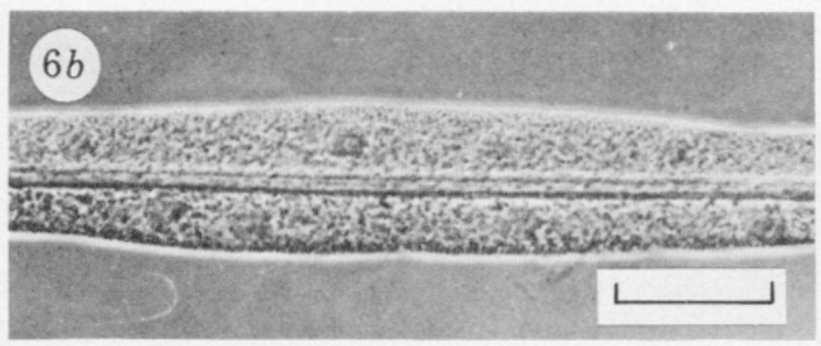
FIGURE 2. Electron micrograph of a piece of an endotube preparation from *H. contortus* to show its apearance in thin section. The endotube (e) is perforated with fenestrations (f); on its basal surface there is a layer of microfibrils. Scale bar 1 μm.

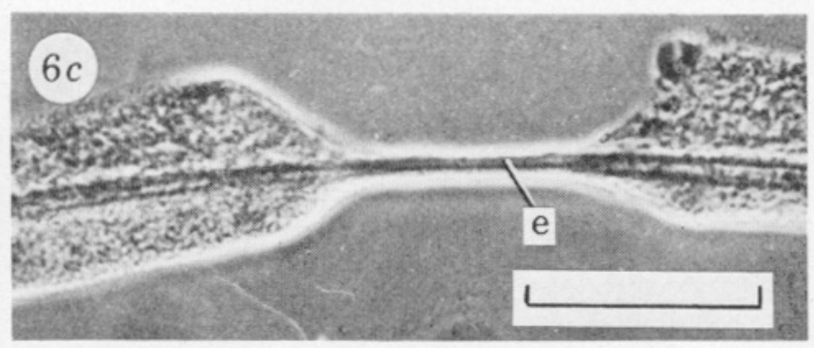
FIGURE 3. Phase contrast light micrographs of (*a*) the intact intestine of *Ostertagia circumcincta*. The surface of the lumen is visible (arrows); (*b*) partially dissected intestine to show the endotube protruding from the end of the basal cytoplasm. Scale bars 10 μm.

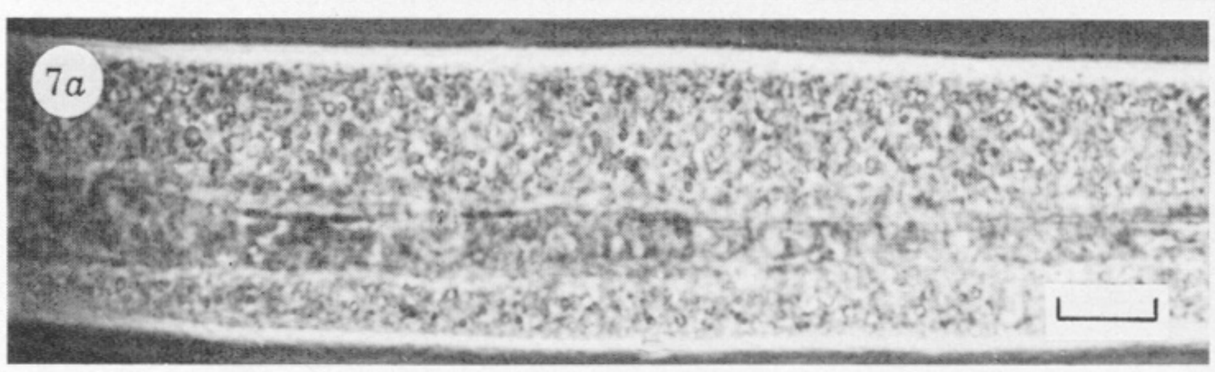


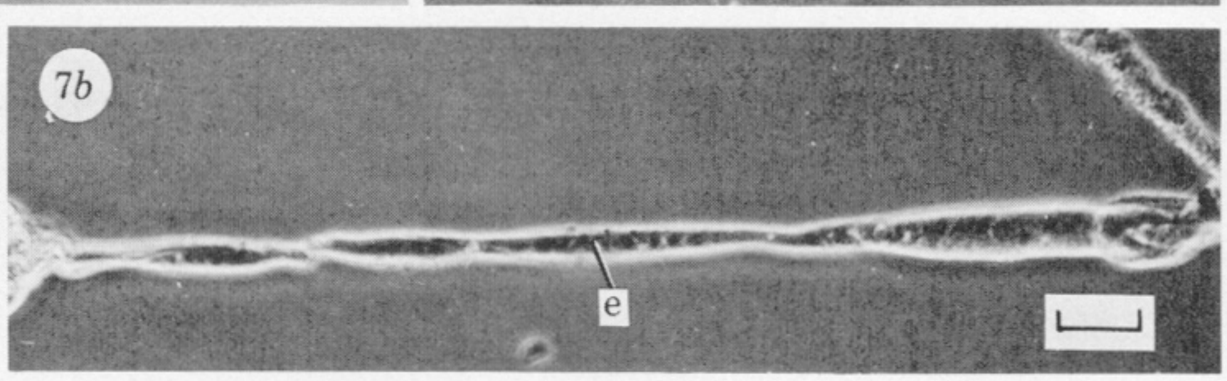


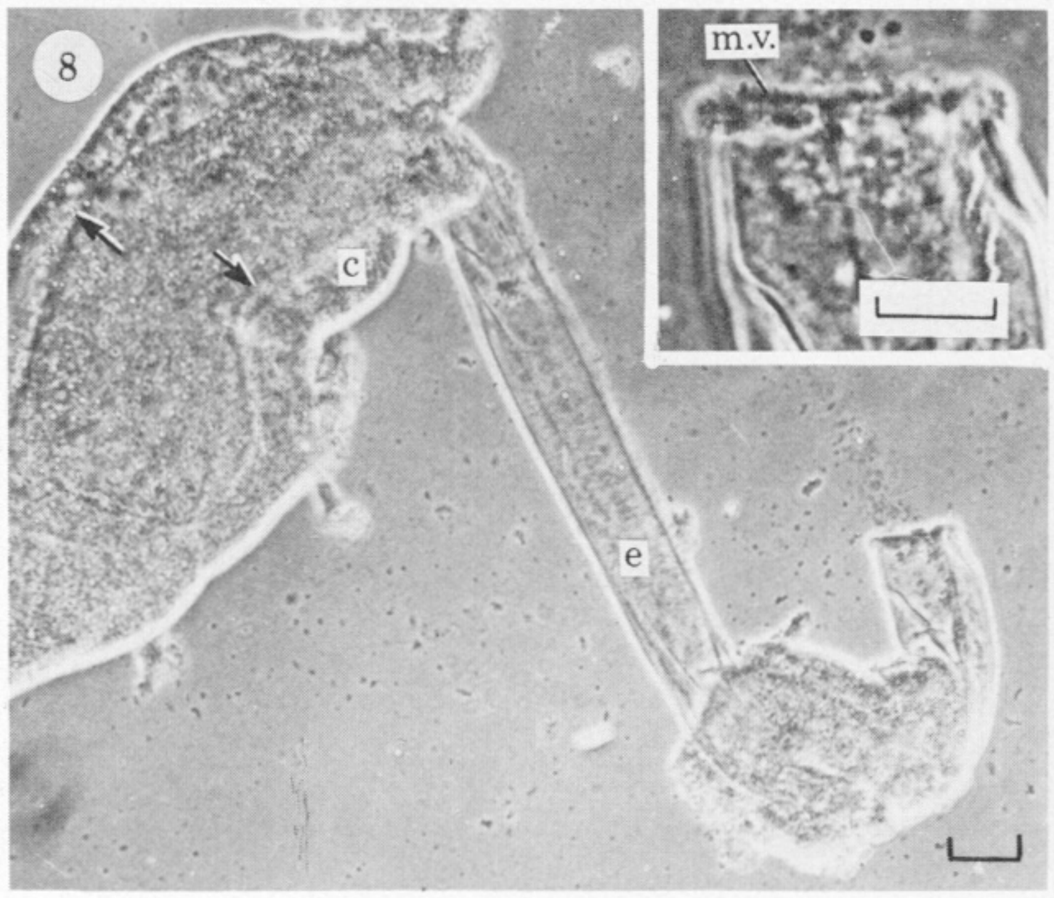












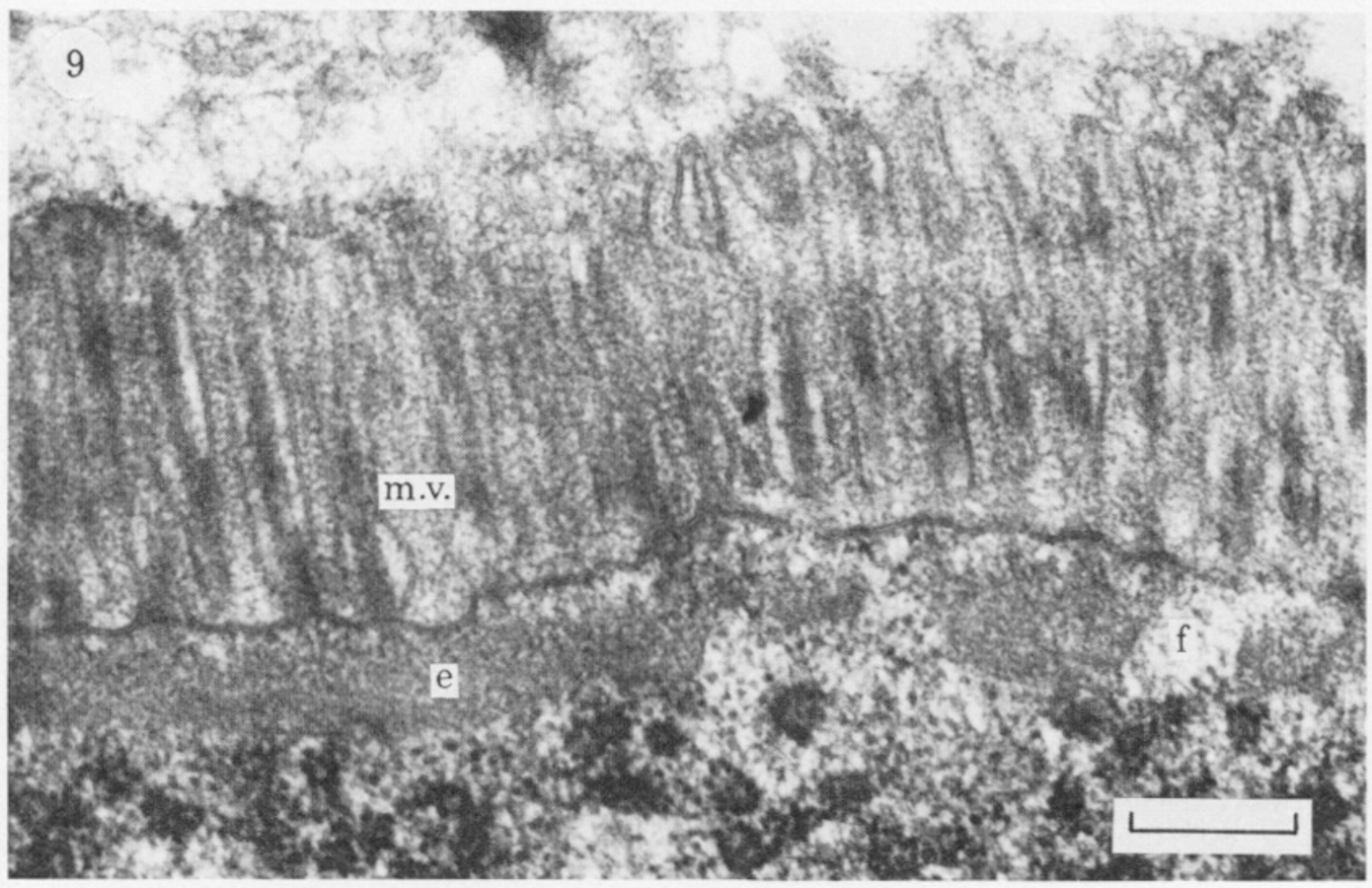


FIGURE 4. Electron micrograph of a cross-section of an endotube–brush-border complex obtained from *Ostertagia*. Two terminal bars (t) and small amounts of basal cytoplasm are retained. Scale bar, 1 μm.

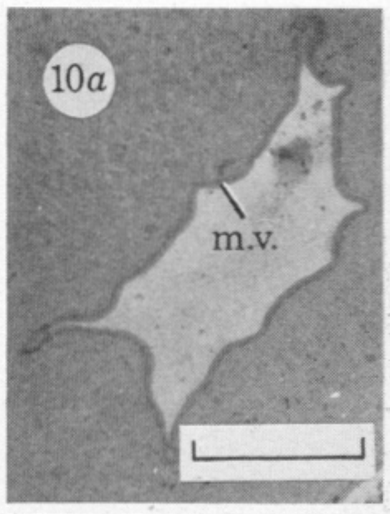
FIGURE 5. Electron micrograph of part of a transverse section of intestine of *Ostertagia* to show the extension of the endotube (e) along the terminal bar (t). Scale bar, 0.5 μm.

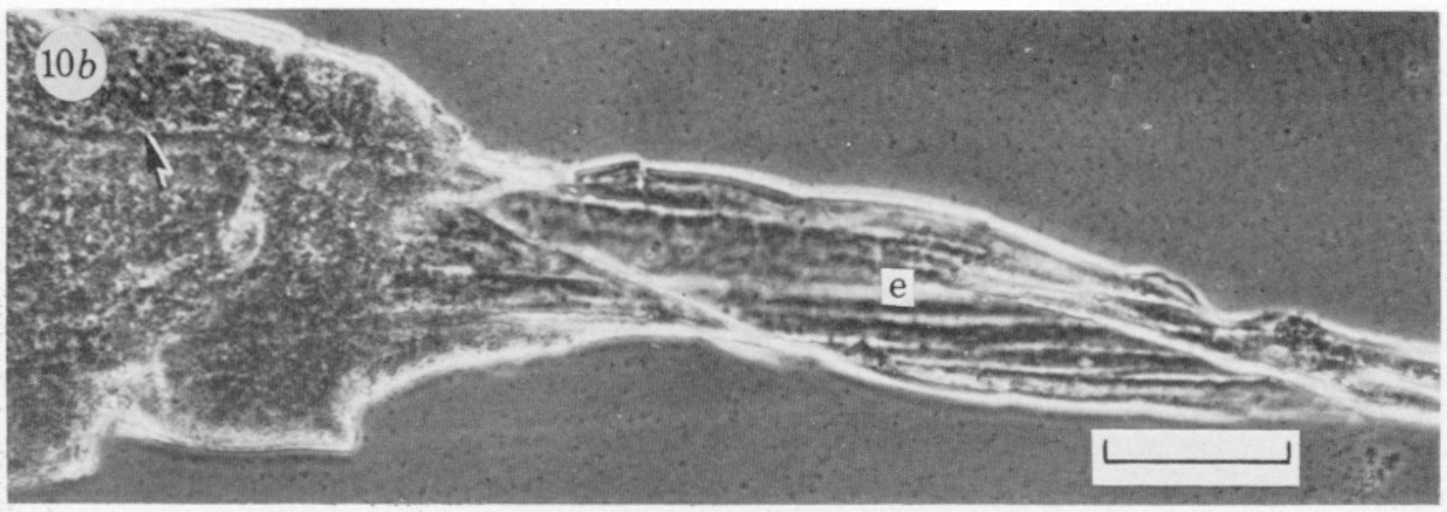
FIGURE 6. Phase contrast light micrographs of (*a*) posterior, (*b*) anterior regions of intact intestine from *Nematodirus*. The luminal surface is clearly visible and, (*c*), is continuous with the endotube exposed by blunt dissection. Scale bars, 5 μm.

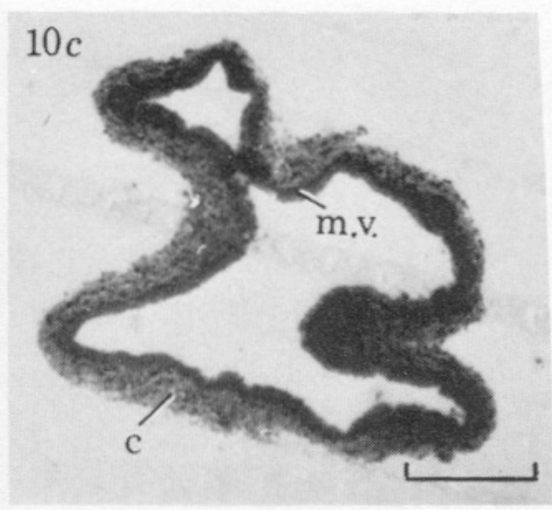
FIGURE 7. Phase contrast light micrographs of (*a*) intact intestine of *Trichostrongylus* and (*b*) a piece of endotube dissected from it. Scale bars, (*a*), 10 μm; (*b*), 20 μm.

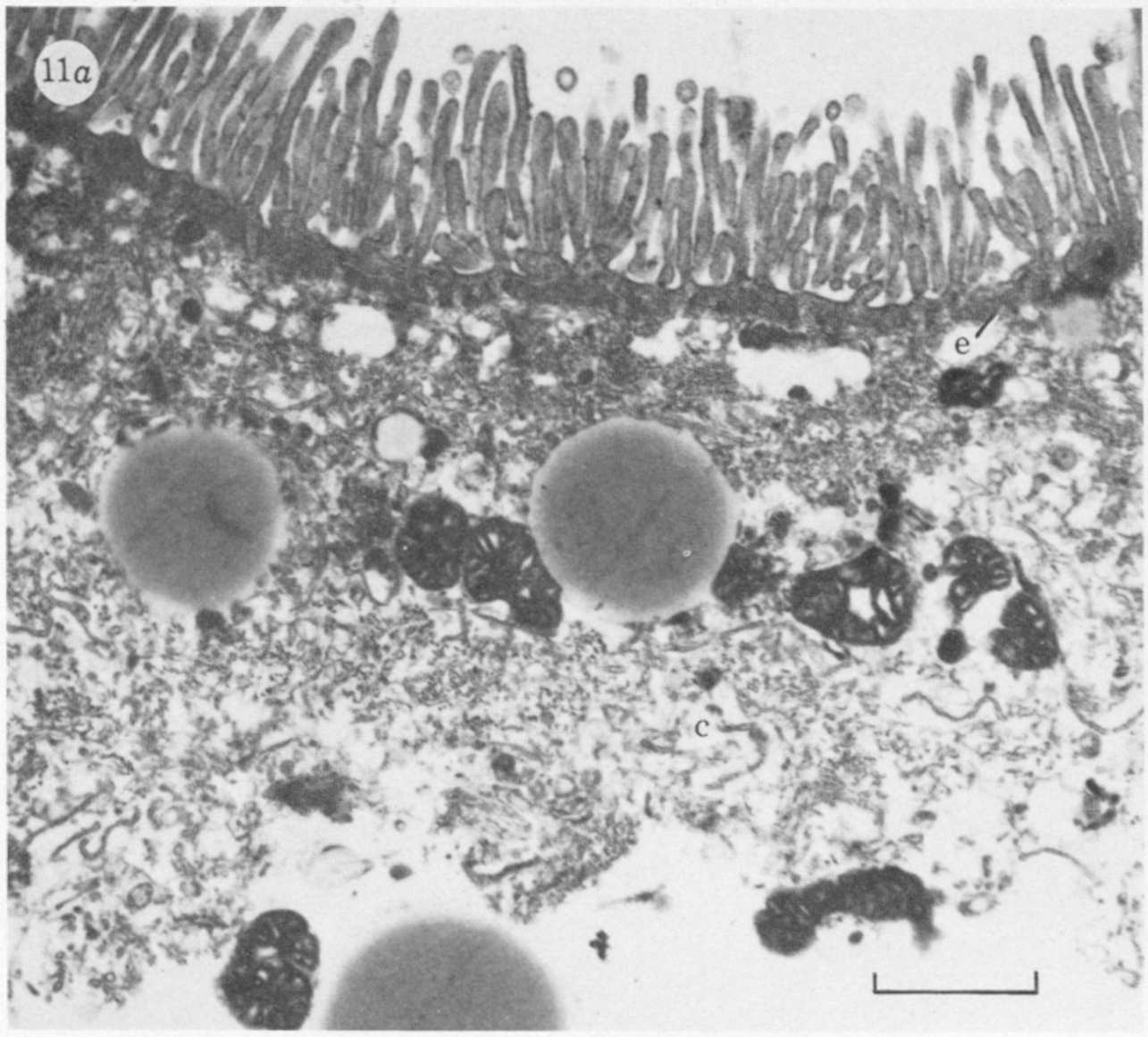
FIGURE 8. Phase contrast light micrograph of partially dissected intestine from *Nippostrongylus brasiliensis*. The surface of the lumen is visible within the intact portion of the intestine (arrows) and is continuous with a short length of exposed endotube. A fringe of microvilli can just be discerned at the free end of the endotube (shown at higher magnification in the inset). Scale bar, 20 μm (inset, 10 μm).

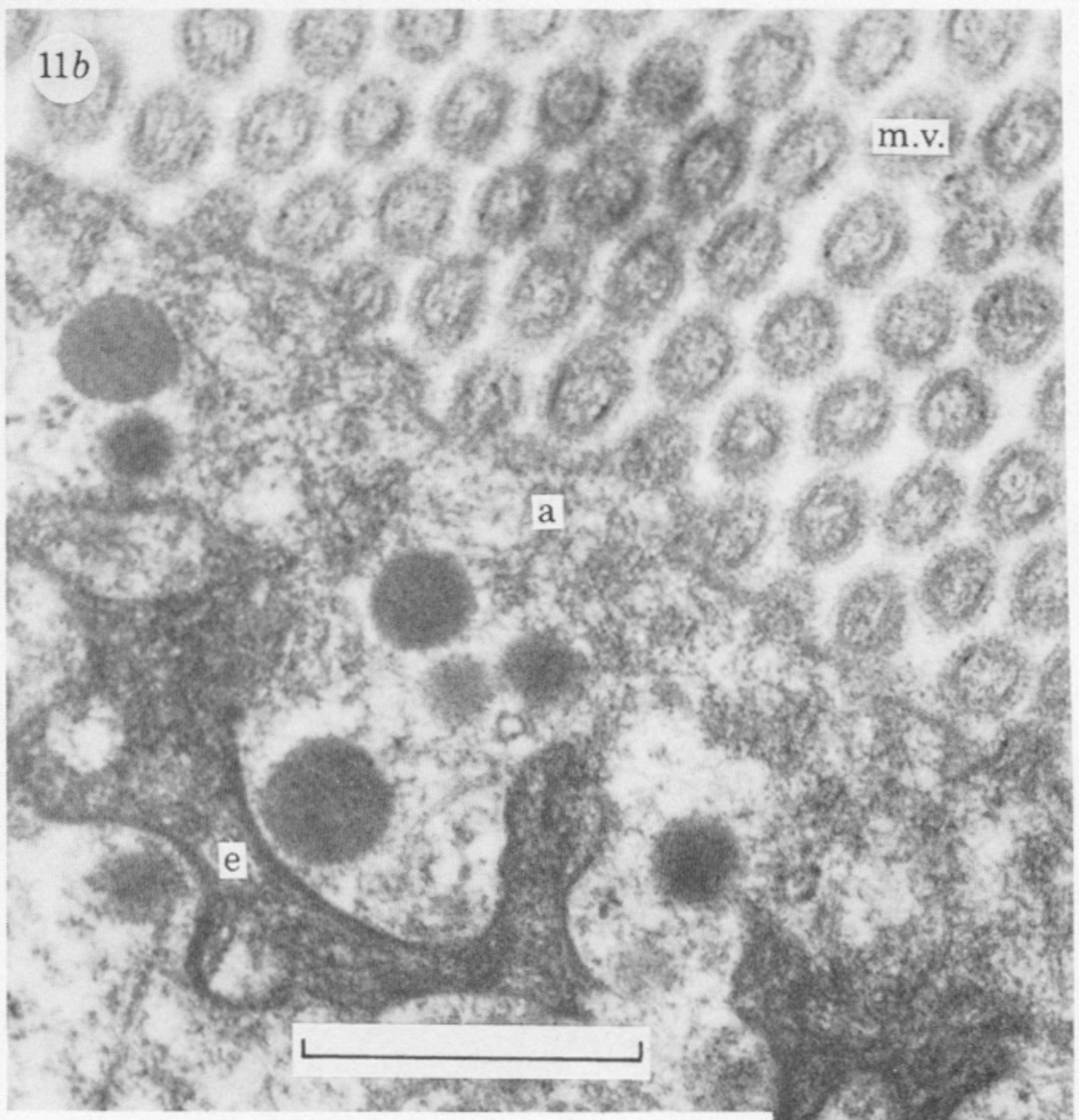
FIGURE 9. Electron micrograph of a section of part of the intestine of *N. brasiliensis*. Scale bar, 0.25 μm.

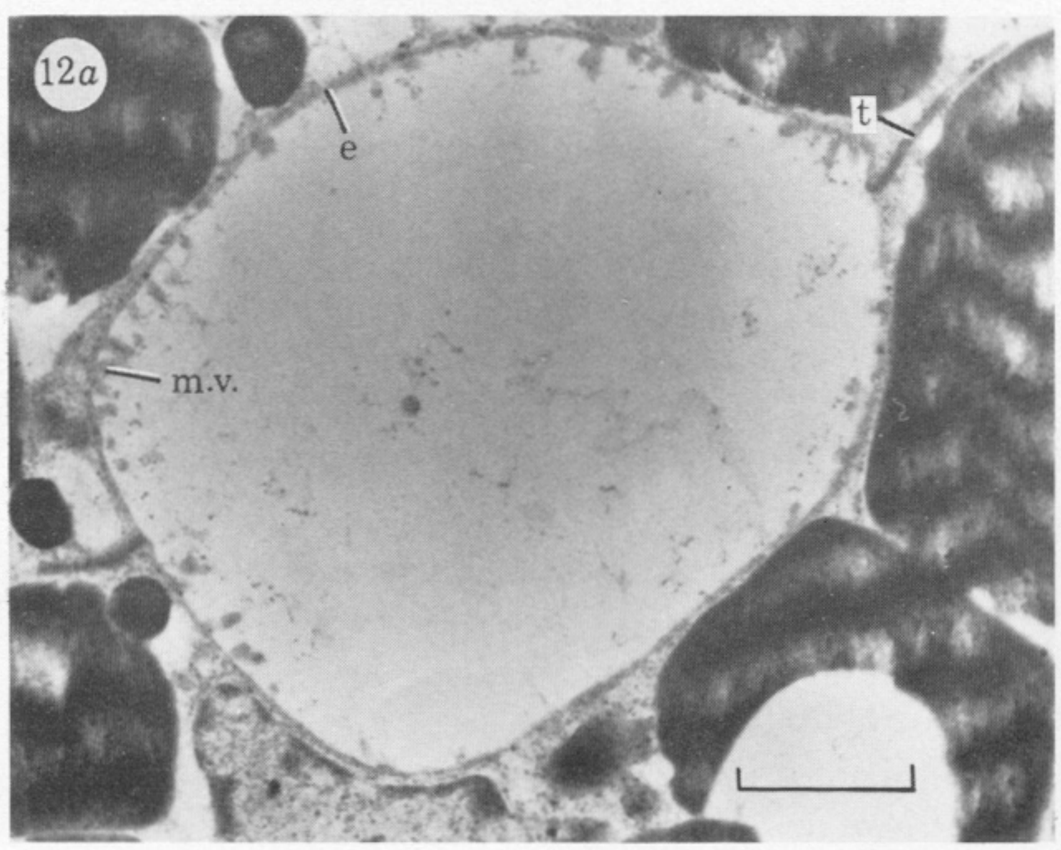












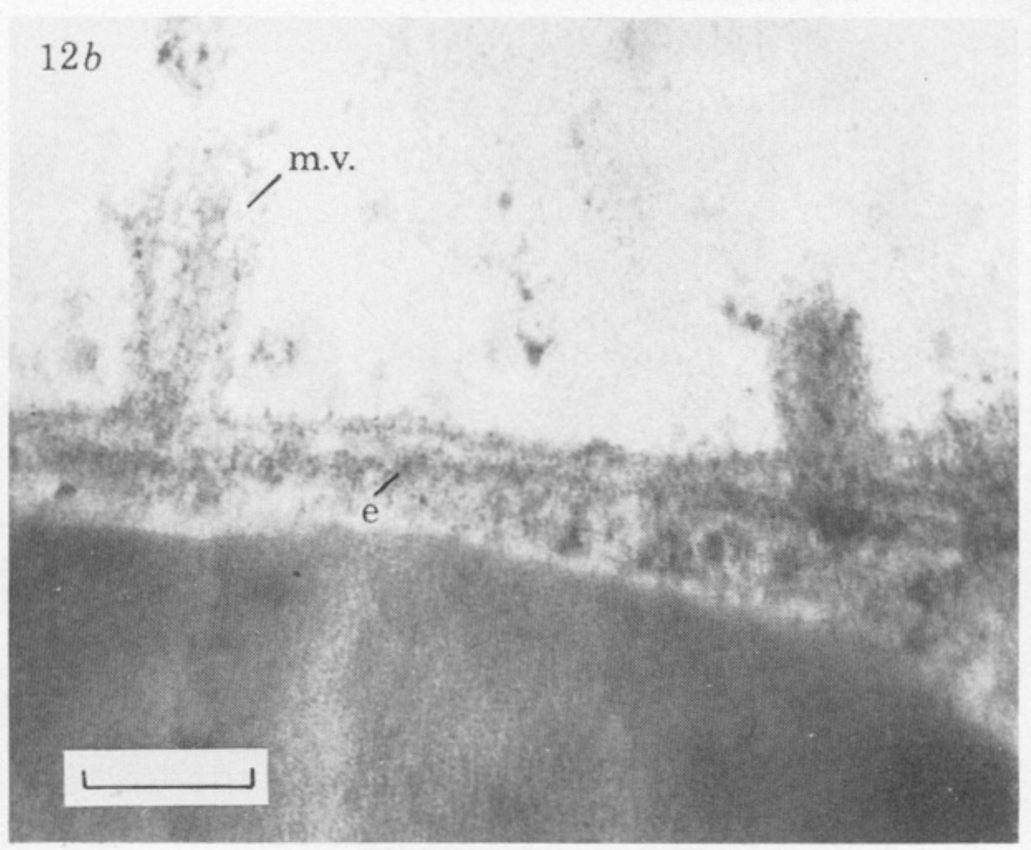
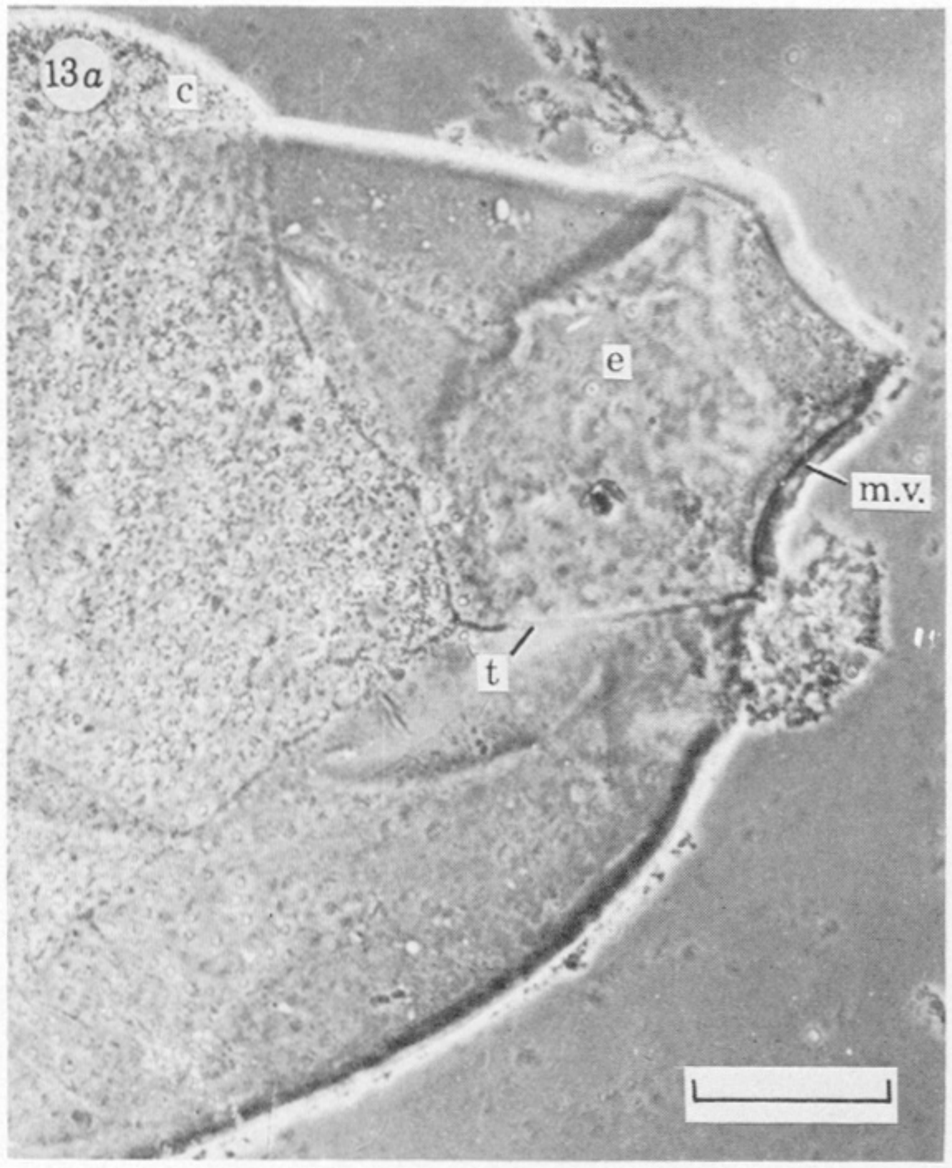
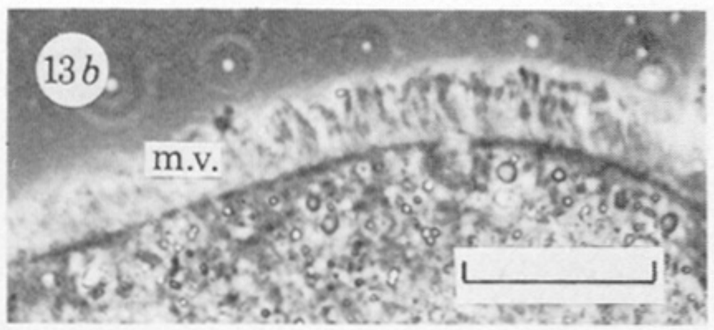


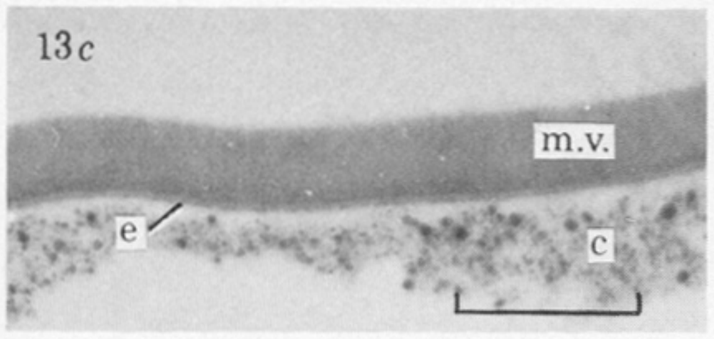
FIGURE 10. Light micrographs of (*a*) cross-section of part of the intestine, (*b*) partially dissected intestine showing the protruding endotube to be continuous with the lumen surface (arrow) and (*c*) cross-section of the endotube–brush-border complex with associated layer of basal cytoplasm, from *Dictyocaulus viviparus*. Scale bars, (*a*, *b*), 50 μm; (*c*) 10 μm.

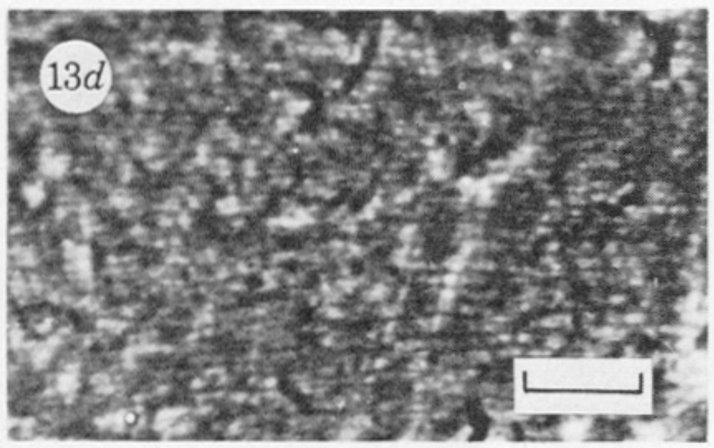
FIGURE 11. Electron micrographs of parts of endotube preparations from *Dictyocaulus viviparus*. (*a*) This is from a thin section adjacent to the 0.5 μm thick section shown in figure 10*c*. (*b*) This is to show the basal prolongations of the endotube with which microfilaments are associated. Scale bars, (*a*) 1 μm; (*b*) 0.5 μm.

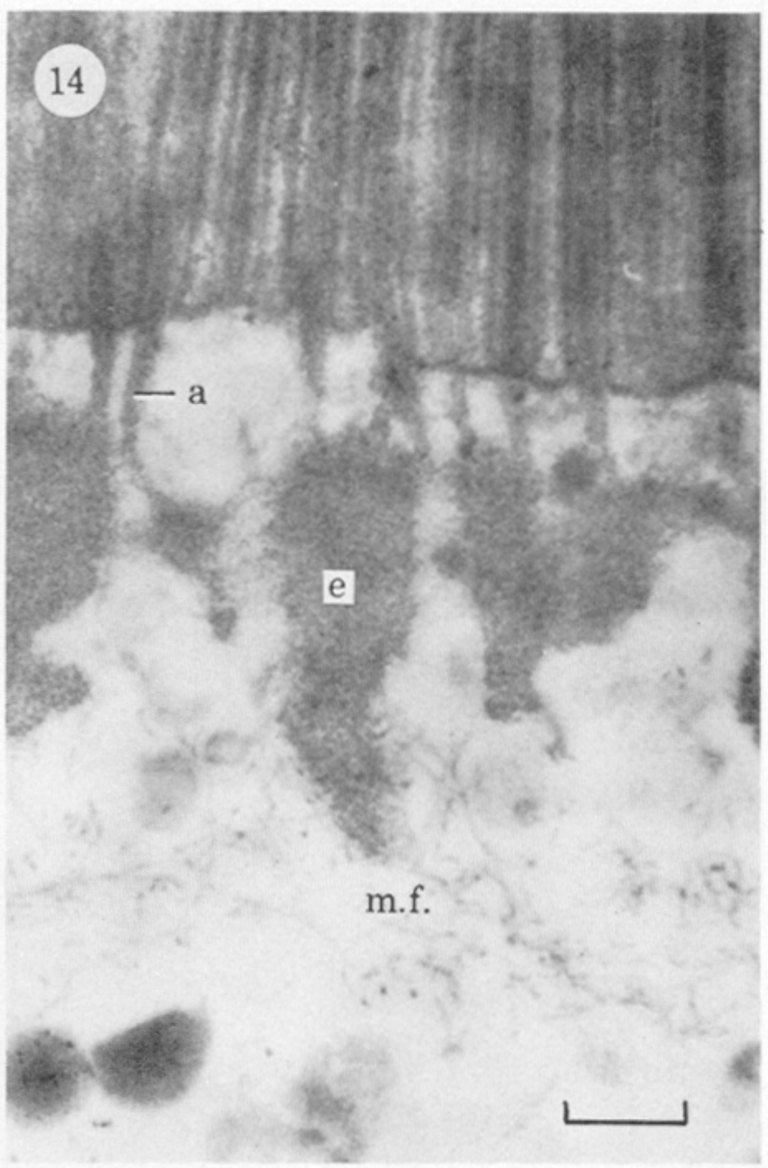
FIGURE 12. Electron micrographs of parts of this transverse sections through the intestine of *Muellerius*. (*a*) The oval shaped lumen is lined with short, sparse microvilli the axial cores of which join to the thin continuous layer immediately below the plasma membrane as shown at higher magnification in (*b*). The adjacent cytoplasm is packed with large, heavily staining granules. Scale bars, (*a*) 1 μm; (*b*) 0.1 μm.

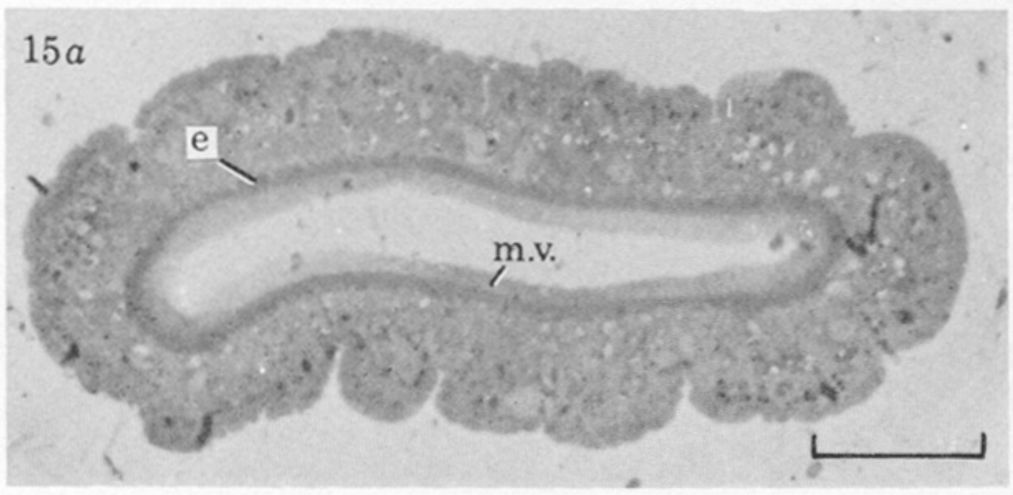


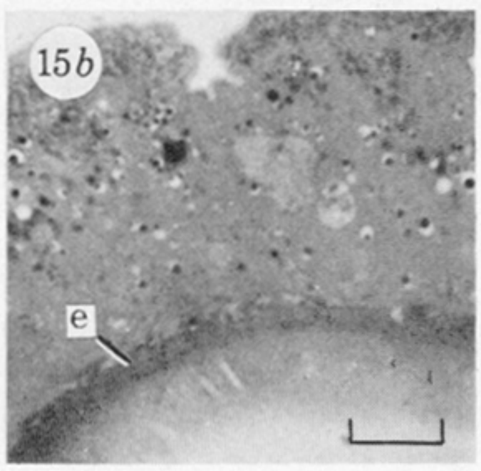


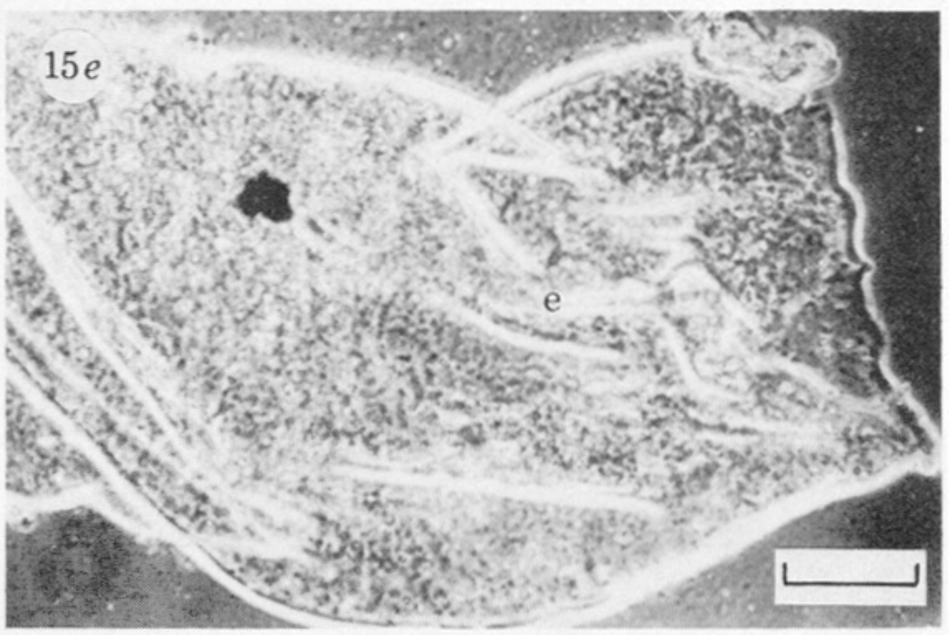


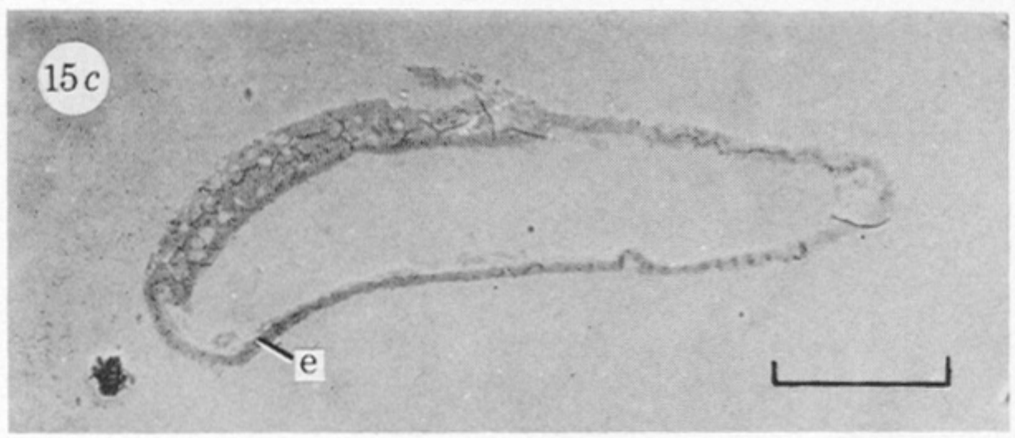


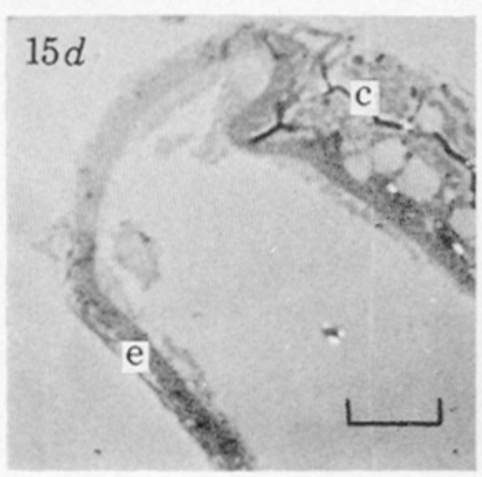












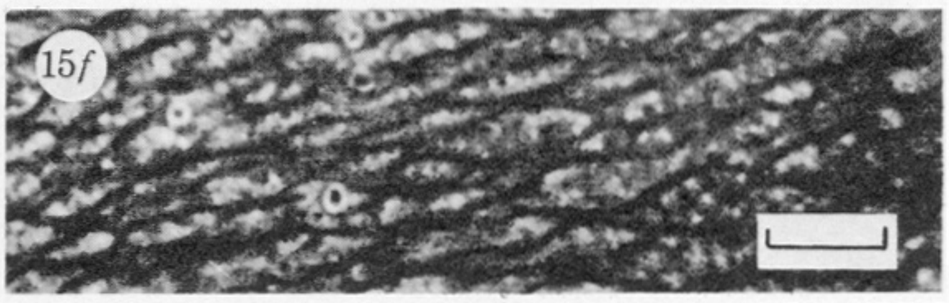
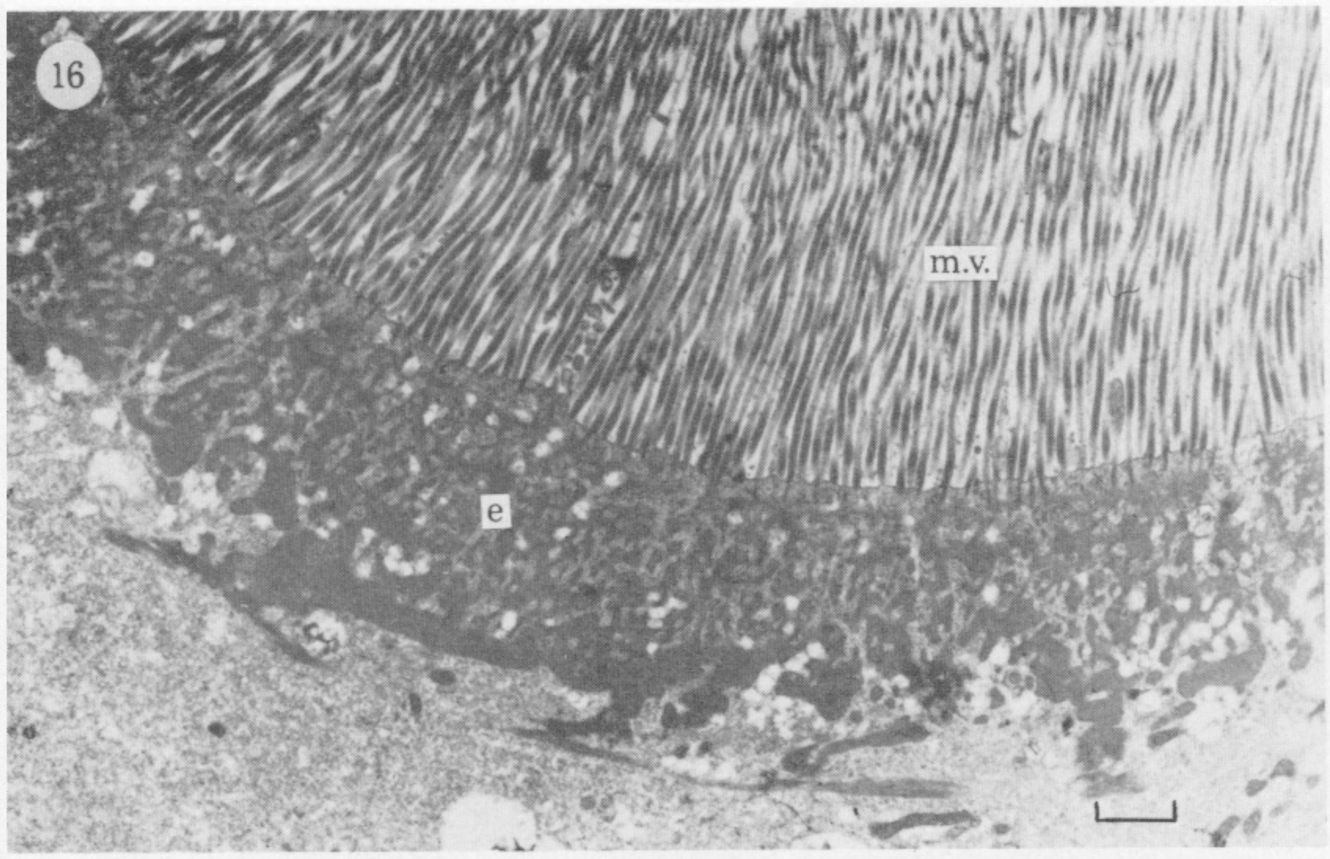
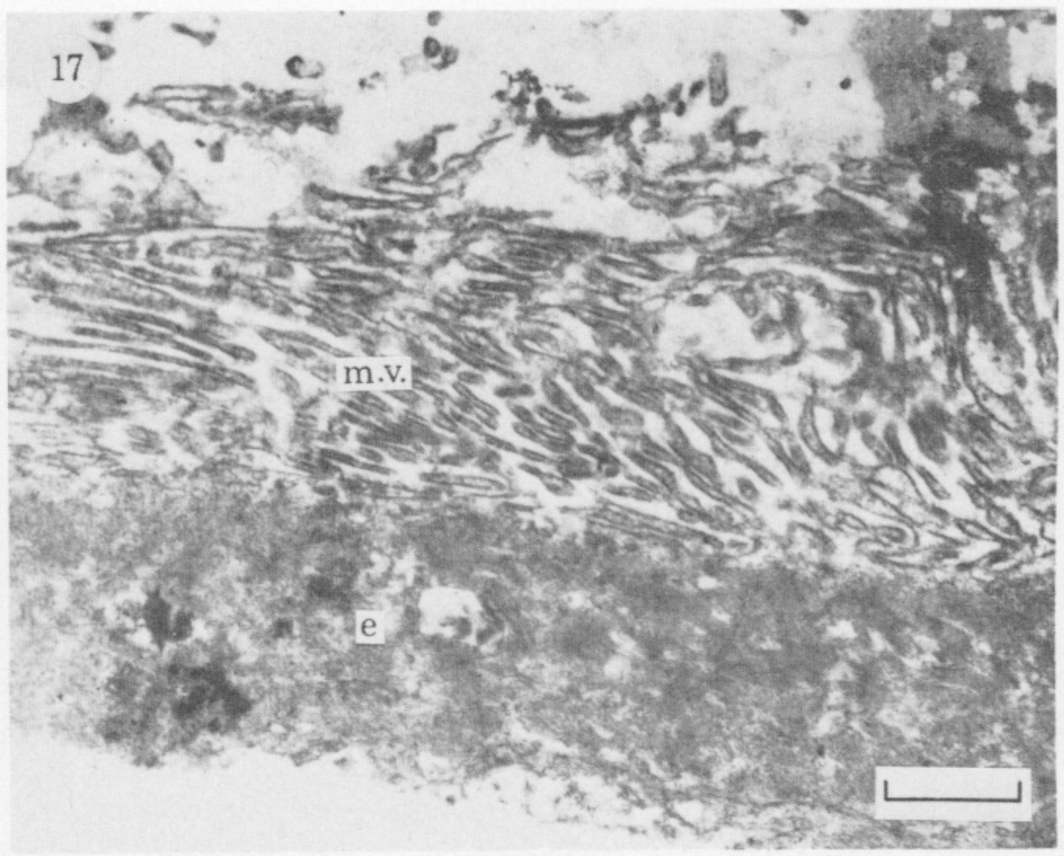


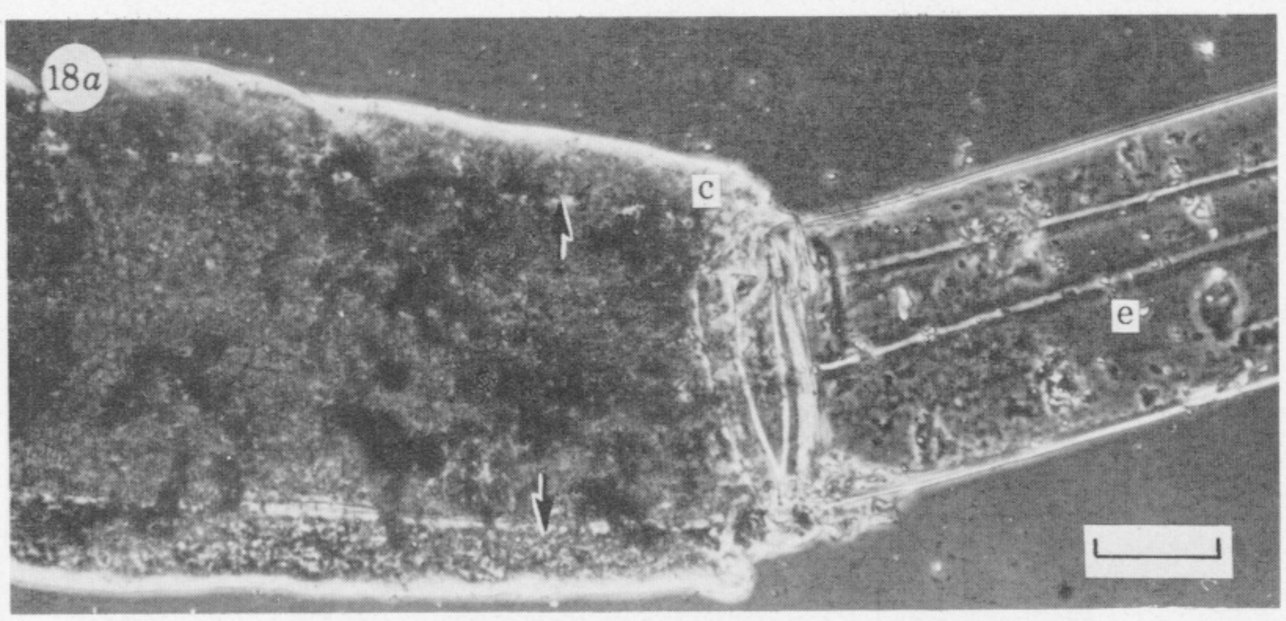
FIGURE 13. Light micrographs of (*a*) partially dissected intestine, (*b*), (*c*) a fringe of microvilli and (*d*) the reticulum on the basal side of an endotube from *Ancylostoma caninum*. (*a*), (*b*), (*d*). These are phase contrast micrographs; (*c*) is a micrograph of a section of an endotube preparation. Scale bars, (*a*) 50 μm; (*b*,*c*) 20 μm; (*d*) 10 μm.

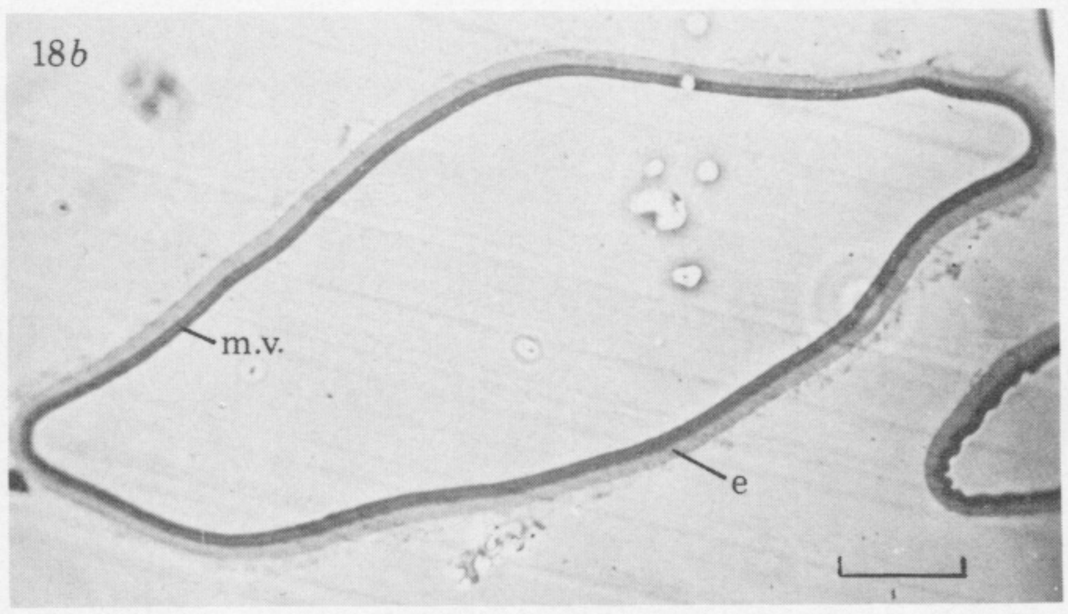
FIGURE 14. Electron micrograph of a thin section of an endotube preparation from *Ancylostoma* showing connections to the axial cores of the microvilli and, on the basal side, the microfibrils. Scale bar, 0.2 μm.

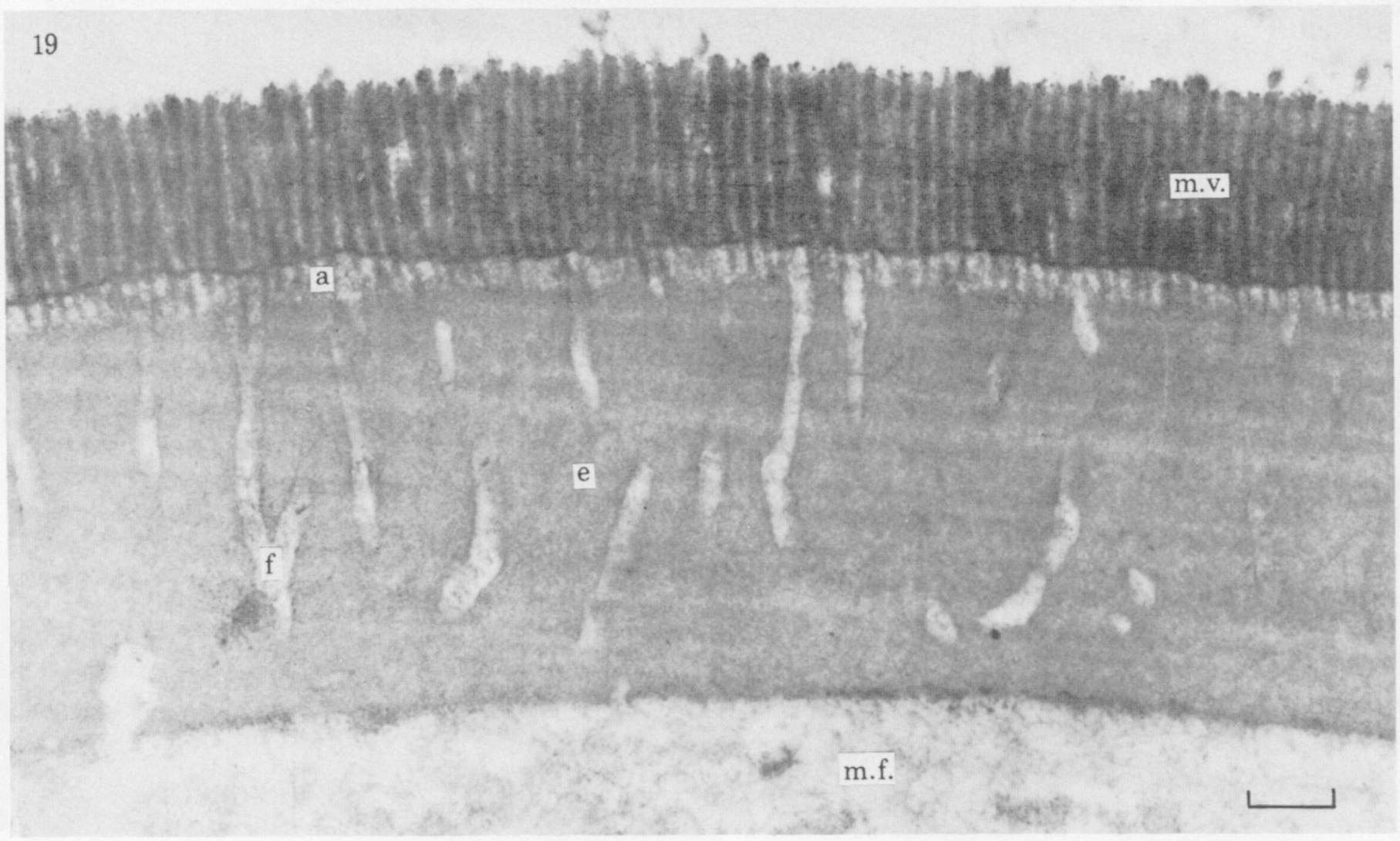
FIGURE 15. Light micrographs of (*a*), (*b*) a transverse section of the intestine of *Syngamus trachea*, (*c*), (*d*) a transverse section of an endotube preparation obtained from an intestine kept at 4 °C for 6 d. (*e*) Phase contrast light micrograph of an endotube preparation from an intestine aged as in (*c*) and (*d*), and (*f*) the reticulum on the basal surface of a piece of endotube from fresh intestine. Scale bars, (*a*,*c*), 50 μm; (*b*,*d*,*f*), 10 μm; (*e*) 100 μm.

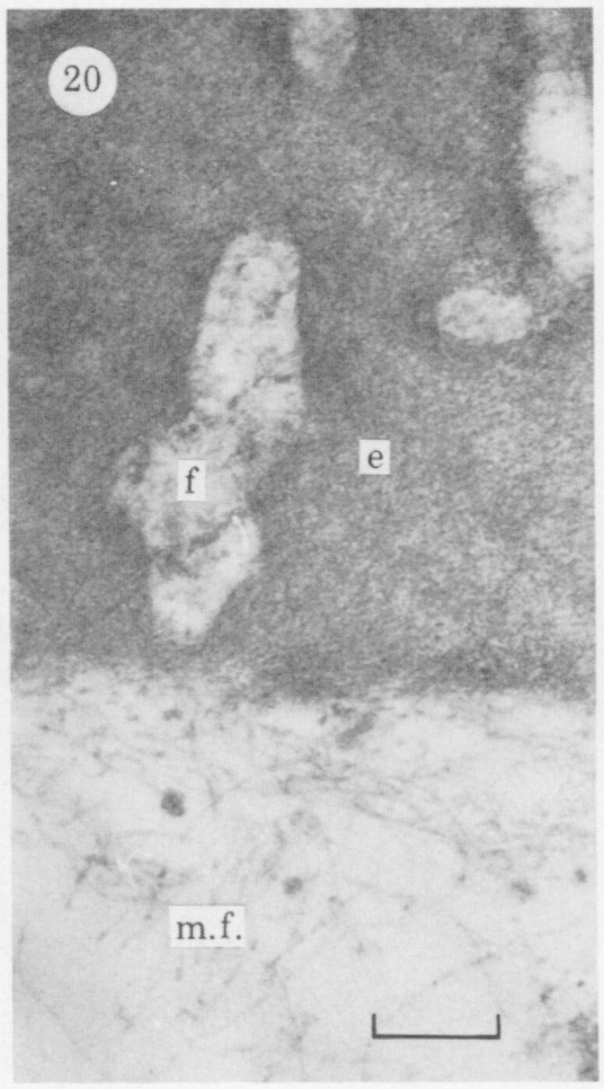




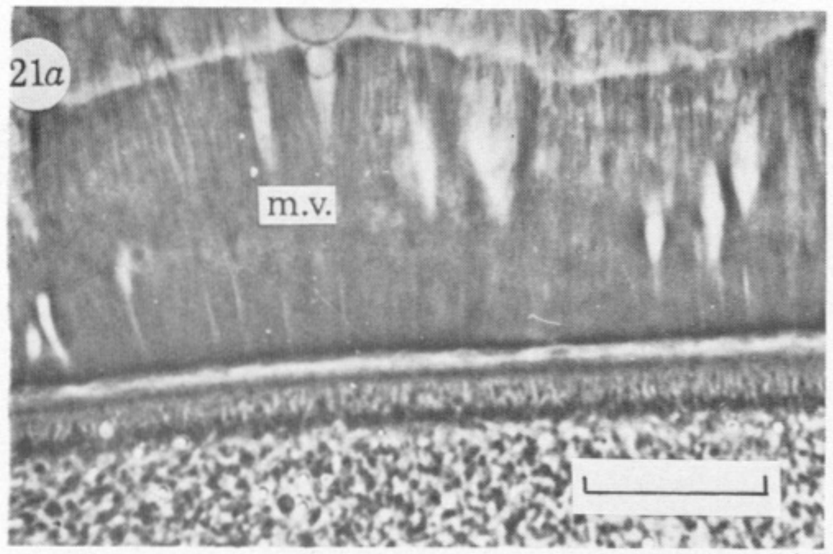


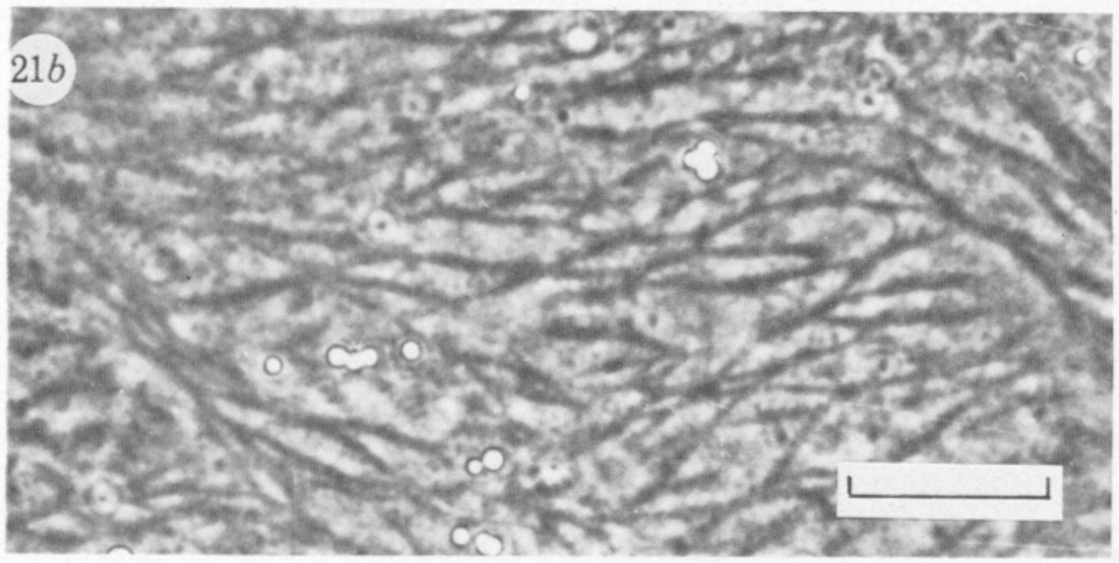


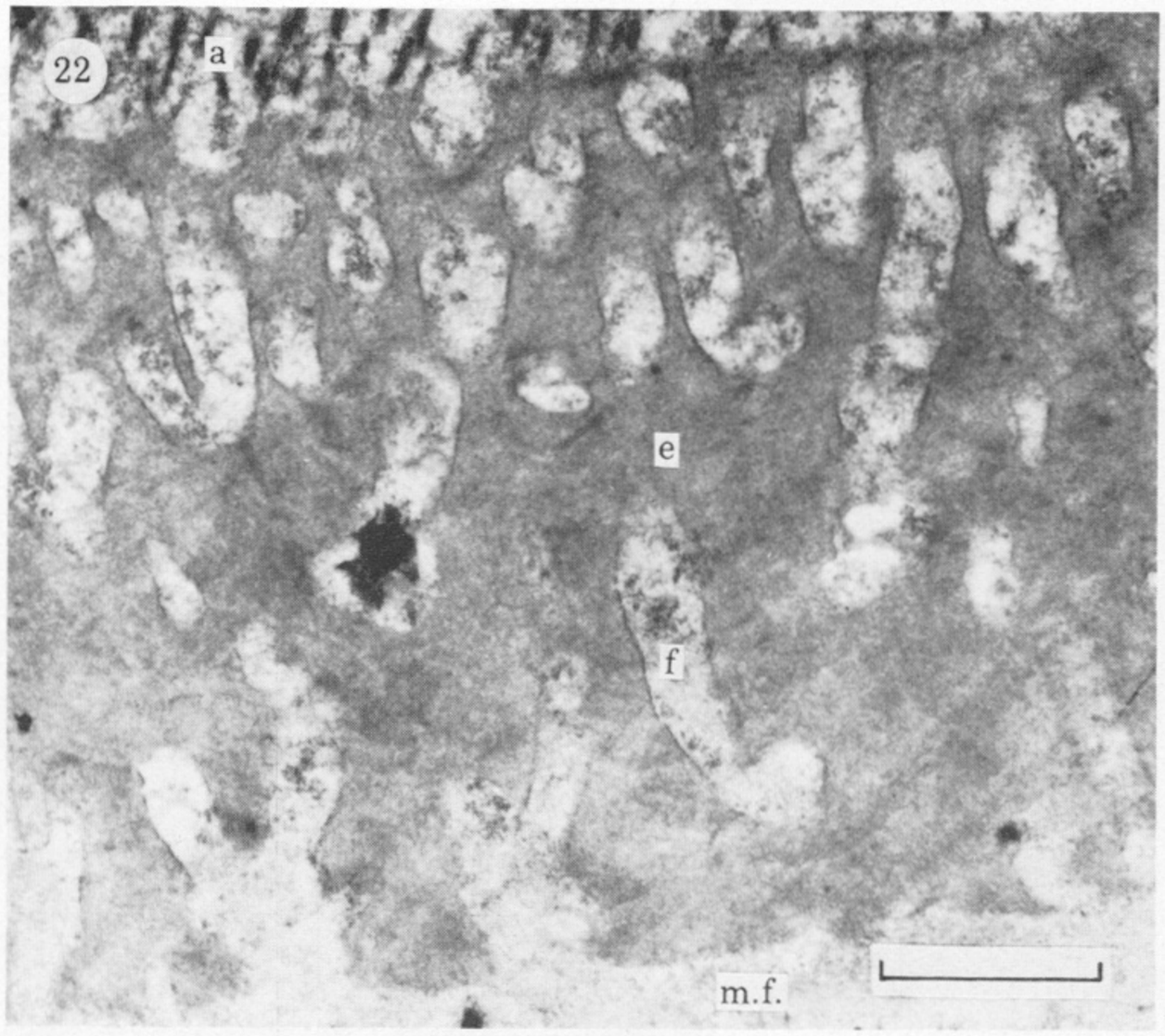


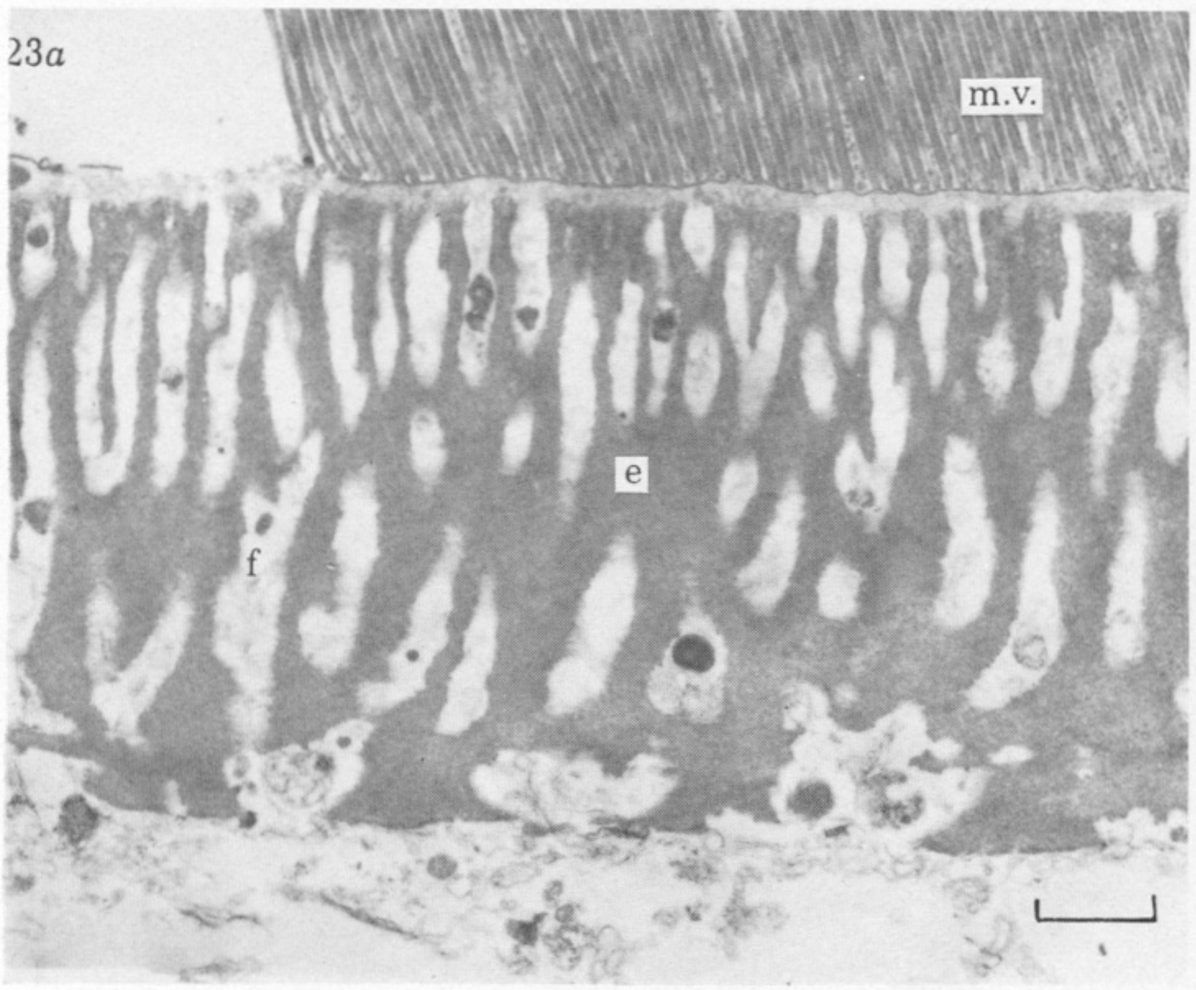


FIGURES 16–20. For description see p. 8.









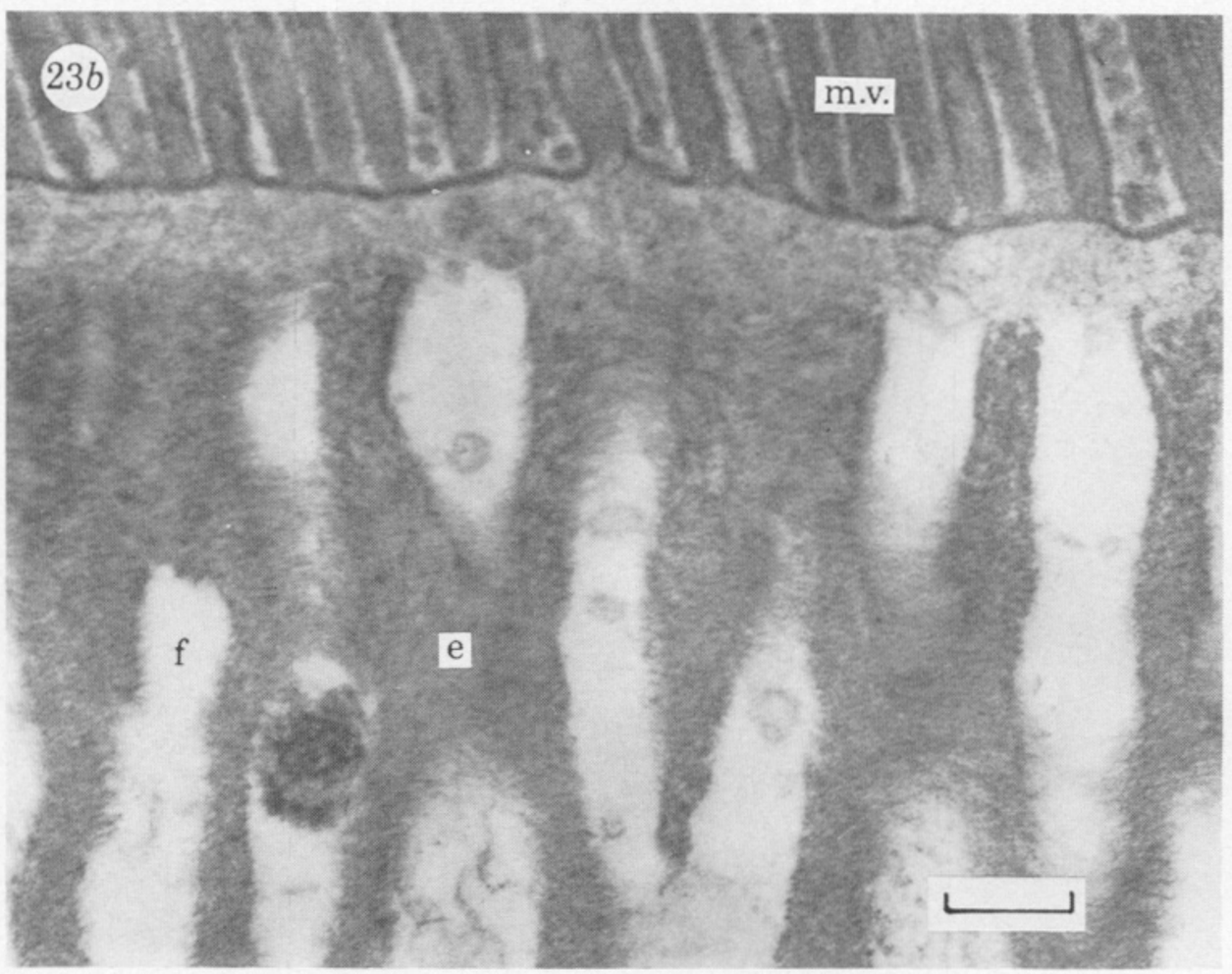
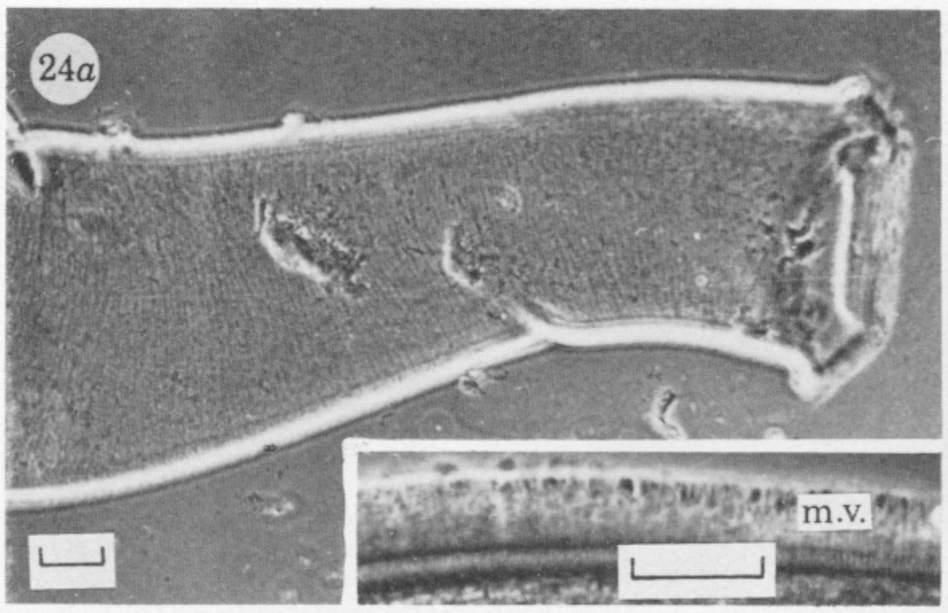
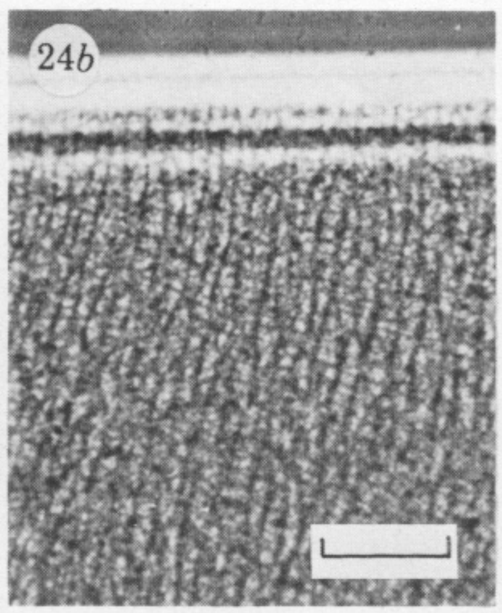


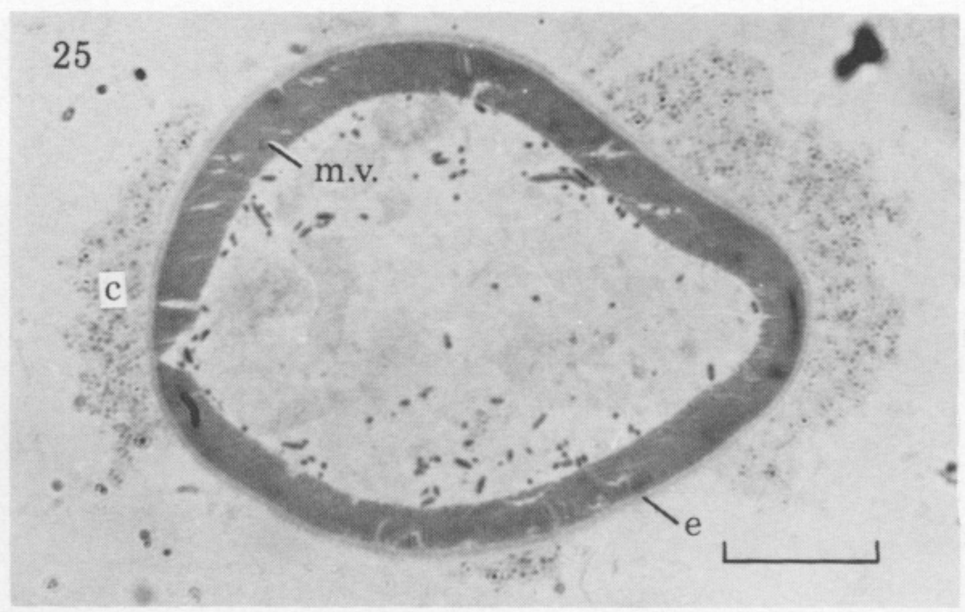
FIGURE 21. Phase contrast light micrographs of parts of endotube preparations from the intestine of *Strongylus endentatus* showing (*a*), the long microvilli, numbers of which are ballooned, and (*b*), the reticulated appearance of the basal surface. Scale bars, (*a*) 20 μm; (*b*) 10 μm.

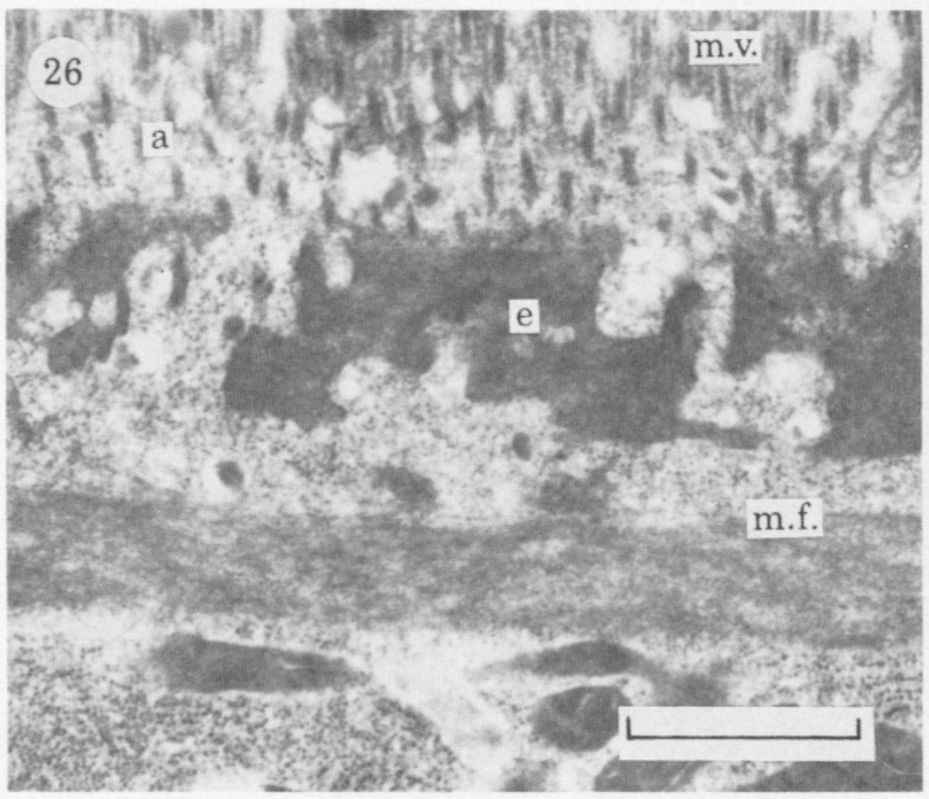
FIGURE 22. Electron micrograph of a thin, slightly oblique section of an endotube in *S. edentatus* intestine. Scale bar, 1 μm.

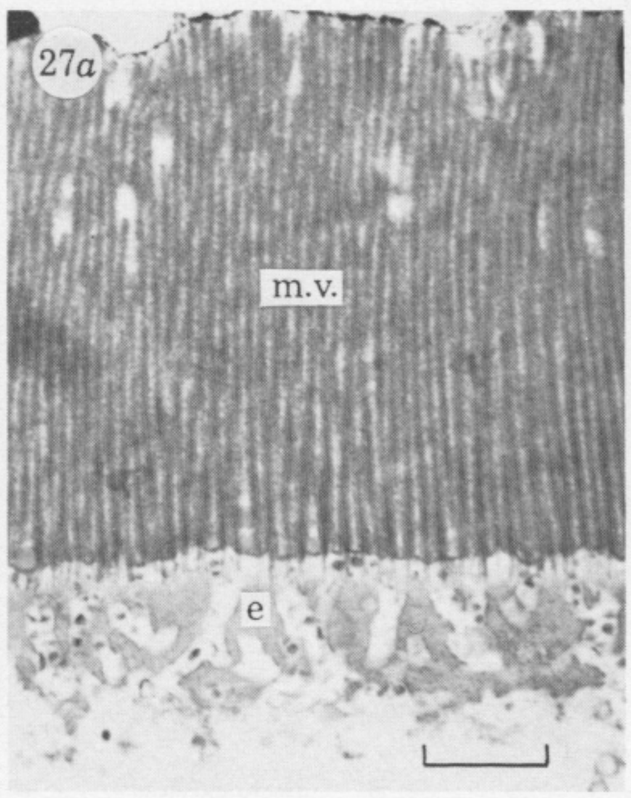
FIGURE 23. Electron micrographs of an endotube preparation from *S. edentatus* showing (*a*) partial loss of microvilli, and (*b*) loss of axial cores from remaining microvilli, and fibrous nature of the endotube. Scale bars, (*a*) 1 μm; (*b*) 0.25 μm.

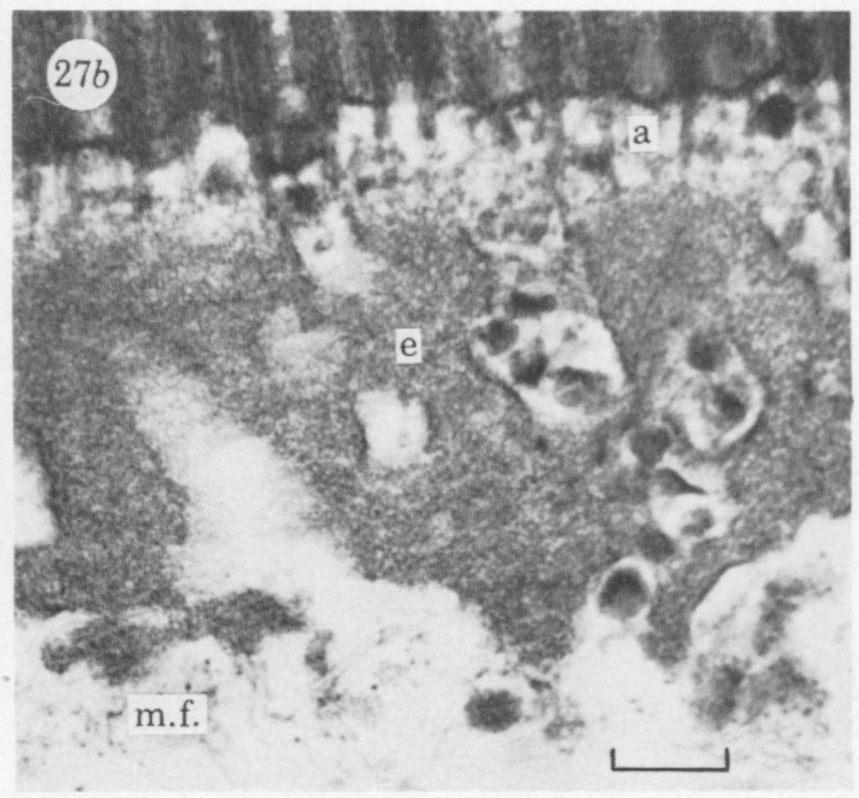


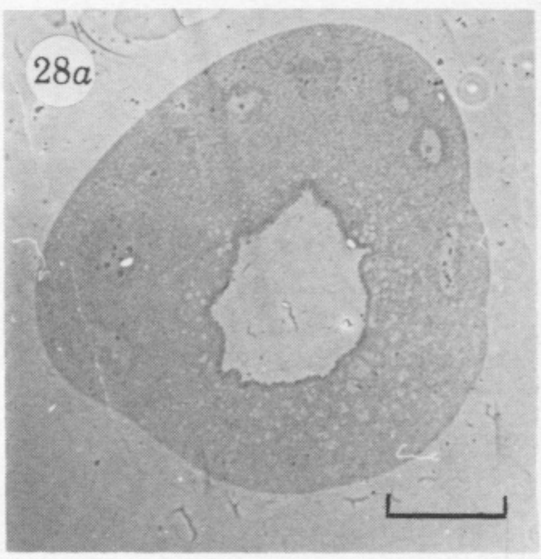


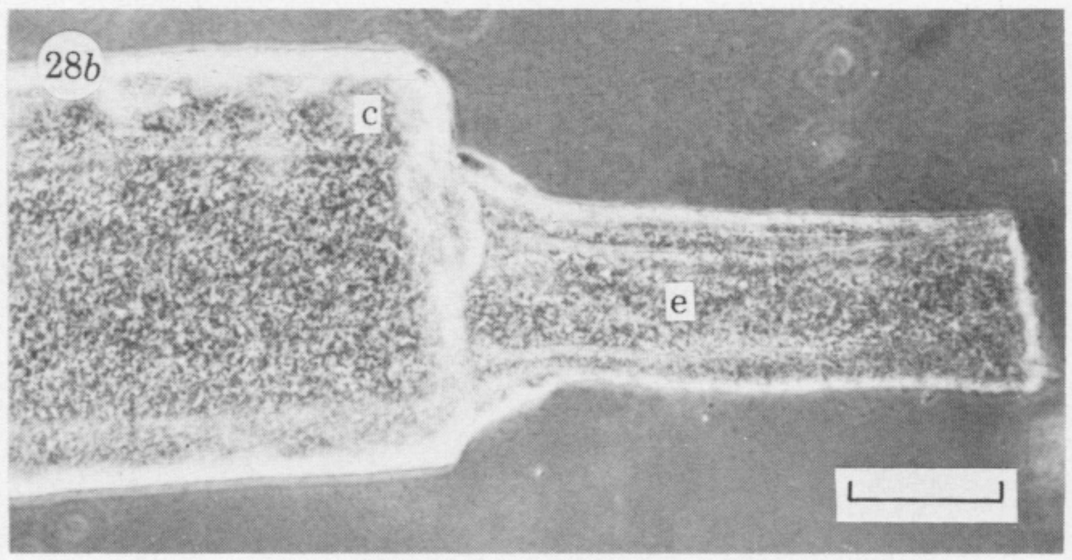


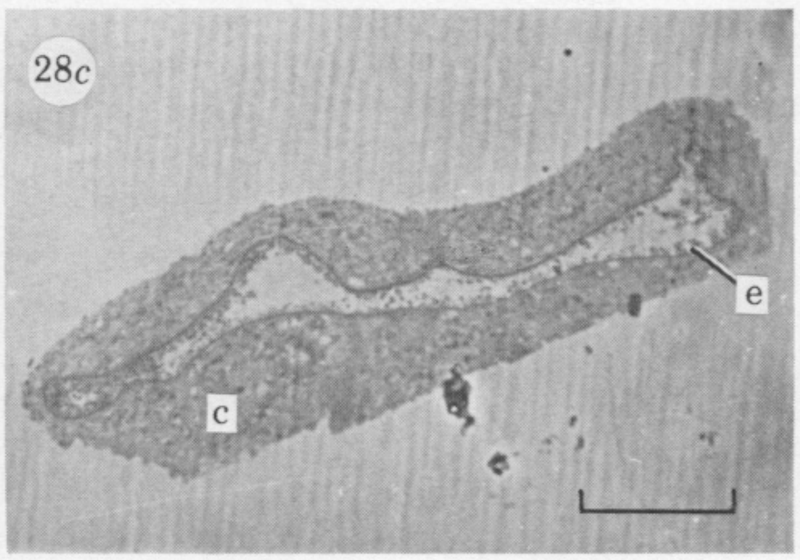


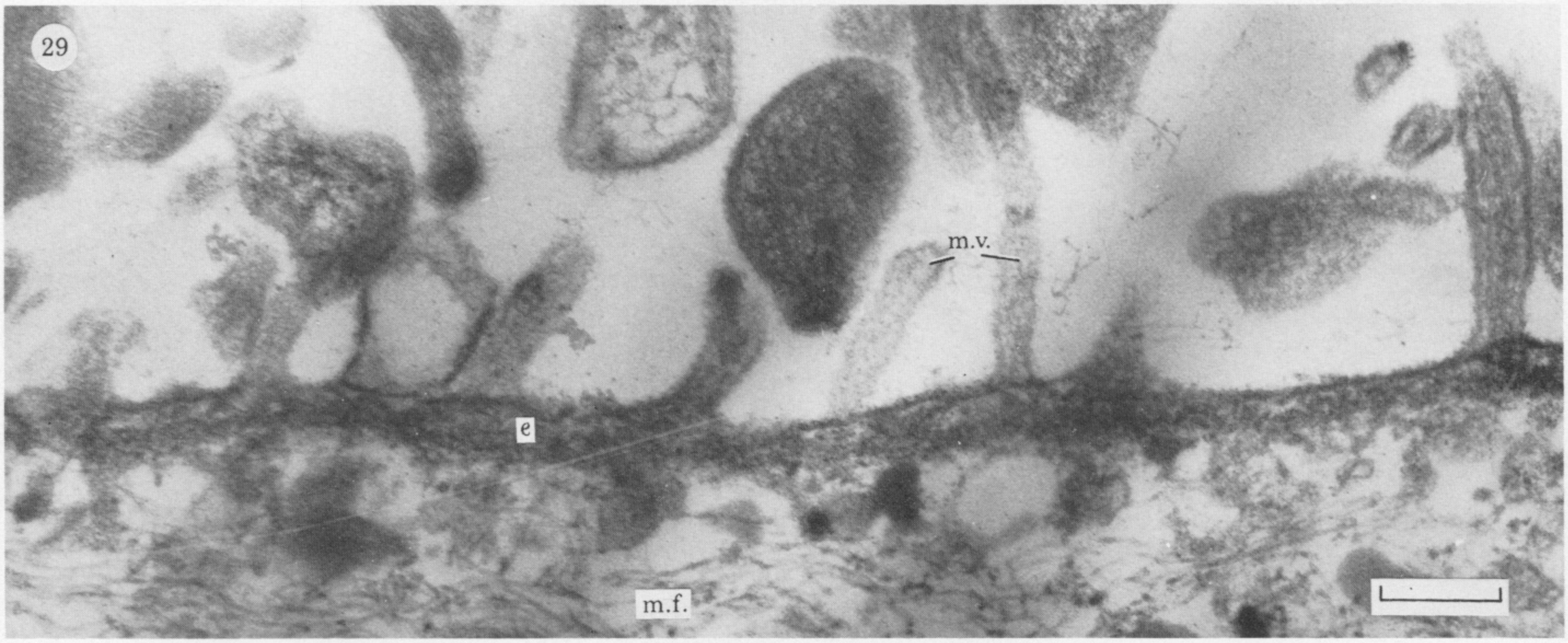




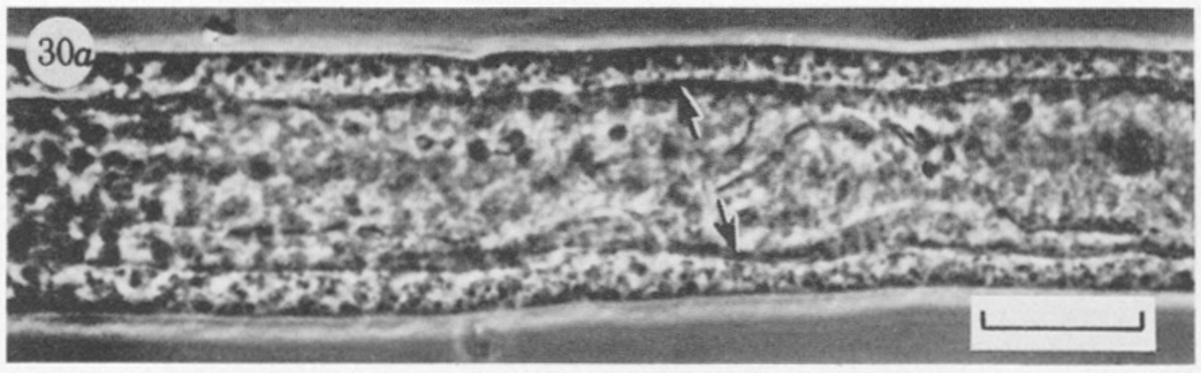


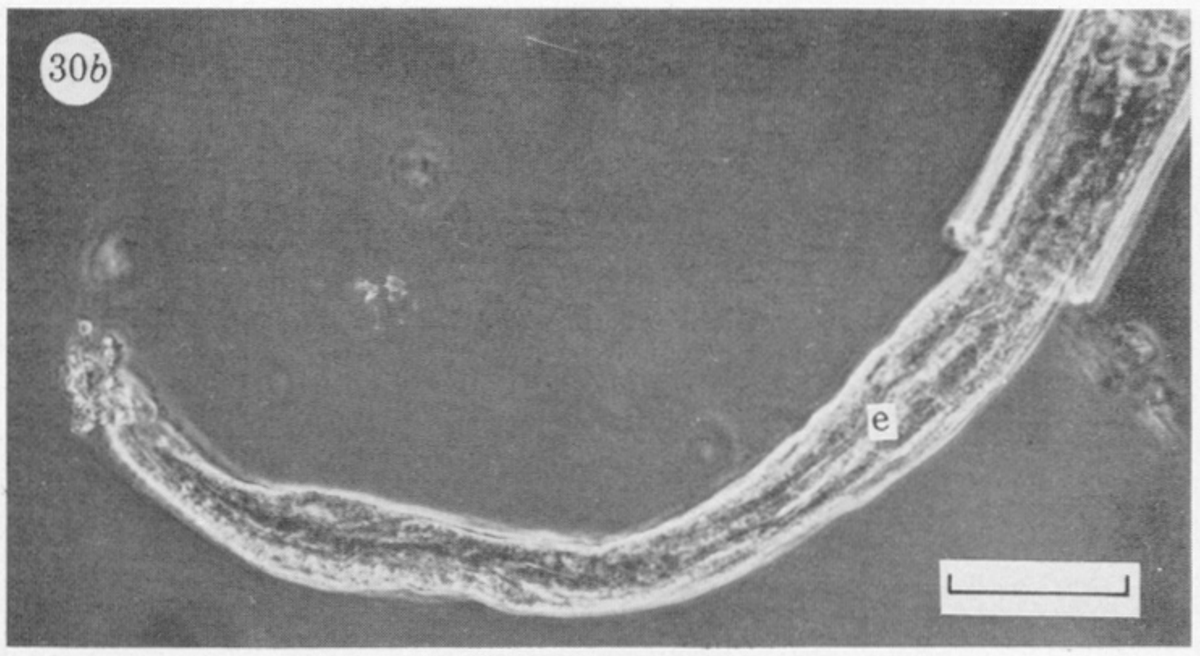


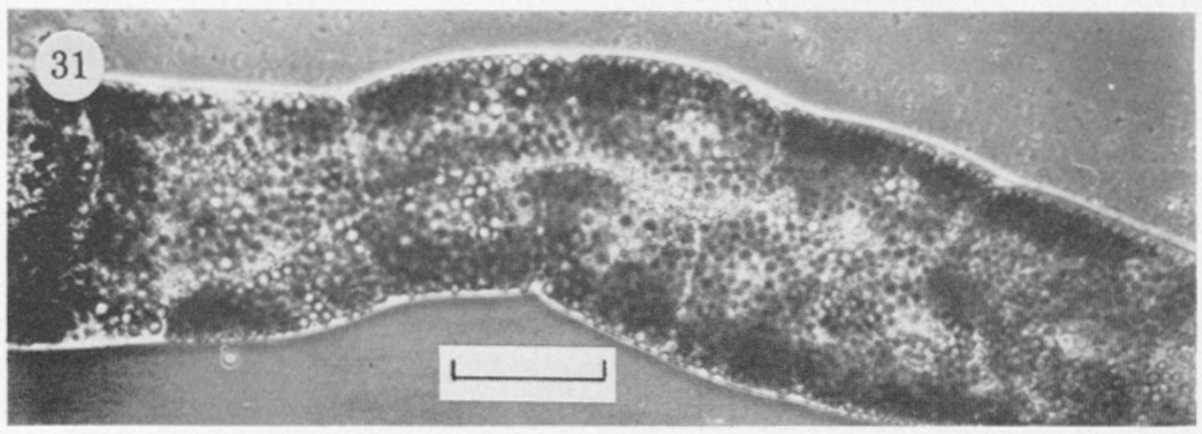


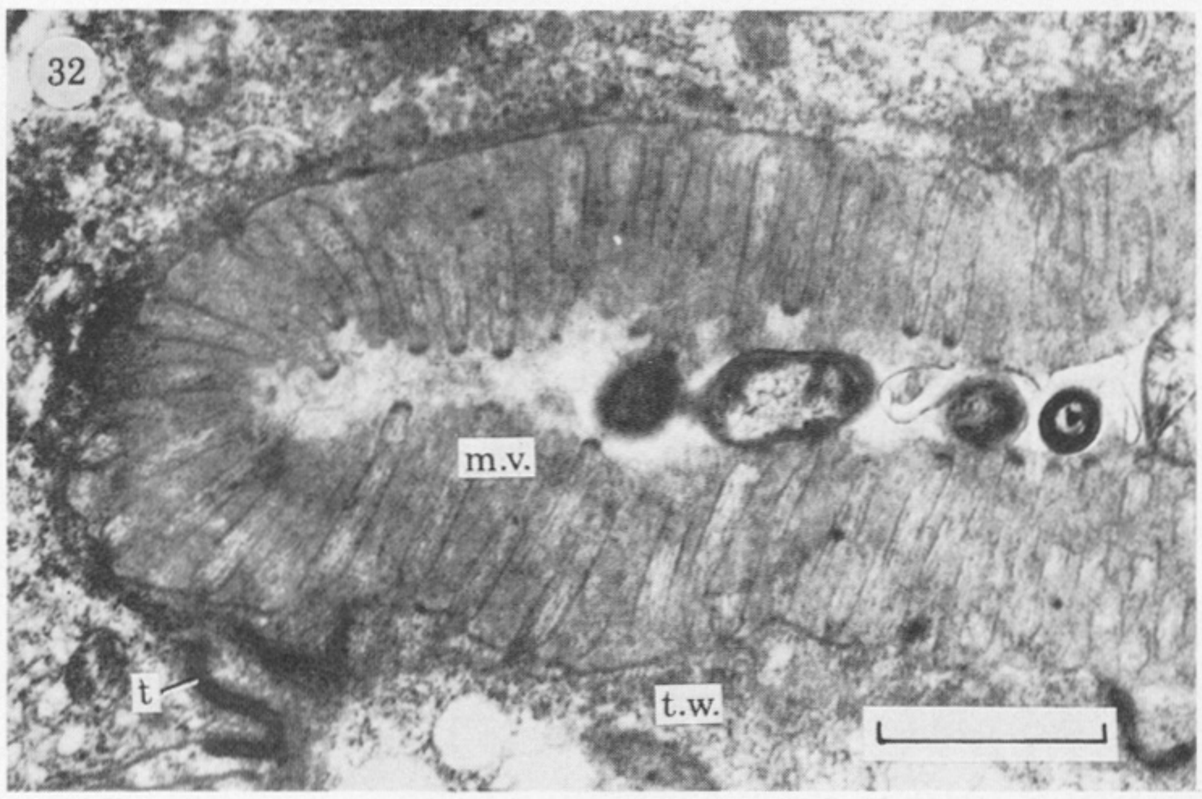


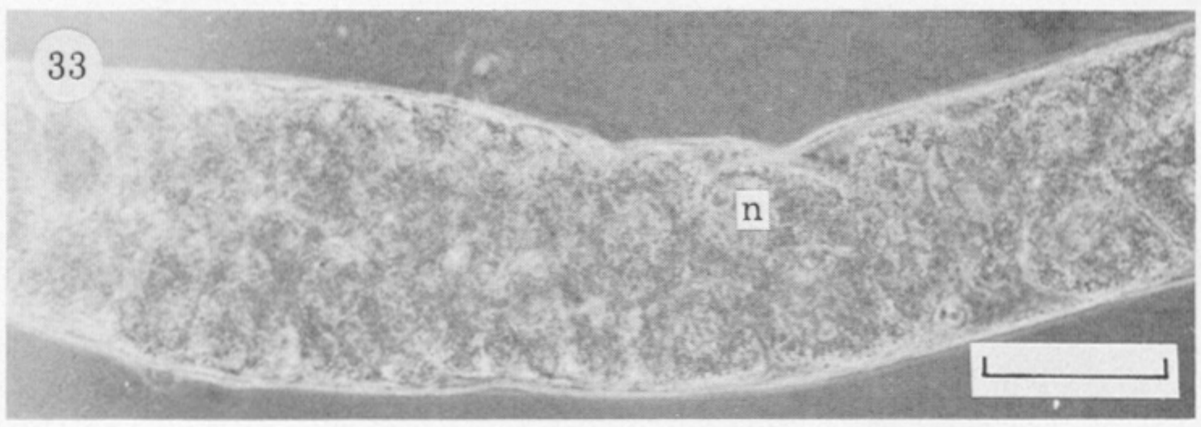
FIGURES 24–29. For description see over.

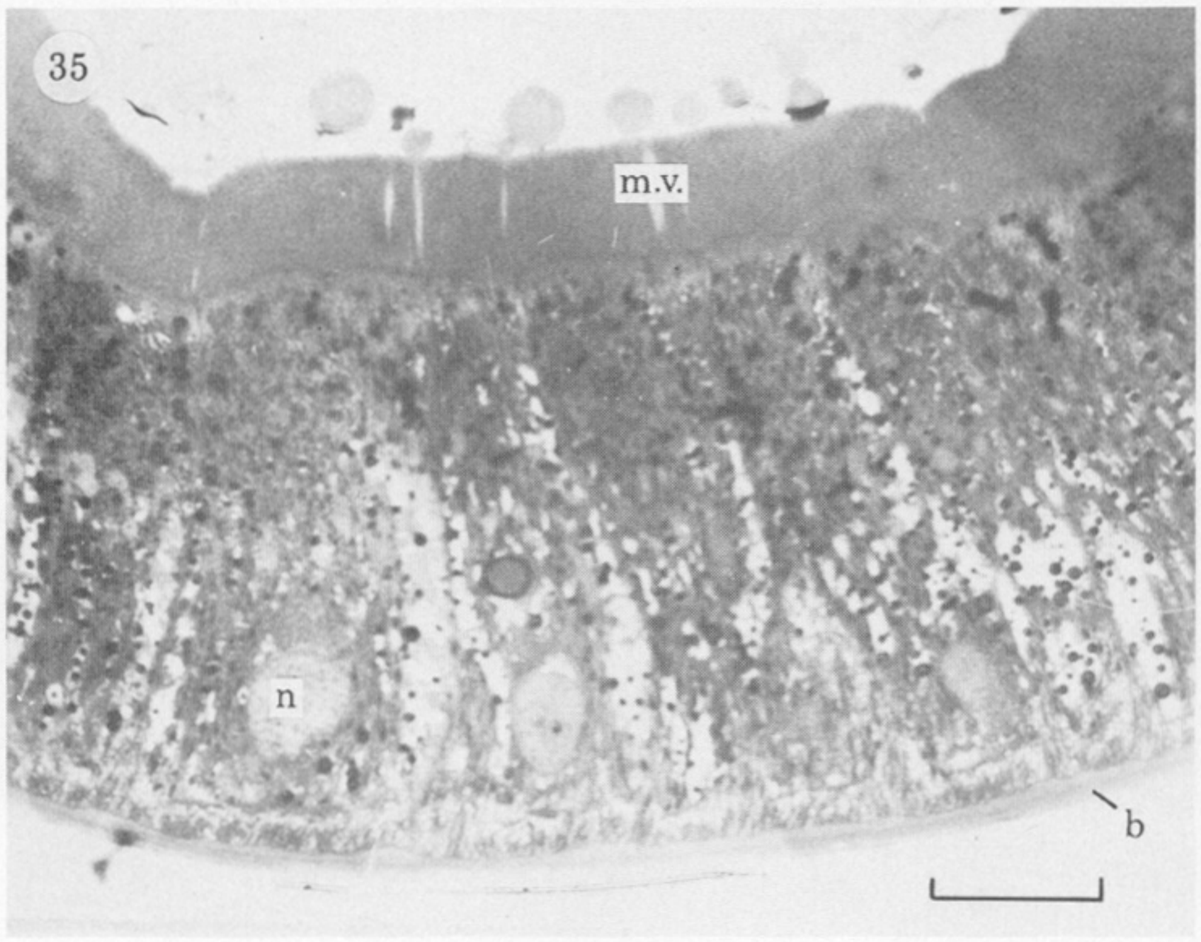


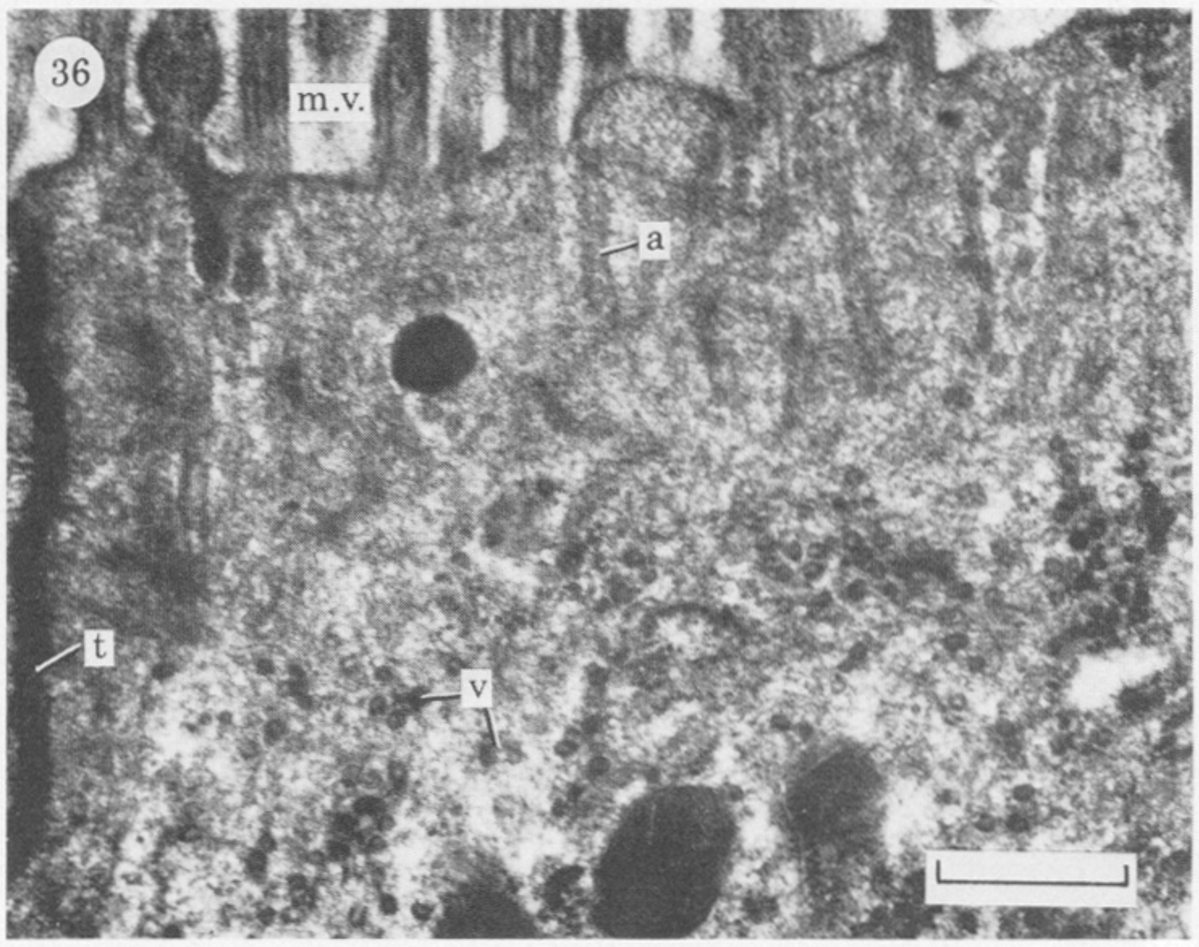




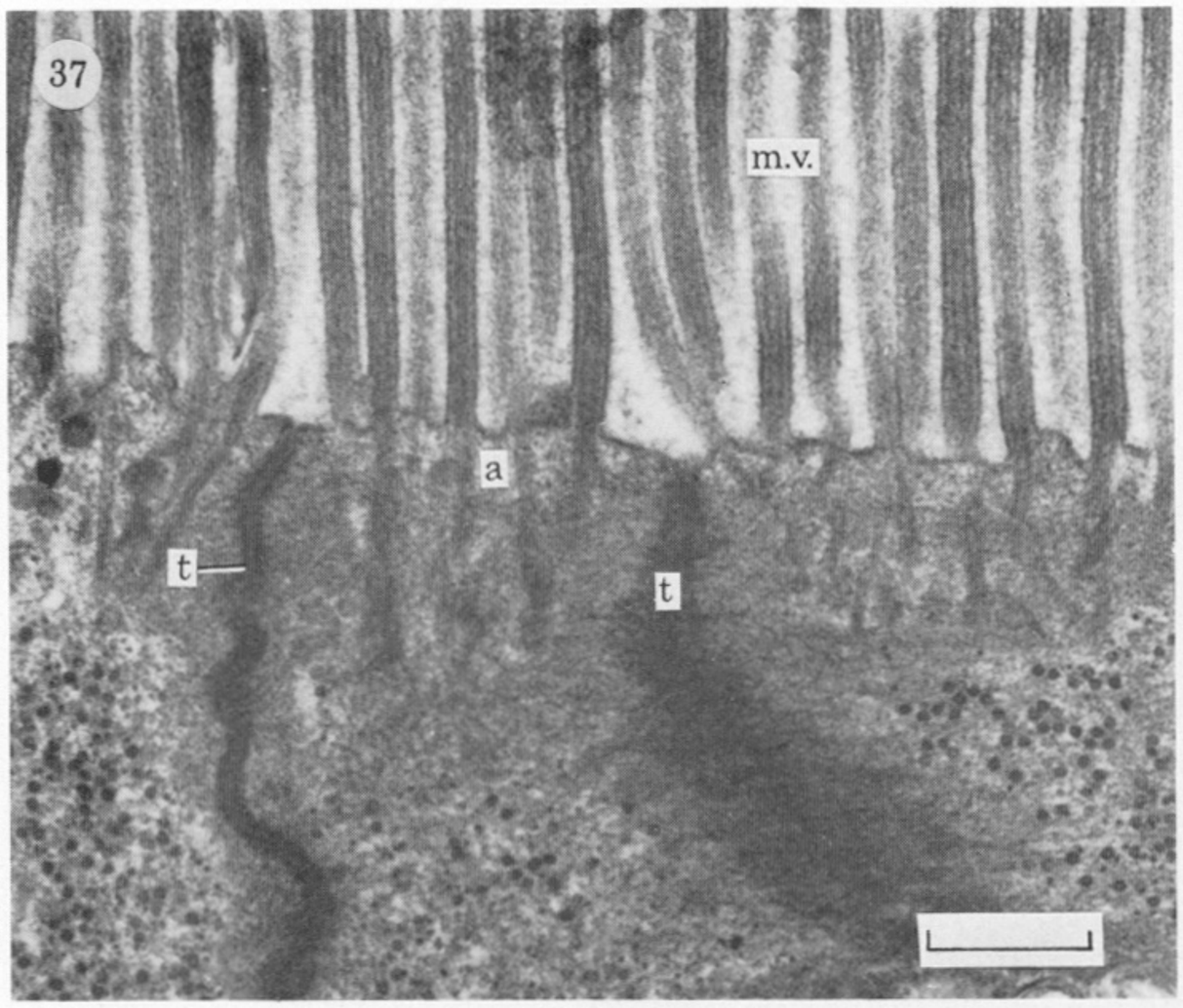


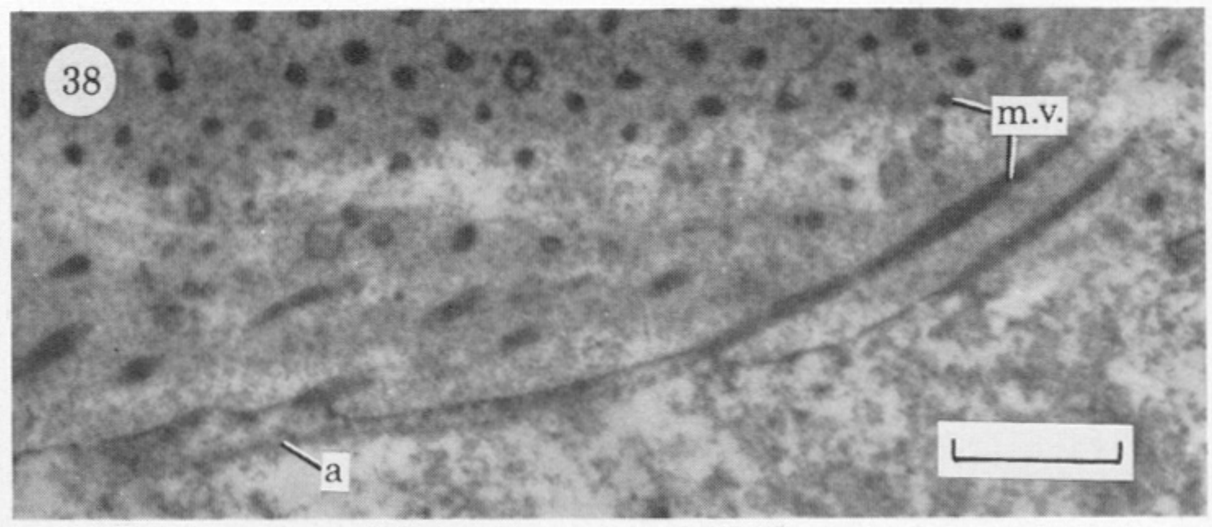


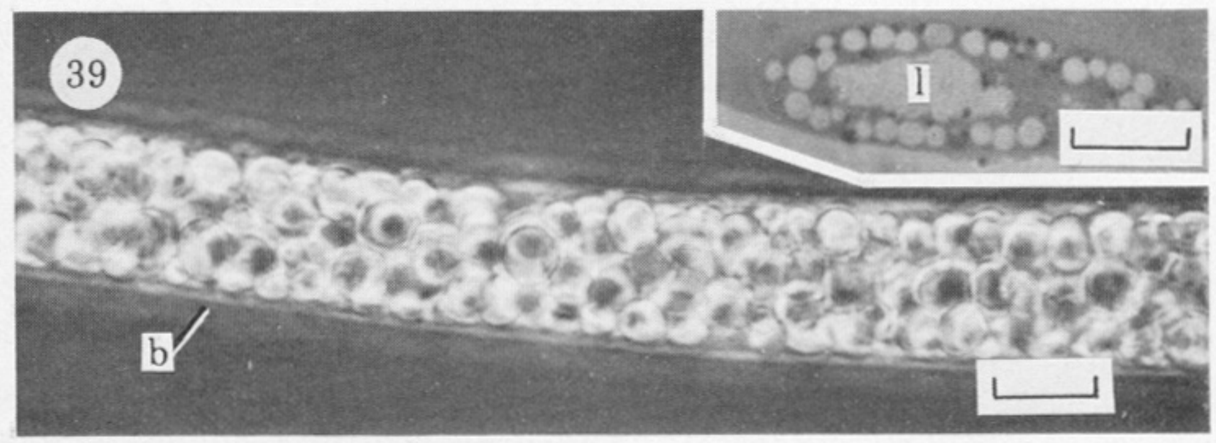


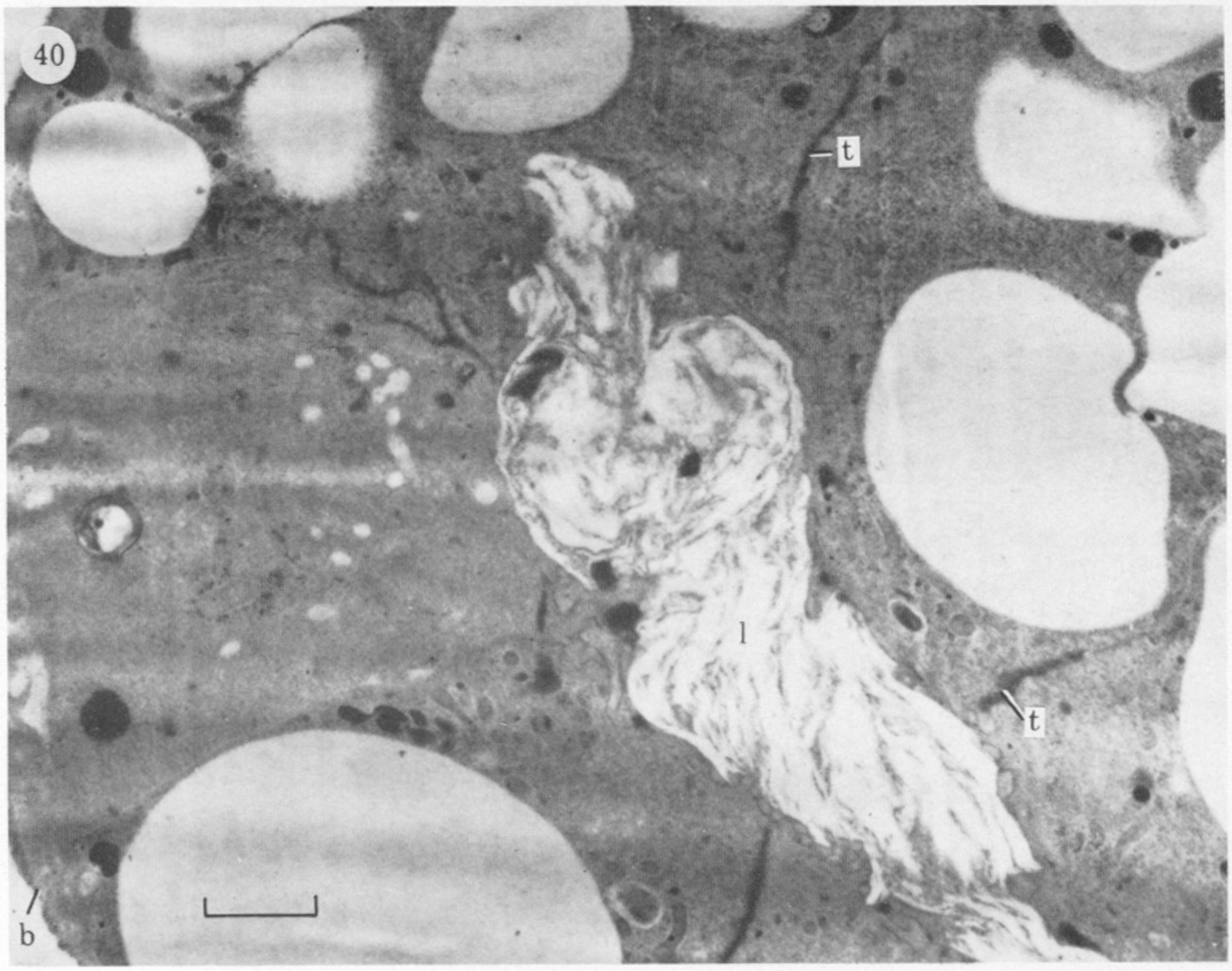


FIGURES 30–36. For description see opposite.









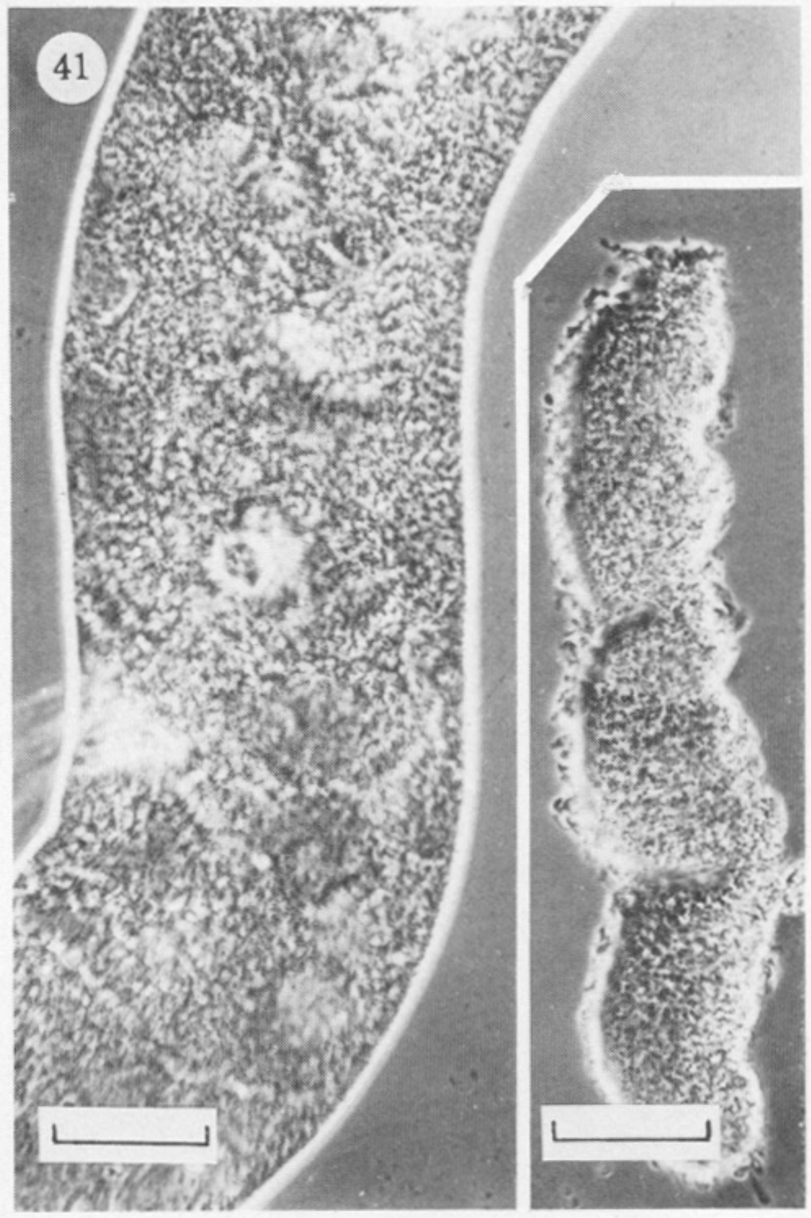


FIGURE 37. Electron micrograph of parts of the apical region of intestinal cells from *Ascaris suum* showing one terminal bar in vertical section and one in oblique section. Scale bar, 0.5 μm.

FIGURE 38. Low power electron micrograph of the luminal surface of the intestine of *Heterakis gallinae*. Scale bar, 1 μm.

FIGURE 39. Light micrographs of intestine from *Litomosoides carinii*. (*a*) Intact intestine by phase-contrast; *inset* section. Scale bars, 20 μm.

FIGURE 40. Electron micrograph of a section through part of the intestine of *L. carinii*. Scale bar, 1 μm.

FIGURE 41. Phase contrast light micrographs of the intestine of *Trichuris ovis* and (inset) three cells obtained therefrom. Scale bar, 100 μm (inset 50 μm).