FILAMENTS IN THE MICROVILLOUS BORDER OF INTESTINAL CELLS

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Previous electron microscope studies have suggested that the core of the microvilli of intestinal columnar cells is composed of fibrils or filaments (9, 16, 19). Recent improvements in techniques for preserving and embedding tissue (6, 11) make it possible to demonstrate more clearly the morphology, disposition, and arrangement of these filaments.

MATERIALS AND METHODS

Adult Sprague-Dawley rats were perfused through the left ventricle first with balanced salt (10) to remove the blood. They were then fixed by perfusion with a mixture of 6.5 per cent glutaraldehyde and 2 per cent acrolein in a phosphate buffer (pH 7.5) (11). After 15 minutes, the duodenum was removed, fixed for an additional hour in the same aldehyde mixture, and rinsed in Veronal-acetate buffer for 15 minutes. It was then postfixed in Veronal-acetate–buffered 2 per cent osmium tetroxide (8) and embedded in Epon (6). Sections were stained with lead according to Karnovsky's method A (3) and/or uranyl acetate (17), and examined in a Siemens Elmiskop I.

OBSERVATIONS

Longitudinal sections of the apical portion of absorptive cells show delicate filaments which extend vertically from the apex of the microvillus into the cytoplasm where they end as rootlets (Fig. 1). Each filament is approximately 60 A in diameter and as many as eight can be seen clearly in a longitudinal section. The filaments are quite straight and can be followed for considerable distances in the section, in contrast with the filaments of the terminal web below. Within the microvillus, branching of the filaments is not seen, although cross-linking of the filaments cannot be ruled out. In some places, the filaments are quite uniform in diameter, while in other places they have a nodular appearance. The plasma membrane of the cell is separated from the core of filaments by a

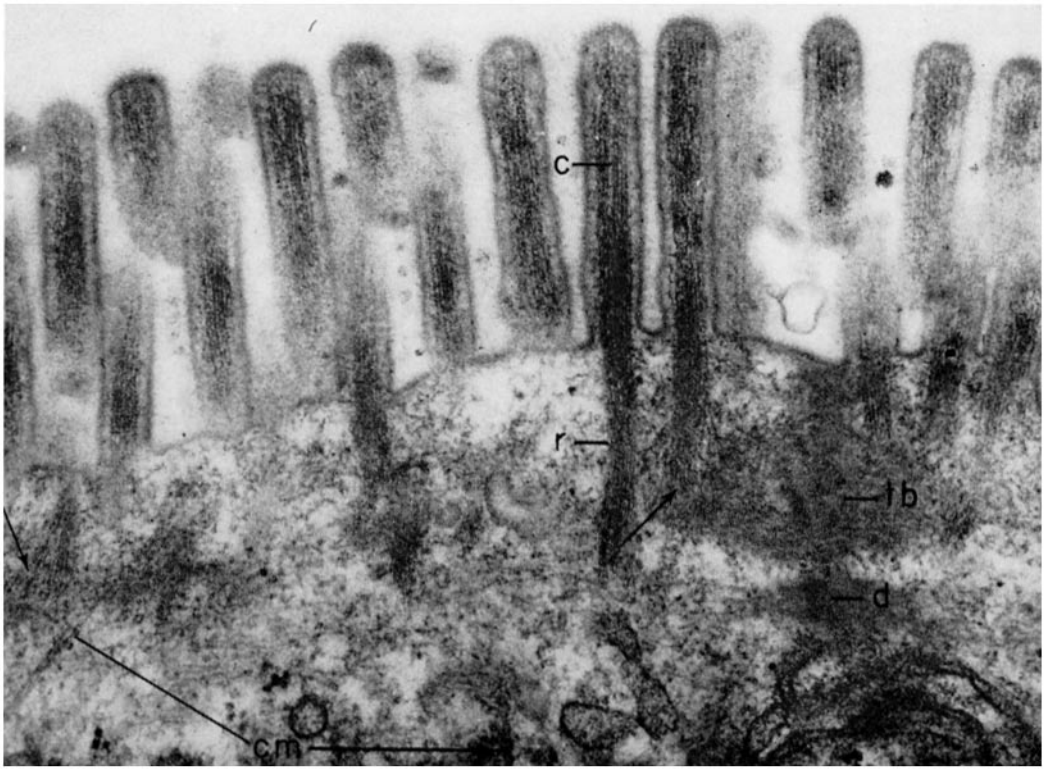

FIGURE 1 Electron micrograph of a longitudinal section through the striated border of an absorptive cell of a duodenal villus. The longitudinally arranged central core (*c*) is composed of straight filaments. These continue into the apical cytoplasm as the footlet (*r*). To the right, a terminal bar (*tb*) and apical desmosome (*d*) have been sectioned obliquely. The terminal web is on the same level as the terminal bar. Similarly, the desmosomal web is on the same level as the desmosome. Arrows indicate angulations of rootlet filaments in the region of the terminal web. Cytoplasmic microtubules (*cm*) can be seen near the terminal web. × 52,500.

filament-free zone approximately 200–300 A in thickness, except at the apex of each microvillus. At the apex the filaments appear to end in the accumulation of dense material adjacent to the inner leaflet of the plasma membrane (Fig. 2). The lower ends of the filaments terminate in the apical cytoplasm as rootlets.

Immediately below the striated border lie the transversely oriented filaments of the terminal web which attach to the *zonula adhaerens* of the terminal bar (2). Beneath this dense web lies the desmosomal web, a looser zone of filaments which attach mainly to the most apical desmosome. In certain places, angular bends of the rootlet filaments suggest that there is direct continuity between the rootlets and the terminal web. In other places, the rootlet filaments diverge and form a cone-shaped structure. Still other rootlets continue past the terminal web and associate with the desmosome web. Microtubules are also seen in the apical cytoplasm.

In a transverse section of the microvillous border and the apical cytoplasm, filaments are observed in oblique- and cross-section (Fig. 3). Each microvillus contains a central core of filaments surrounded by a homogeneous zone of medium density. The surrounding plasma membrane is seen to have the trilaminar, unit membrane structure except where sectioned obliquely. The filaments have an average diameter of 60 A. There are approximately 50 filaments per core, although the number varies depending on the size of the microvillus. In several locations, the filaments are arranged in a hexagonal packing with a center-to-center spacing of approximately 100–150 A.

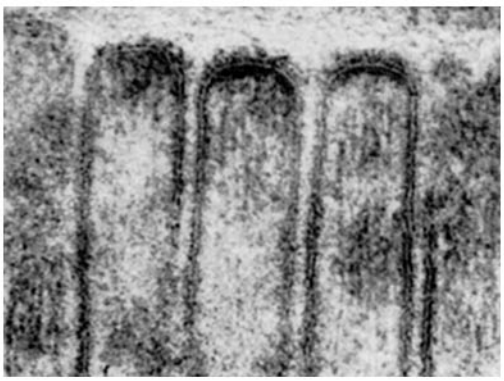

FIGURE 2 Higher magnification electron micrograph of the tips of microvilli of the duodenum. On the outer surface of the apical plasma membrane are seen irregular accumulations of densely stained material. In the cytoplasm within the tip of the microvillus, a zone of increased density is present. Filaments are embedded in this inner dense material. Attachment to the membrane itself cannot be resolved. × 105,000.

The appearance of the filaments in rows is probably due to a slight tilting of the core or rootlet.

DISCUSSION

Most studies of the intestinal epithelium have shown only a suggestion of an organized structure within microvilli (1, 4, 12, 14, 15).

Zetterqvist (19), in his study of the small intestine, showed that jejunal microvilli contain a longitudinal fibrillar component. He also described a continuation of this component into the apical cytoplasm which he called a rootlet. Palay and Karlin (9), in a study of the rat jejunum, described the microvillous core as composed of a "dense fibrillar meshwork" and noted that each fibrillar core was continuous with the "filamentous substance of the terminal web underlying the striated border." However, they attributed the presence of rootlets to faulty fixation. Millintogn and Finean (7) observed that the rootlets only

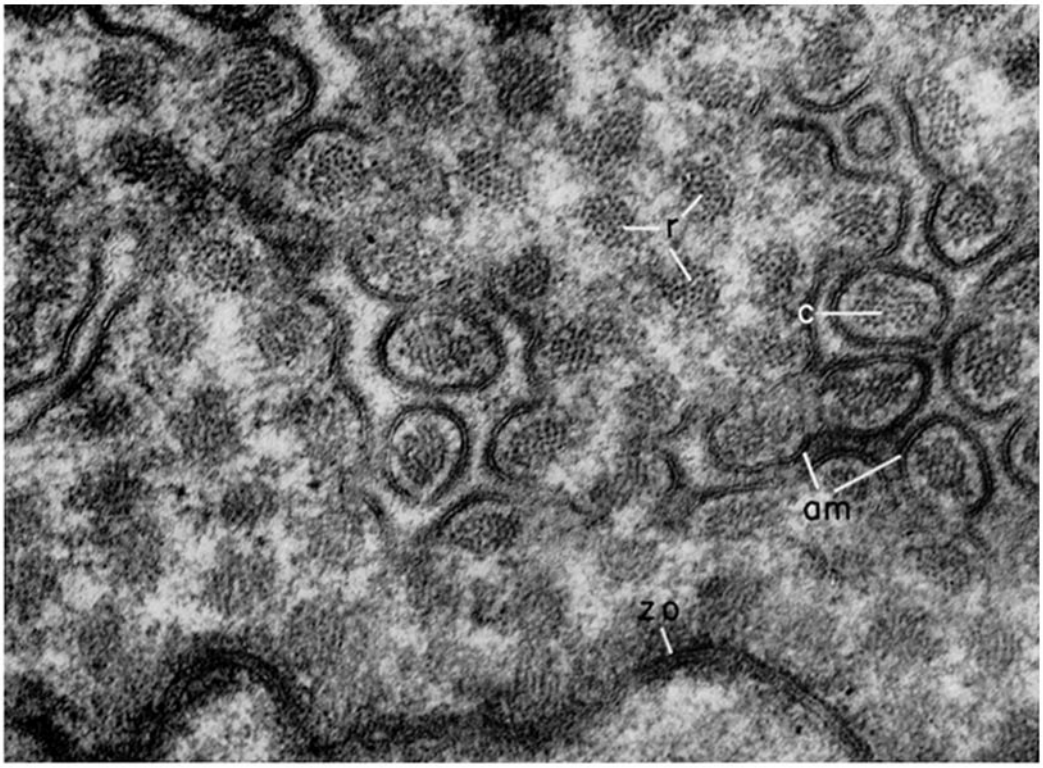

FIGURE 3 Electron micrograph of a cross-section through microvilli and rootlets in the apical ectoplasm of a cell of the duodenal villus. The apical plasma membrane (*am*) can be seen encircling the microvilli, with a zone of lesser density between the membrane and the filamentous core. In other areas the plasma membrane can be seen adjacent to the ectoplasm in which many rootlets (*r*) can be identified. The *zonula occludens* (*zo*) of the terminal bar between the apices of adjacent cells is illustrated. × 105,000.

became distinct in villous cells after small changes in tonicity, pH, or early postmortem autolysis. It is possible that the accentuation of the rootlets in this material was due to the decrease in density of the background cytoplasm which obscured them in the cells fixed in isotonic solutions. The rootlets can be seen in all of their photographs but less clearly in those of preparations fixed in isotonic solutions. Zamboni (18) has described the appearance of the core filaments as chicken-wire–like. Trier (16) has documented the presence of fine filaments in microvilli of crypt cells in the human jejunum.

None of the aforementioned studies have presented conclusive evidence that these structures are truly filamentous. The suggestion that the cores might be composed of small tubules instead of filaments (7) seems to be negated by a comparison with the cytoplasmic microtubules (5, 11, 13) seen in the same section. From the evidence of the longitudinal- and cross-sections and from the comparison of cytoplasmic microtubules with the structures in the core of the microvillus, we suggest that the core structures are filamentous. It seems likely that these filaments represent an extension of filaments from the terminal web.

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