[Jpn. J. Parasitol., Vol. 37, No. 1, 44–50, February, 1988]

Toxocara canis: Scanning and Transmission Electron Microscopy of the Apical Intestinal Epithelium with Special Reference to the Brush Border

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(Received for publication; October 1, 1987)

Abstract

Scanning and transmission electron microscopy (SEM and TEM) were used to study the apical intestinal epithelium of *Toxocara canis.* SEM observations of the epithelial surface revealed the presence of cilia-like extensions, fibrous projections along the cell boundaries limited to the area of the lateral margins of the intestine and secretory blebs in addition to microvilli. The cilia-like extensions and fibrous projections have not been reported previously. TEM of the cross sections of the brush border showed the presence of unusually large microvilli, round, dense structures with lamellar coat and concentric lamellar structures among the usual microvilli.

Key words: SEM, TEM, ultrastructure, *Toxocara canis,* intestine, brush border

Introduction

Electron microscope investigations have shown that the intestinal cells of nematodes are similar to each other, having a common ultrastructure for secretion as well as food absorption. The intestine is a simple tube consisting of a single layer of columnar epithelial cells with numerous microvilli on the apical surface. These microvilli are usually finger-like with an electron-dense central cytoplasmic core which is made up of longitudinal filaments (Sheffield, 1964; Bruce, 1966; Miller, 1967; Lee and Miller, 1969; Jenkins and Erasmus, 1969; Davidson, 1983).

Despite of considerable work on the ultrastructure of intestinal cells in nematodes, SEM has not been used to observe three-dimensional features of the luminal surface of the intestine. Little information is available on the fine structure of the brush border of the apical epithelial cells, especially on microvilli variation and structures in the microvilli. In this investigation SEM is coupled with TEM to describe the ultrastructure of the apical intestinal epithelium of *Toxocara canis,* with special attention to some unusual structures in the microvilli.

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Materials and Methods

For SEM observations, adult worms of *Toxocara canis* were removed from dogs. The worm intestine was dissected and separated into anterior, middle and posterior regions before it was opened longitudinally. The open intestine was pinned on a silicone board, washed by gentle flow of saline followed by buffer, and then fixed for 2 hours in chilled 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3. After fixing in 1% osmium tetroxide the intestinal strips were dehydrated with ethanol and dried in a carbon dioxide critical point drying apparatus. The specimens were coated with gold and viewed under a JEOL JSM-U3 scanning electron microscope operated at 15 kV.

For TEM, the dissected intestine was cut into small pieces and fixed in Karnovsky's (1965) fixative. After washing in 0.1 M

cacodylate buffer, pH 7.2, the tissues were postfixed in buffered 1% osmium tetroxide, dehydrated in ethanol and embedded in Epon 812. Sections were double stained with uranyl acetate and lead acetate prior to examination in a Hitachi HS-9 electron microscope at an accelerating voltage of 75 kV.

Results

The intestine was a flat tube with a narrow lumen. The epithelial cells were tall with the undulating brush border (Fig. 1).

No marked differences from the anterior to posterior regions were observed in the surface structure of the intestinal epithelium. The epithelium consisted of mound-shaped cells whose luminal surface was marked by numerous thickly packed microvilli. The central area of each cell was slightly higher than the peripheral (Fig. 2). In some part of the intestine, mostly along the lateral margins of the intestine, fibrous projections of various lengths and of uniform thickness were seen only along the cell boundaries (Figs. 2 and 3). These projections occasionally occurred in bundles (Fig. 3). Fine cilia-like extensions were also observed along the border between the cells limited to the lateral margins of the intestine (Fig. 4). Small round secretory bodies and holes, possibly associated with secretion, were seen in the intestinal surface near the lateral margins (Fig. 5).

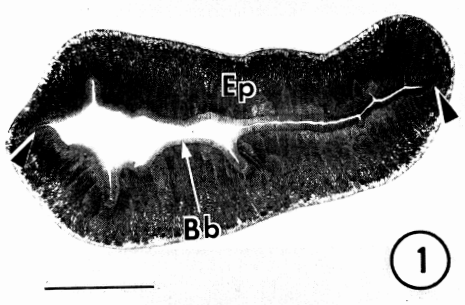


Fig. 1. A light microscope picture showing a cross section of the middle part of the intestine. Arrowheads indicate the lateral margins. Ep: Epithelium; Bb: Brush border. Bar = 0.2 mm

Microvilli appeared as finger-like projections, round or oval in cross section, and varied in size, ranging from 0.10 to 0.25 μm. The outer plasma membrane was provided with a fluffy coat. Inside the microvilli were core filaments whose number and arrangement varied from one microvillus to another (Figs. 6 – 9). The core filaments were arranged occasionally in a circle with the outer and inner structureless area. Large microvilli which measured approximately 0.80 μm in diameter and lacked core filaments (Figs. 6 inset and 7) were seen infrequently. Some groups of microvilli were seen in the lumen far from the apical layer of the usual microvilli (Fig. 7). Round or oval electron-dense structures, which were bigger than the typical microvilli, also occurred in the microvilli. They ranged from 0.25 to 0.55 μm in diameter, had no core filaments or fluffy coat, and were surrounded by loosely connected lamellae (Fig. 8). Other concentrically and irregularly wound lamellar structures, which varied in shape and size, were also found among the microvilli (Fig. 8).

The terminal web area consisted of a fibrous network, within which the rootlets of the core filaments terminate (Fig. 9). Just below the apical plasma membrane small pinocytotic vesicles were scattered. Some of them were seen in close contact with the membrane at the bases of the microvilli. Microgranules measuring approximately 45 nm, multivesicular bodies, mitochondria, free ribosomes and rough endoplasmic reticulum, were observed just below the terminal web.

Discussion

Cross sections of the intestine through microvilli showed that the microvilli are considerally variable in size, ranging from 0.10 to 0.25 μm in diameter. Unusually large microvilli with an approximate diameter of 0.8

μm, which are devoid of core filaments, are rarely found. The core filaments in *Toxocara canis* are loosely gathered in the center of the microvilli and are similar to those of *Ascaris suum* (Sheffield, 1964) and *A. lumbricoides* (Kessel *et al.*, 1961). In the large microvilli, the core filaments are much more in number than in the usual ones. Unlike the above species *Ancylostoma caninum* has the core filaments gathered compactly in the center of the microvilli (Miller, 1967; Andreassen, 1968). Although the microvilli of nematodes are usually finger-like, those of *Metastrongylus* sp. are marked by dilated, balloon-like tips which appeared to be budded off into the lumen (Jenkins and Erasmus, 1969).

SEM observations revealed the presence of some unusual structures on the luminal surface of the intestinal epithelium, which have not yet been reported in previous investigations. Fibrous projections that were found along the border between the cells, protrude into the lumen of the intestine out of the luminal surface of the microvilli. In TEM observations, cross sections of round electron-dense structures bearing lamellar coats occur occasionally among the microvilli. These structures at low magnification appear to be distributed in a line among the microvilli. This may suggest that these unique structures are arranged along the cell boundaries and correspond to the fibrous projections observed by SEM. Although cell inclusions containing crystals in nematode intestines have been reported previously (Chitwood and Chitwood, 1950; Blitz and Gibbs, 1971; Bird *et al.*, 1978), the above structures do not appear to correspond to any of them.

Fine cilia-like extensions are also found along the cell borders of the intestinal lateral margins. It is possible that these structures are unusually extended microvilli-like stereocilia, which are usually present in mammalian epididymis (Nicander, 1965) and certain sense organs (Spoendlin, 1964; Barber and Emerson, 1980). This may be supported by the observation that some groups of microvilli in cross sections were seen in the lumen of the intestine far from the apical layer of the usual microvilli. No previous reports have demonstrated cilia or cilia-like extensions in the intestinal epithelium of nematodes (Bird, 1971). Further study would clarify their detailed structure and why they occur along the cell boundaries of the lateral margins.

A few groups of secretory bodies were seen in the luminal surface of the intestine. Small holes, from which a secretion had probably occurred, were also observed over the surface of the microvilli near the secretory bodies. There has been a report of blebs secreted from the luminal surface of the microvilli in *A. lumbricoides* by Kessel *et al.* (1961). Borgers and de Nollin (1974), however, commented that these blebs may be morphological alterations due to the storage of the material *in vitro* before fixing. Sheffield (1964) also observed some blebs in one of the intestinal cells of *A. suum*, and mentioned that these blebs originated from the apical membrane and were responsible for the excretion of their contents into the lumen.

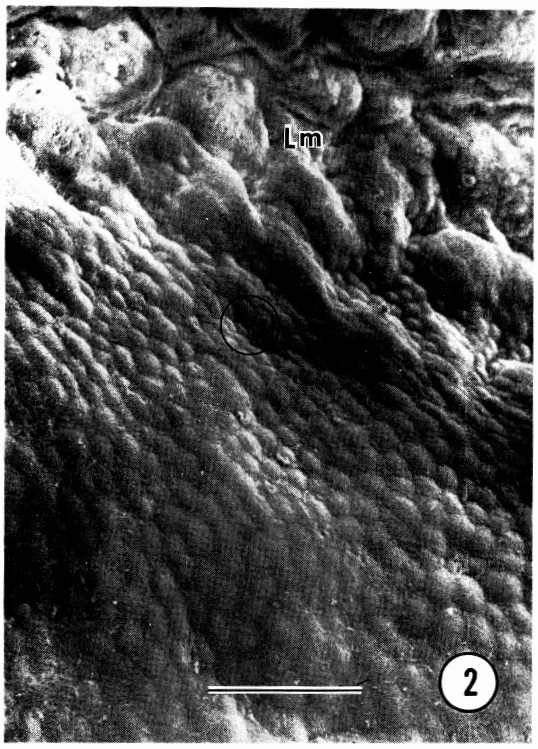
The structure of the apical epithelial area of *T. canis* is similar to that of *A. suum* (Sheffield, 1964) in having abundant pinocytotic vesicles just below the plasma membrane at the bases of

Fig. 2. A low-power SEM micrograph showing the epithelial surface near the lateral margins (Lm) of the middle part of the intestine. The epithelium consists of mound-shaped cells which are characterized by packed numerous microvilli and fibrous projections along the border of the cells. A circle indicates the area at which the enlarged micrograph of Fig. 3 is taken. Bar = 50 μm

Fig. 3. An enlarged SEM micrograph of fibrous projections which are located at the circular area of Fig. 2. Some projections are gathered in a bundle along the border between the cells (arrowhead). Mv: Microvilli. Bar = 1 μm

Fig. 4. An enlarged SEM micrograph of cilia-like projections on the microvilli. Mv: Microvilli. Bar = 1 μm

Fig. 5. A low-power SEM micrograph showing secretory bodies and small holes, from which secretion probably occurred on the microvilli. Bar = 10 μm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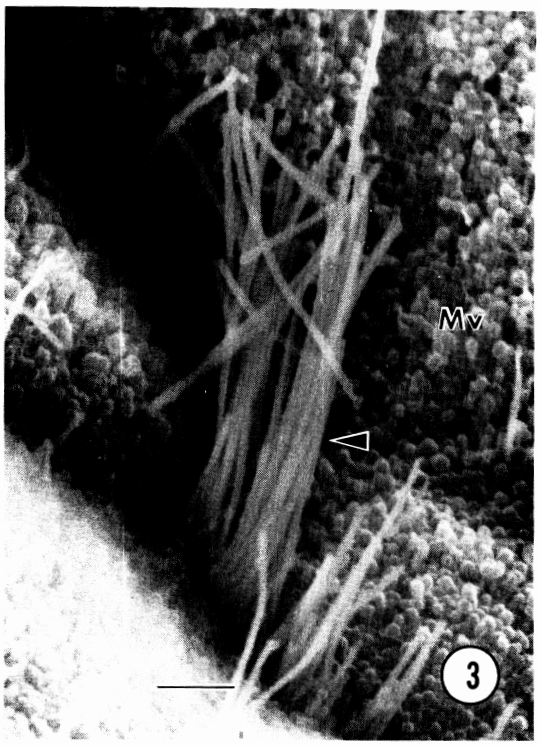
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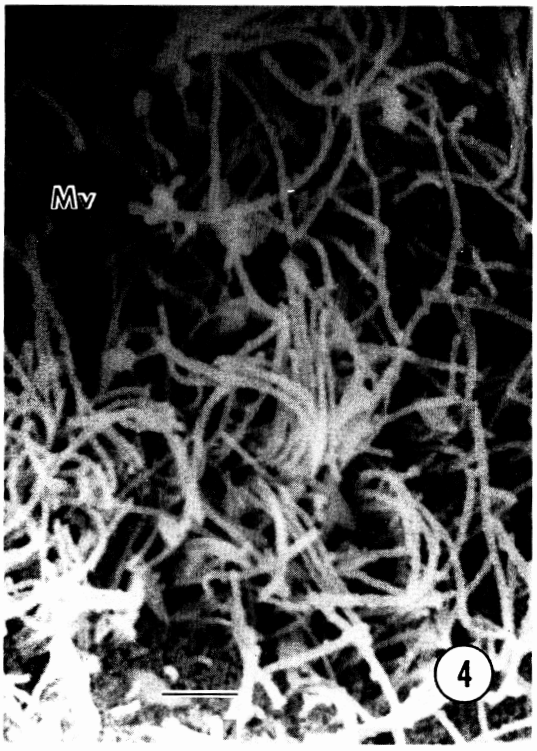
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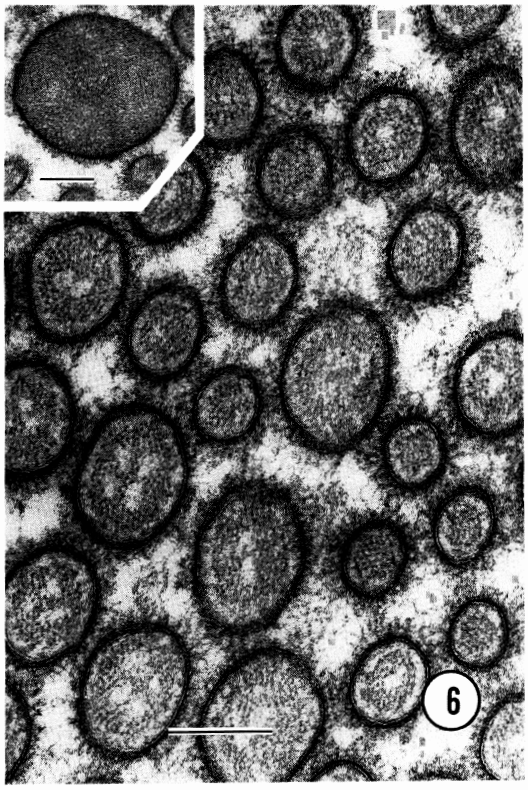

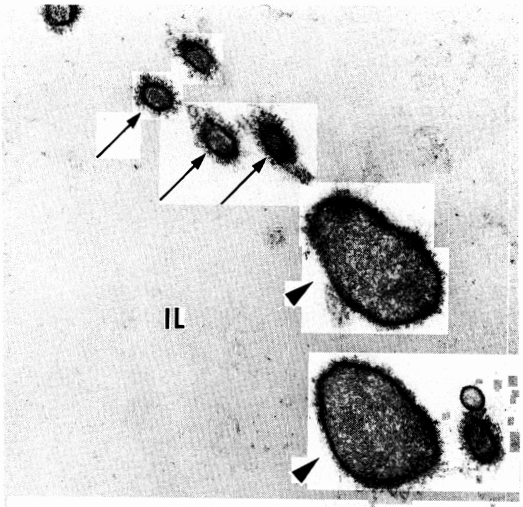
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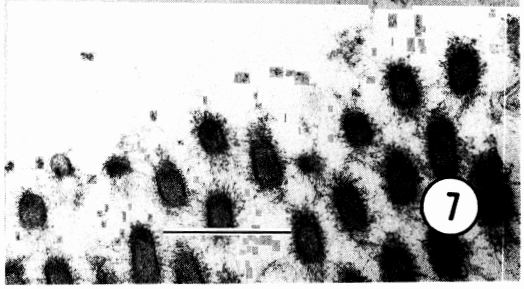

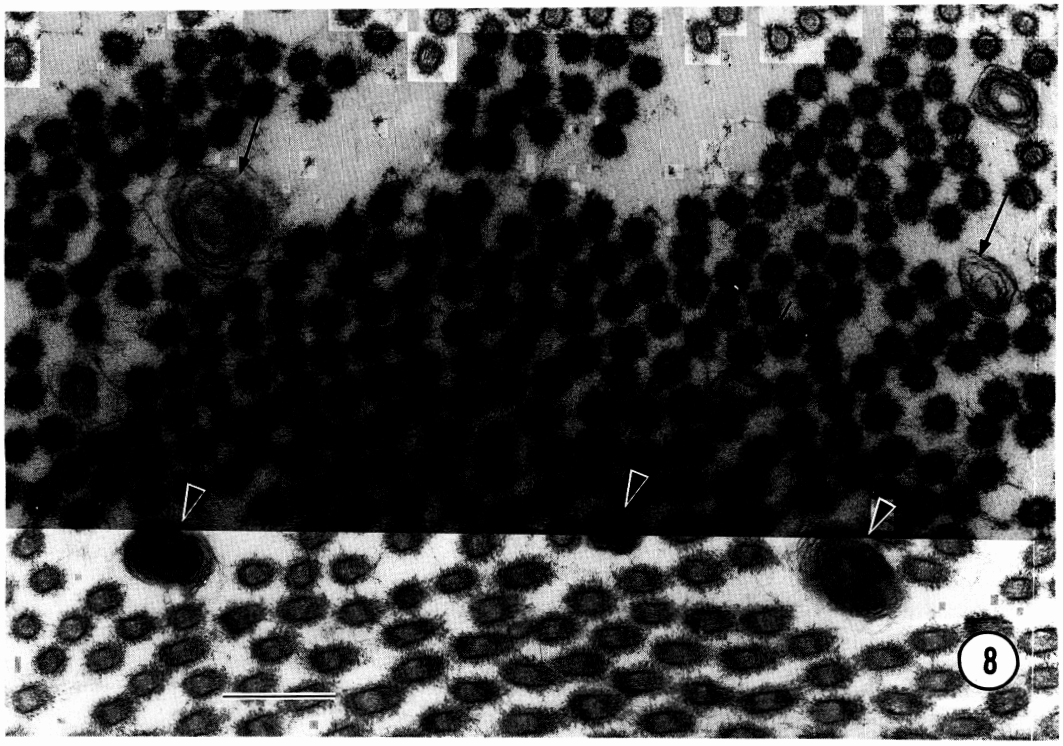

the microvilli and other organelles just below the terminal web area. The abundant pinocytotic vesicles suggest an active absorption of fluid or particulate foods for nourishment of the worm.

Acknowledgement

The authors express their sincere thanks to Dr. B. Fried of Lafayette College for his interest and critical reading of the manuscript.

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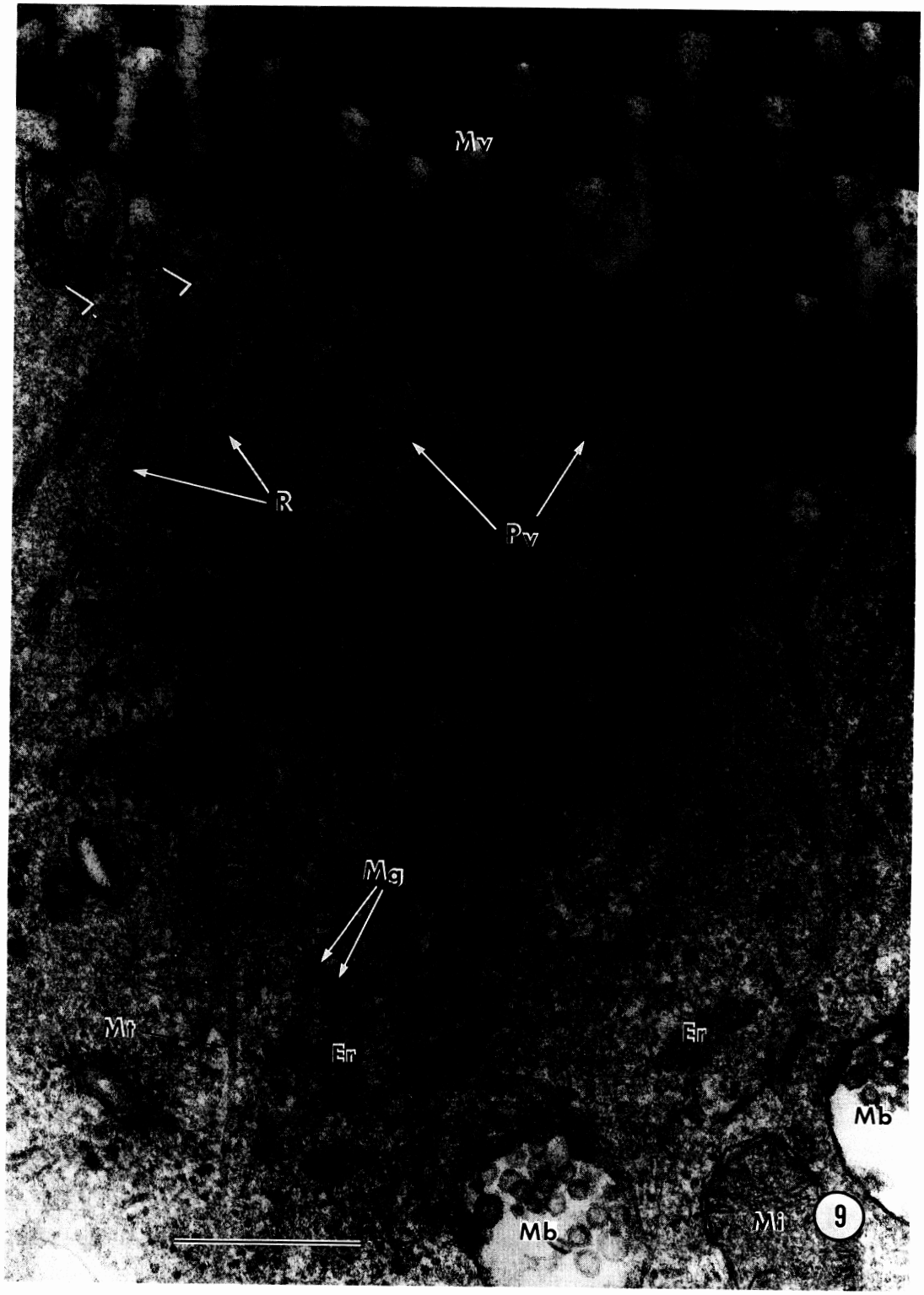
Fig. 6. A TEM micrograph of a cross section through the microvilli. Each microvillus has a cytoplasmic core which is made up of longitudinal filaments and surface fuzzy coat. Note the differences in the size of the microvilli and varied arrangement of the core filaments in the microvilli. Bar = 0.2 μm.
Inset is a micrograph showing an unusually big microvillus without core filaments. Bar = 0.2 μm

Fig. 7. A TEM micrograph of a cross section through the apical ends of the microvilli. Arrowheads indicate unusually large microvilli without core filaments. A group of the microvilli is seen in the intestinal lumen far from the apical layer of the microvilli (arrows). IL: Lumen of intestine. Bar = 0.5 μm

Fig. 8. A TEM micrograph of the microvilli in a cross section. Arrowheads indicate round or oval uniformly dense structures with loosely surrounding lamellae. Other lamellar structures of irregular shape are also seen among the microvilli (arrows). Bar = 0.5 μm

Fig. 9. A TEM micrograph of the apical part of the epithelium. The terminal web area consists of fibrous network, within which rootlets of core filaments (R) terminate. Pinocytotic vesicles (Pv) are marked just below or in close contact with the plasma membrane (arrowheads); Mb: Multivesicular body; Mg; Microgranule; Mi: Mitochondrion; Mt: Microtubule; Mv: Microvillus; Bar = 0.5 μm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