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Filamentous Microbes Indigenous to the Murine Small Bowel: A Scanning Electron Microscopic Study of Their Morphology and Attachment to the Epithelium

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Abstract. Segmented, filamentous prokaryotic microorganisms colonize and attach to the cells in the epithelium of the mucosa of the small bowels of mice and rats. Scanning electron micrographs, derived from specimens of mouse small intestine, reveal microbial filaments of at least two types. One type is thin (0.8 μm) with only faint lines suggesting septa; the other is thicker (1.4 μm) and has distinct segments with pronounced septa. Most of the segments are rounded; a few are thin and elongated. Immediately surrounding the attachment site of these organisms, the surface of the epithelial cells appears roughened and occasionally stringy. The filaments may differ morphologically because they represent different phases in the life cycle of a single microbial type. Alternatively, however, they may differ because they are the cells of different microbial types colonizing the same epithelial habitat.

Introduction

The gastrointestinal tract of laboratory mice and rats contains many indigenous microorganisms localized in distinct habitats. Each habitat is colonized by populations of microbes of one or more types (9). Some of the microorganisms are known to associate with the epithelial surface in the habitat they colonize. Some may attach physically to the epithelium. The mechanisms by which they associate with or attach to epithelia are poorly understood (9).

Segmented, filamentous microorganisms are known to colonize and attach to the epithelium of the small bowel in both mice (2,6,10) and rats (1–4,8). A microbe of similar morphology has also been seen attached to the epithelium in the small intestines in chickens (5). The identity of these organisms has not been established. Light and transmission electron microscopy have been used to detail some of their ultrastructure and the habitat they colonize (1–4,6,8). In this article, we amplify that information with findings from a study with the scanning electron microscope (SEM) of the attachment and morphology of the organisms in mice.

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Materials and Methods

Animals

Male Swiss white mice, 10 to 12 weeks old, were obtained from the vivarium of the Memorial Medical Center in Springfield, Illinois. They had been housed in plastic cages (Isocage, Bectin-Dickinson Co.), fed commercial mouse food (Lablox, Wayne, Allied Mills, Chicago, Ill.), and given tap water to drink.

Scanning Electron Microscopy

The mice were killed with ether or chloroform. Their abdominal cavity was opened to expose the gastrointestinal tract. The distal ileum was rapidly flushed, by interluminal perfusion, with cold 2.5% gluteraldehyde in phosphate buffer (0.02M) at pH 7.3. Then 25 to 30 mm of the ileum was isolated between hemostats and perfused further with fresh, cold fixative. The segment was then removed from the animal and placed in a Petri dish filled with fixative. The specimen was cut into 3-mm^2 pieces. The pieces were placed in fresh, cold fixative and allowed to stand for 1 hour. Some of the pieces were processed with post-fixation in osmium tetroxide, followed by dehydration in ethanol. Others were processed through the O-T-O-T-O method (7) with alternating changes in aqueous solutions of 1.5% osmium tetroxide and saturated thiocarbohydrazide, followed by dehydration in ethanol. All specimens were dried from 100% ETOH in a Bomar Critical Point Dryer, and glued to scanning electron microscope stubs with silver paint. Samples only post-fixed with osmium tetroxide were coated with carbon-gold-palladium on a tilting omnirotary stage in a Denton DV-502 vacuum evaporator. Samples prepared with the O-T-O-T-O method were not coated.

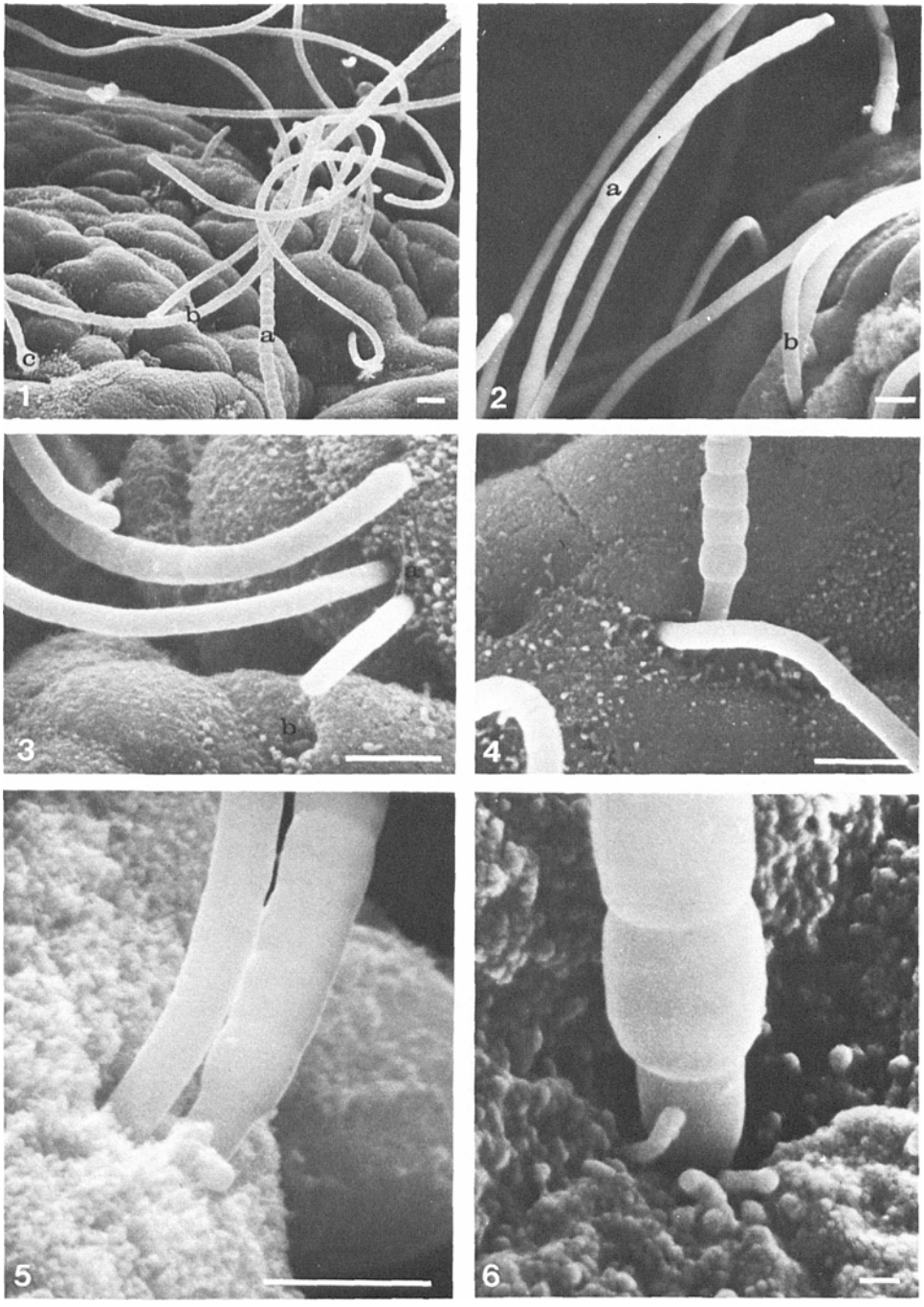
The specimens were examined in a Mark II Stereoscan SEM (Cambridge Instrument Co., Ltd., Cambridge, England) or in a Hitachi S-500 (Hitachi, Ltd., Tokyo, Japan). The micrographs were taken at 20 kv with Polaroid PN/55 film.

Results

Types of Filaments, Intrasegmental Bodies, and Terminal Bodies Found Attached

Two types of filaments could be seen attached to the epithelium. One type was thick, by comparison with the other, and displayed plump, rounded, distinct segments with defined septa (Figs. 1–10). This organism commonly had a thin tapered structure at the site of attachment in the epithelial cell (Figs. 4–6). The filament extending from that structure consisted mostly of segments about 1.4

Fig. 1. Overview of segmented filamentous microbes attached to the ileocecal mucosa showing two distinct types of filaments. A thick, heavy one with defined septa is shown at **a** and a slender filament with less defined septa is indicated at **b**. A terminal, bulbous structure is shown at **c**. Scale = 5 μm (1380×). **Fig. 2.** Intersegmental body in the thick filament is shown at **a**. Two types of filaments extending from what appears to be a common attachment site at **b**. Scale = 5 μm (1820×). **Fig. 3.** Stringy rough appearance of the mucosa at an attachment site is shown at **a**. Hole with debris in it is indicated at **b**. Scale = 5 μm (4600×). **Fig. 4.** Both types of filamentous microbes attached to the mucosa. The nipple-shaped end segment of the thicker filament can be seen. Scale = 5 μm (4600×). **Fig. 5.** High magnification of the two types of filaments attached to the mucosa. Scale = 5 μm (8645×). **Fig. 6.** High magnification of the nipple-shaped end segment attached to the mucosa. Scale = 0.5 μm (27,573×).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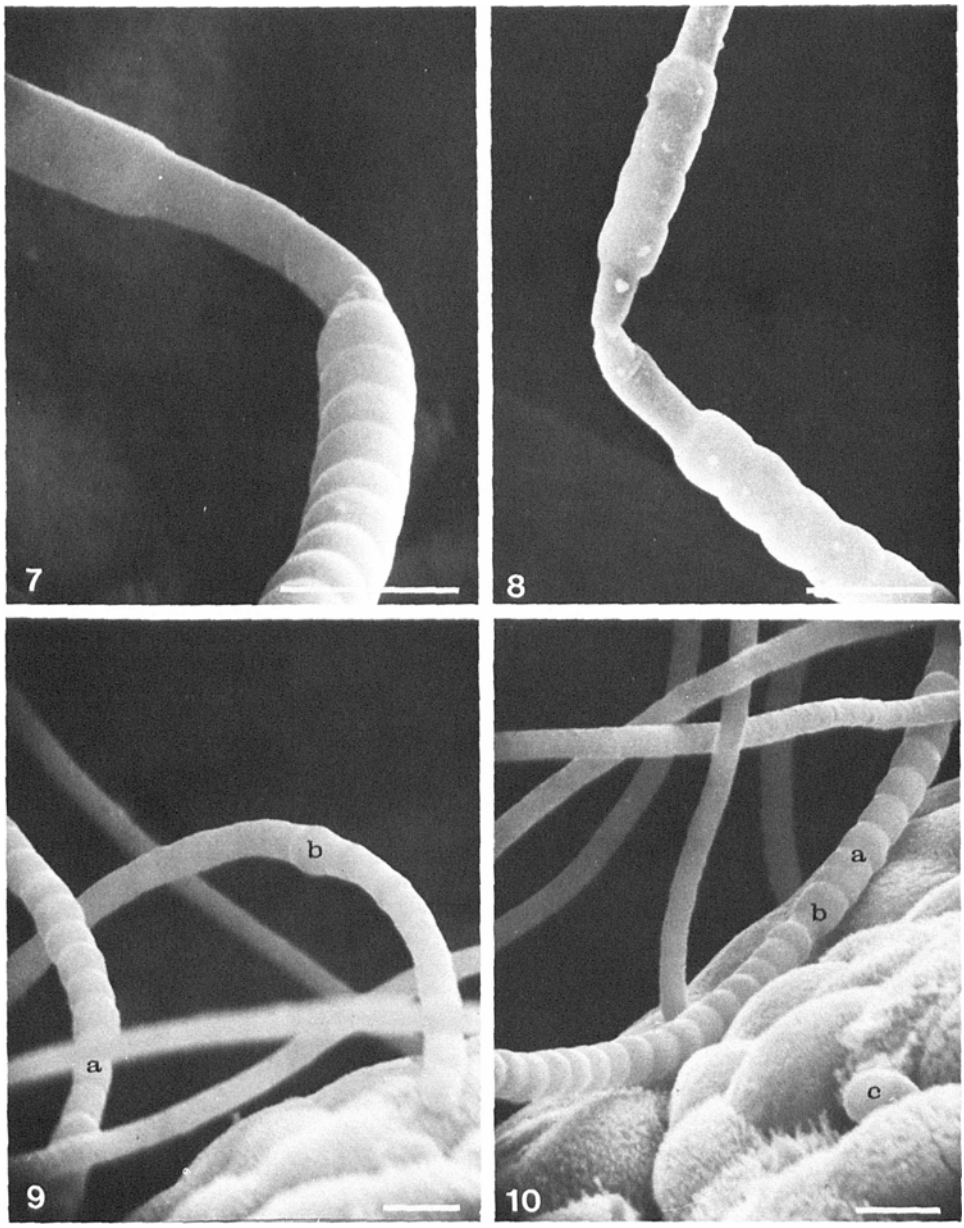

Fig. 7. Nipple-shaped intrasegmental body. Scale = 5 μm (8645×). **Fig. 8.** Nipple-shaped intrasegmental bodies showing separation between two segments. Scale = 5 μm (5915×). **Fig. 9.** Thin segments within thick filament are at **a.** Large barrel-shaped intrasegmental body is at **b.** Scale = 5 μm (3867×). **Fig. 10.** Short segments within thick filament are at **a.** Larger segment with smaller, thin segment immediately following are at **b.** Bulbous, spore-like structure attached at junction of epithelial cells is at **c.** Scale = 5 μm (3867×).

μm in diameter. Frequently, however, segments averaging 2.0 μm in diameter were interspersed between the predominating ones (Figs. 2 and 9). Nevertheless, a few segments within the filament itself were just as thin as the attaching structure and were even similarly tapered. Some of these tapered elements were apparently separated by a septum (Figs. 7 and 8). Some quite short, thin segments were also often seen between the predominating ones (Fig. 10). Some of these thick filaments had terminal spherical bodies averaging 1.6 μm in diameter (Figs. 1, 14, and 15).

The other type of filament was thinner than the first, averaging 0.8 μm in diameter. It did not have clearly defined septa, a tapered first segment, enlarged segments, or terminal spherical bodies as were seen in the thicker filaments (Figs. 1–5, 9, and 10).

Bulb-shaped Structures Attached to Epithelial Surface

In addition to the long filaments of the two types, bulb-shaped structures were seen attached to the epithelial surface (Figs. 10, 11–13, and 18). These structures were 1.8 μm in diameter and were often seen attached at the junction of four mature epithelial cells (Figs. 10–12). One of these bodies had attached to it, or in close proximity, another much smaller, bean-shaped structure (Figs. 12 and 13). In addition, one of them displayed a ring on the luminal side of the bulb. The ring was of the approximate diameter of a segment of the microbe (Fig. 18). These bulbous structures resembled the terminal bulbs observed on the thick filaments (Figs. 1, 14, and 15).

Attachment Sites

As noted, the thick, heavy filaments have tapered end segments that penetrate the outer surface of the microvilli of the epithelial cells (Figs. 4–6). The thin filament also penetrates between the microvilli but does not have a specialized, apparently differentiated end segment (Figs. 3–5). Frequently, dark holes were observed in the area of the distal ileum where the heavier populations of the filamentous organisms are found (Fig. 15). At lower magnifications, these holes often appeared to be empty, but when observed at higher magnifications they proved to have structures in them (Figs. 3 and 15–17). Some of the structures had rings that could be interpreted to be scars remaining after detachment of filaments (Figs. 16 and 17).

Epithelial Cell Surface Modification

Surrounding the attachment sites of both types of filamentous microbes, the epithelial cell surface was modified ultrastructurally (Figs. 2, 4, 6, and 15–18). In these areas, the cell surface appeared rough and sometimes stringy, where the microvilli were rearranged or enlarged.

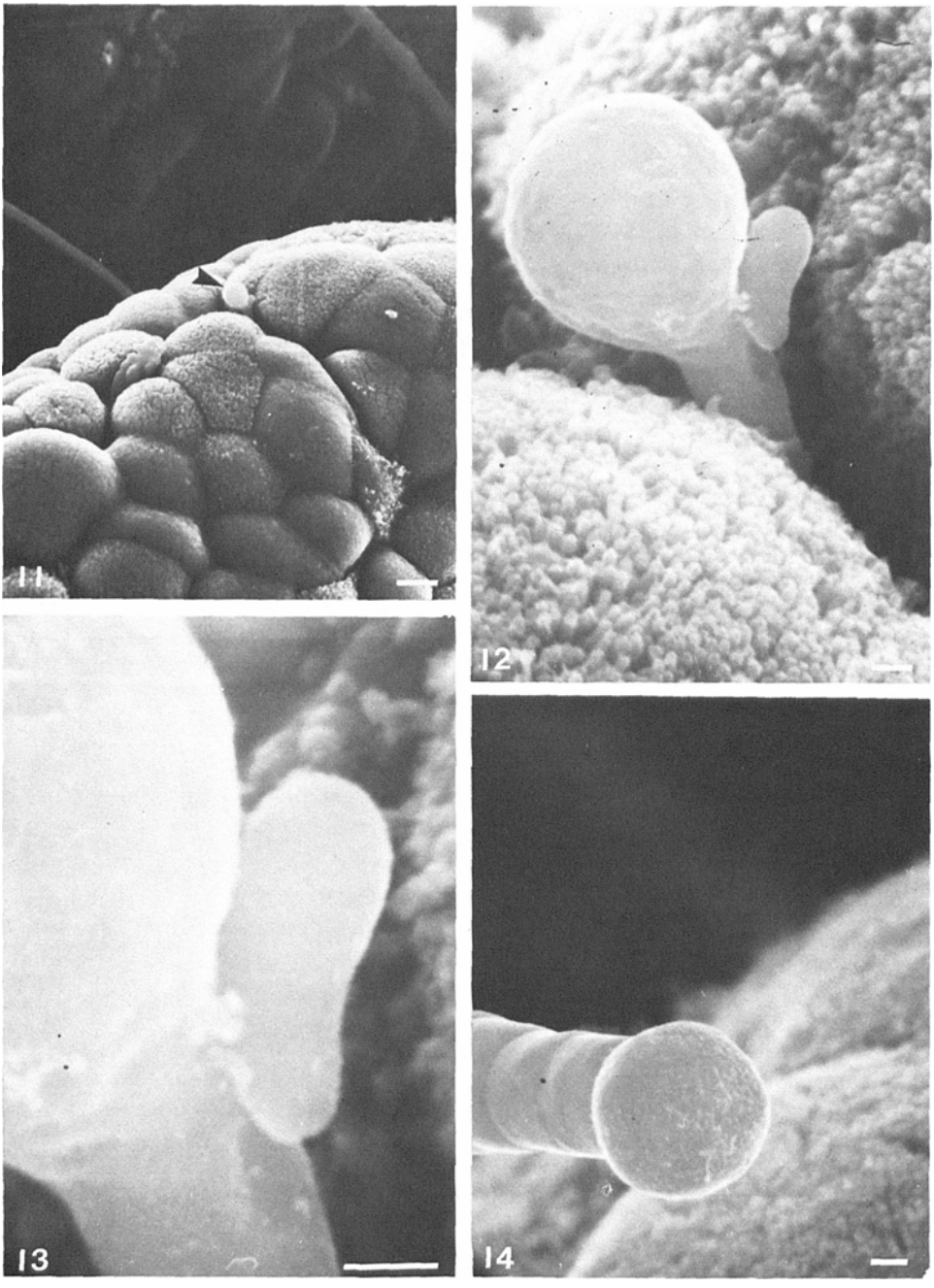

Fig. 11. Arrow indicates a bulb-shaped structure attached at the junction of epithelial cells. Scale = 5 μm (1820×). **Fig. 12.** Higher magnification of structure shown in Fig. 11 with a nipple-shaped attachment organ and a curved rod-shaped structure in close proximity. Scale = 0.5 μm (18,-200×). **Fig. 13.** Higher magnification of the curved rod-like structure shown in Fig. 12. Scale = 0.5 μm (45,500×). **Fig. 14.** Distal end of a thick, segmented filament showing a bulbous structure followed by modified segments. Scale = 0.5 μm (15,925×).

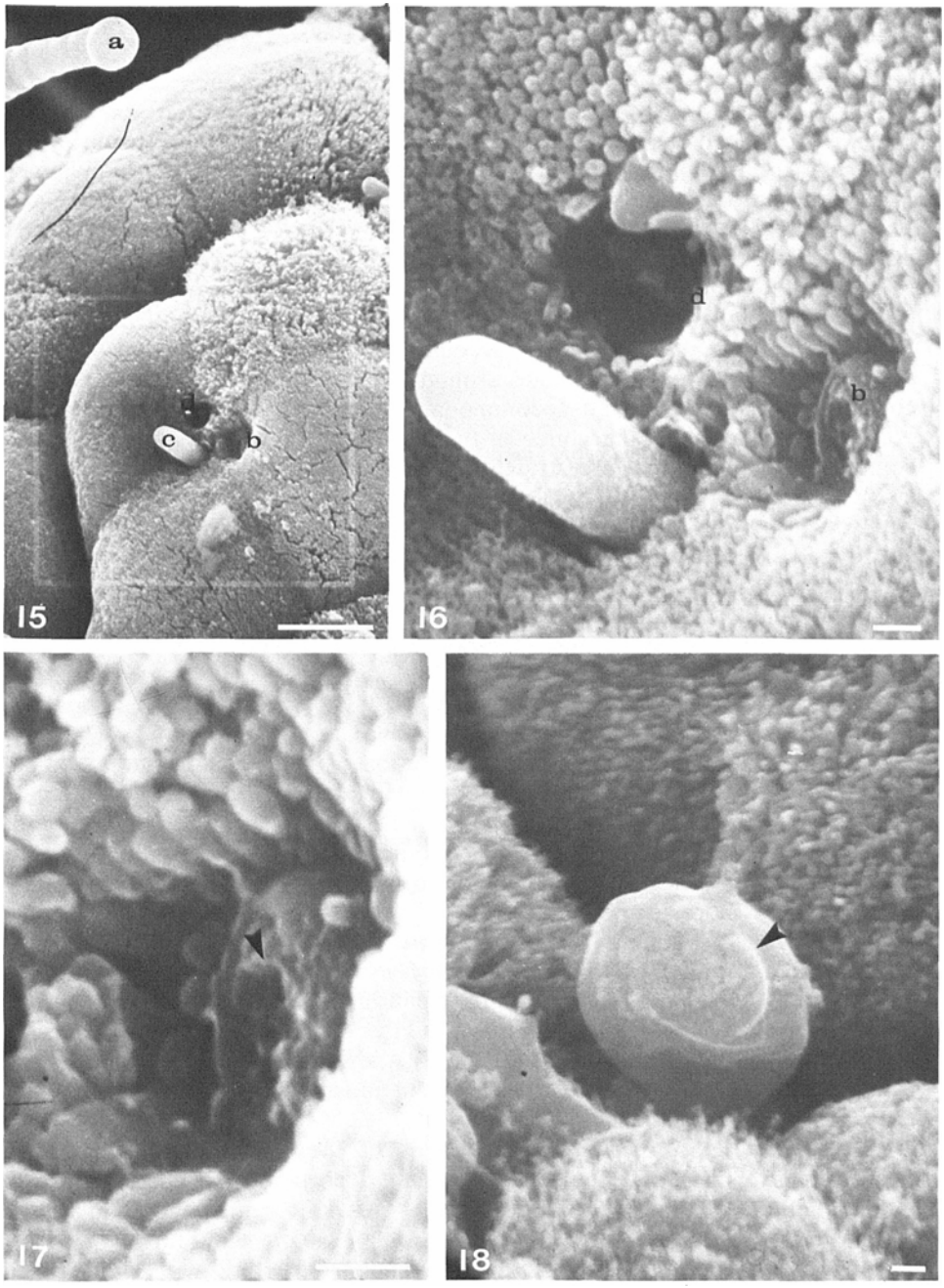

Fig. 15. Low magnification of the field from which Fig. 14 was taken. The bulbous structure and modified cells at the distal end of a microbe are at **a.** Debris in what may be a former attachment site is at **b.** What appears to be a newly attached, undifferentiated microbe is at **c.** What may be a dark, empty former attachment site is at **d.** Scale = 5 μm (4550×). **Fig. 16.** Higher magnification of **b** and **d** from Fig. 15 showing the debris in both sites. The rearrangement and hypertrophy of the microvilli is especially evident around **b.** Scale = 0.5 μm (25,000×). **Fig. 17.** Higher magnification of the debris in **b,** Fig. 15. Arrow shows the remnants of what is probably a septal ring at the ostensible point of detachment of a filament. Scale = 0.5 μm (22,750×). **Fig. 18.** Bulbous structure attached at the junction of four epithelial cells. Arrow indicates remnants of an apparent septal ring at the point of detachment of the filament. Scale = 0.5 μm (11,830×).

Discussion

Findings from several studies of the microbial flora of the murine gastrointestinal tract have confirmed that filamentous microorganisms colonize the small bowel, populating most heavily the distal region near the ileocecal junction (1,2,8,10). Moreover, light and transmission and scanning electron micrographs indicate conspicuous modification of the microvillus border of the absorptive epithelial cells at the point of attachment of these microbes (2,4). The microvilli appear rearranged or hypertrophied, surrounding the area of the point of attachment. Plasma membrane alterations could account for the hypertrophied appearance of the area (2). However, the changes around the attachment site could be due to contraction of the microvilli to accommodate attachment of the organisms (4).

The microbes are prokaryotic (2). In rats, two types have been seen (1): one consists of filaments that are straight and narrow with smoothly rounded free ends; the other consists of long filaments with smooth free ends and shorter ones with rough, irregular free ends (1). We too have seen two types of filaments. So both types can be found in mice as well as rats.

The nipple- (2) or teardrop-shaped (1) end segment of the thick filaments has been described as a holdfast produced as an intrasegmental body and then released to attach and produce another filament (1). Our evidence suggests another source of holdfast development in which the nipple-shaped structure (Figs. 7 and 8) develops as part of a filament itself, then divides and separates from the filament to attach and produce a new one.

Bulbous-shaped bodies on the distal portion of mature filaments have been described (1). We have observed similar structures attached to smaller, tapered, and less defined segments (Figs. 1, 14, and 15). These structural units may be able to detach from the main filament and act as reproductive units, with the smaller segments acting as attachment organs and the bulbous structure functioning to produce more segments.

Intrasegmental bodies in these filamentous microbes have been considered to be survival or reproductive forms (1,2). These bodies have prokaryotic cell structure (2). They have been said to be similar in origin to endospores (1). They appear to us to be nearly the same size as the bulbous-shaped bodies we see attached to the epithelium (Fig. 12). Thus, they may indeed be reproductive forms able to escape from the mother segment and attach to the epithelial surface to generate a new filament (Figs. 1 and 2).

What may be former attachment sites of these segmented filamentous microbes have been described (3). Some are reported to be empty, some to contain debris (3). We observed both types. A dark hole appeared empty at the lower magnification, but did show signs of some sort of remnant at higher magnification. The hole close to it contained a structure with the circular remnants of what appeared to be a septal ring at the point of detachment of a main filament. The area surrounding this hole also exemplified enlarged, rearranged microvilli.

As has been suggested by others (1), the life cycle of this strange segmented, filamentous microbe could be quite complicated involving several diverse morphological forms. Alternatively, however, such morphological diversity may

exist because more than one taxonomic group of the organism exists. This problem may not be resolved until the organisms have been cultured *in vitro*.

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