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Habitat, Succession, Attachment, and Morphology of Segmented, Filamentous Microbes Indigenous to the Murine Gastrointestinal Tract

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Some indigenous microorganisms localize on epithelial surfaces in various areas of the digestive tracts of animals. One of these, a segmented, filamentous microbe, localizes on the epithelium in the small bowels of mice and rats. These filamentous microbes colonize mice at weaning time and persist in adult animals for at least 2 months. Results of the study of light and electron micrographs suggest that the microorganisms are procaryotic, and that they interact with small bowel epithelial cells to form an attachment site. This site consists of modified epithelial cell membrane and apical cytoplasm adjacent to the attached bacterium. The microbe fills the site with part of its first segment. This segment has a nipple-like appendage on the end inserted into the epithelial cell. The other segments, which compose the rest of the filament, are usually separated by septa. Many of the individual segments contain intrasegmental bodies that appear to be procaryotic cells. Some of these intrasegmental bodies are similar in morphology to the first segment of each filament inserted into an epithelial cell. These intracellular bodies may be components in the life cycle of the microorganism. The organism has not yet been cultured in recognizable form. Therefore, such a hypothesis cannot be proved as yet, nor can the microbe be classified with certainty. Because it localizes in an epithelial habitat in the small bowel, however, it may be a particularly important microbial type in the gastrointestinal ecosystem of laboratory rodents.

Adult murine gastrointestinal tracts contain many types of indigenous microorganisms living in relatively stable communities localized in specific regions of the tract (5, 7, 10, 15, 16, 18). The various microbial types in these communities colonize the tracts of neonatal mice in a characteristic and reproducible succession. The succession in suckling mice has been reported for lactic acid bacteria, coliforms, enterococci, bacteroides, fusiform, and spiral-shaped microbes. The habitats of these microorganisms in suckling and adult mice have been described (4, 7, 17, 19).

Another microbial type can be found on the epithelial surfaces of villi in the small intestines of adult rats (13, 14), mice (11; C. P. Davis, S. Erlandsen, and D. C. Savage, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, p. 57), and chickens (8). This organism has never been identified with certainty. Moreover, little is known about its morphology, habitat, and ecology. In this report, we detail the ultrastructure, habitat, succession, and attachment to epithelial cells of these segmented, filamentous microbes in the small bowels of laboratory rodents.

MATERIALS AND METHODS

Animals. Specific pathogen-free male young adult rats were purchased from four different suppliers (Hilltop Laboratory Animals, Chatsworth, Calif.; National Laboratory Animal Co., Creve Cour, Mo.; ARS/Sprague Dawley, Madison, Wisc.; and Charles River Breeding Laboratories, Inc., Wilmington, Mass.). Male and female mice (5 to 8 weeks old) were obtained from ARS/Sprague Dawley and Charles River Inc. Each mouse strain was bred in our laboratory to provide suckling mice. The rat and mouse strains are listed in Table 1. The animals were housed in plastic boxes covered with filters, both of which were supplied by either Isocage, Carworth (New City, N.Y.) or Wahmann Mfg. Co. (Timonium, Md.). Bedding was supplied by either Allied Mills (Chicago, Ill.) or by American Excelsior Co. (Lombard, Ill.). Lab Blox food (Allied Mills, Chicago, Ill.) and acidified water (6) were given ad libitum.

Histological methods. All animals were killed with 95% alcohol-chloroform-ether vapors (1:2:3, vol/vol/vol). Pieces of ileum (1 to 2 cm), approximately 1 to 5

TABLE 1. *Detection of segmented, filamentous microbes in murine ilea*[a]

Rat strain	Supplier	Rats possessing microbes[b]	Mouse strain	Supplier	Mice possessing microbes[b]
HLA-W	Hilltop Lab. Animals	5/5	Crl:COBS CD-1(ICR)BR	Charles River	33/35
NFL	National Lab. Animals	5/5	C57 BL/6St Crl BR	Charles River	0/14
SPD	ARS/Sprague Dawley	5/5	Sch:ARS Ha(ICR)f	ARS/Sprague Dawley	47/50
Crl:COBS CD(SD)BR	Charles River	5/5	C57 BL/6S chf	ARS/Sprague Dawley	15/15

[a] Segmented, filamentous microbes were detected with light microscopy in Gram-stained frozen sections or by phase microscopy in unstained resin-embedded sections of intestinal tissue.

[b] Ratio represents the number of animals in which the microbes were detected to the number of animals examined.

cm from the ileocecal junction, were removed from mice and rats. In other mice, whole small bowel from the pyloric sphincter to the ileocecal valve was removed. This was measured and cut into four equal parts. Each part was spirally wound onto a microtome cryostat block and frozen, with contents intact, in 2% methyl cellulose in 0.85% saline (17). The tissue was sectioned and fixed while on slides in absolute methanol. Sections were stained with a tissue Gram stain (3). Also, two pieces from 10 mouse and 20 rat ilea were obtained approximately 1 to 5 cm from the ileocecal junction. One piece was processed as above for sectioning with a microtome cryostat. The other piece was cut open, and the lumenal contents were brushed gently aside. Then, a strip about 1 mm wide was cut from the piece of ileum and processed for electron microscopy.

Electron microscopy. The strips of mouse and rat ilea were embedded by either the method of Luft (12) or Spurr (21). Sections 1 to 3 μm thick were cut with glass knives and mounted onto glass slides. Thin sections were cut on a Porter-Blum ultramicrotome with a diamond knife or on a Reichert ultramicrotome with glass knives. Then, sections were stained with 2% uranyl acetate and lead citrate, as previously described (5). Specimens were examined with either a Hitachi HU-8 or HU-11C electron microscope.

Light microscopy. Sections stained by a Gram method for tissues were examined with either a Leitz Ortholux microscope or a Zeiss Universal microscope. Unstained 1- to 3-μm sections of ilea prepared for electron microscopy were examined under phase optics.

Sections of spirally wound Gram-stained ileal tissue were examined by following the spiral and by marking the area in the section where the last segmented, filamentous organism was observed. A wire was used to measure the distance from the ileocecal junction back to the area where the last microbe was detected.

RESULTS

Detection of microbes with light microscopy. Sections of murine ilea, examined with either light or phase-contrast optics, frequently contained gram-variable segmented, filamentous microbes located in close association with murine ileal epithelium. The microbes were observed occasionally to be sticking into an epithelial cell (Fig. 1). The bacteria in both Gram-stained sections and resin-embedded sections appeared to be filamentous and segmented. Some areas within the individual segments, when viewed with light or phase optics, possessed slightly different staining or light-transmitting properties, which suggested that the segments might have had internal structural differences (Fig. 1 and 2). Furthermore, sections of resin-embedded tissue from murine ilea often showed a small light opaque area surrounding each organism where it was apparently attached to an epithelial cell (Fig. 2). This was not observed in the Gram-stained sections.

Rats and mice purchased from several suppliers were examined by the above methods to determine whether or not segmented, filamentous microbes were present in close association with their ileal epithelium. Each of the rats examined contained the bacteria in their ileum. Almost every mouse from two different suppliers also possessed the organisms. However, all of the mice from one strain, C57 BL/6St Crl BR, did not have any detectable segmented, filamentous microbes in their small bowels (Table 1).

Area of the small bowel of mice colonized by segmented, filamentous microbes. Sections of whole mouse bowels were examined for segmented, filamentous microbes. The length of the small bowel in which the microbes localized was determined in males and females from two mouse strains (Table 2). Although the organisms could be detected in a large percentage of the length of the small bowel, they could not be detected uniformly throughout it. The population was most dense in the distal ileum. It increased gradually as the distance from the duodenum increased. Also, the microbes colonized the bowel irregularly; one area of the bowel would contain organisms whereas another area would not have them. Microbes could be separated from each other by uncolonized areas

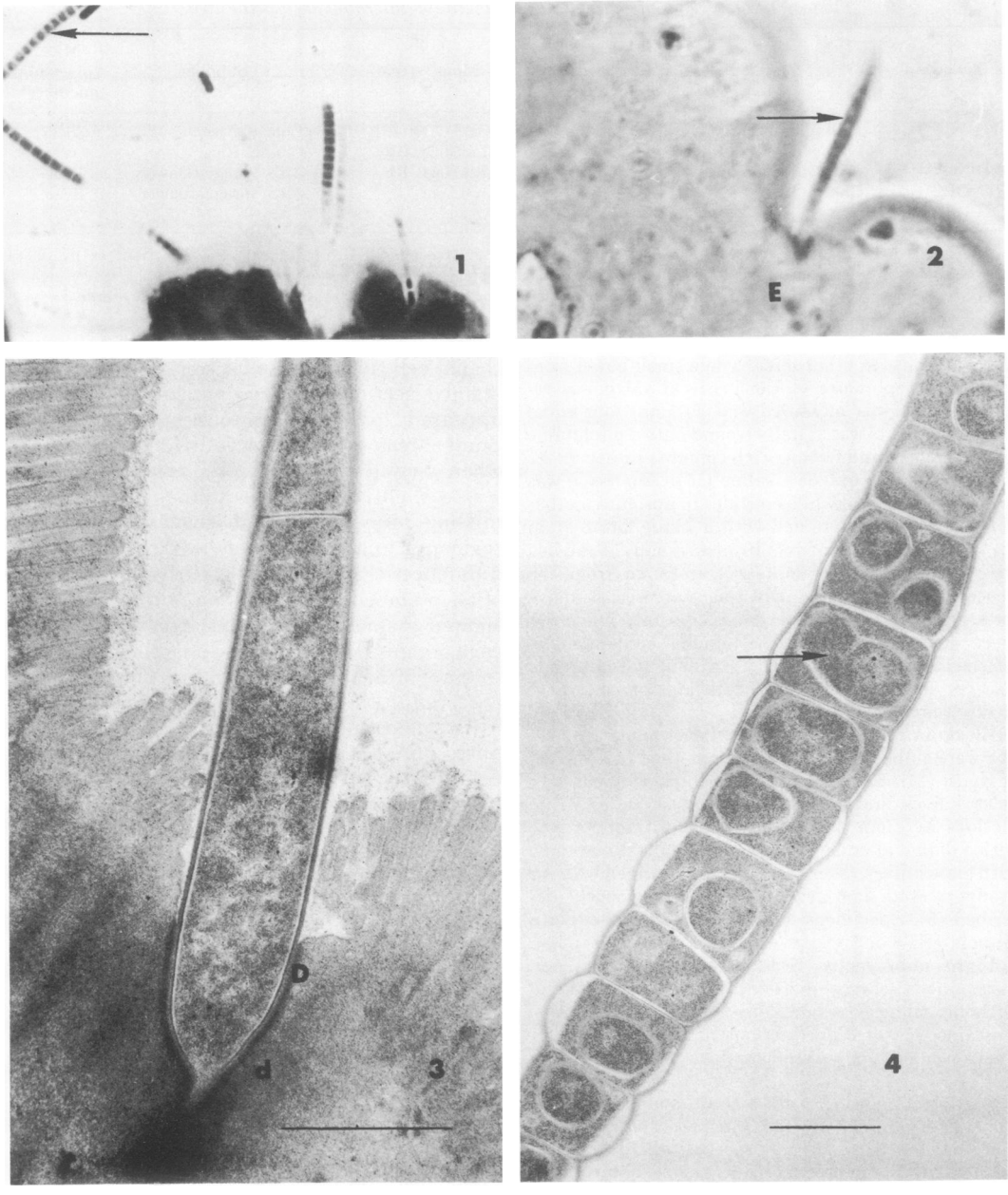

FIG. 1. *Gram-stained section of a rat ileum that shows segmented, filamentous microbes with internal structure (arrow). At the lower right, the microbe and ileal epithelium are closely associated. ×3,840.*

FIG. 2. *Phase-contrast micrograph of a section through Epon-embedded rat ileal mucosa. A filamentous microbe with internal structure (arrow) is attached to an epithelial cell (E). ×5,450.*

FIG. 3. *Electron micrograph of the first segment of a filamentous microbe attached to an epithelial cell. Note the nipple-like appendage, the electron dense area (D) adjacent to the organism, the less electron dense area in the epithelial cell cytoplasm (d), and the variation in the cell wall electron density between the area not associated with the epithelial cell and the area associated with it. Bar represents 1 μm. ×18,400.*

FIG. 4. *Electron micrograph of a section through some segments of a microbe showing intrasegmental bodies. The arrow points to an intrasegmental body that may be dividing. Bar represents 1 μm. ×13,200.*

that ranged from about 2 to 8 cm in length. Furthermore, two animals possessed no detectable segmented, filamentous microbes in the distal 25% of their small bowels, but did have the organisms in more proximal areas.

Males and females of the two mouse strains differed in the percentage of the total length of small bowel populated by the bacteria (Table 2). The organisms colonized less ($P < 0.01$) of the small bowels of CD-1 females than that of CD-1 males and more ($P < 0.01$) of the bowels of Ha(ICR) females than that of the corresponding males.

Colonization of suckling and young adult mice. Charles River CD-1 and ARS/Sprague Dawley Ha(ICR) mice were bred in our laboratory. The offsprings were examined for the segmented, filamentous microbes over a period extending from 7 to 50 days after birth. The microbes began to colonize the ilea of the mice shortly before weaning time (day 21). All of the animals 25 days old or older possessed the bacteria (Table 3).

TABLE 2. *Location of segmented, filamentous microbes in the mouse small bowel*[a]

Mouse strain	Sex	No. of animals	Small bowel containing microbe (mean %)[b]	Standard deviation
COBS CD-1 (ICR)BR	male	15	42.4	13.5
	female	20	16.1	7.1
ARS Ha(ICR)f	male	10	47.2	12.8
	female	10	67.1	14.4

[a] Microbes were detected in Gram-stained frozen sections of the mouse small bowels.

[b] Percentage of the total length of small bowel containing the segmented microbes was calculated by dividing the length of the bowel containing the microbes (measured from the ileocecal valve proximally to where the microbes were first detected) by the total length of the small bowel. The mean values for male and female COBS CD-1(ICR)BR mice are significantly different ($P < 0.01$), as are the values for male and female ARS Ha(ICR)f mice ($P < 0.01$).

Electron microscopy. The segmented, filamentous organisms had essentially the same ultrastructure in both rats and mice. Likewise, ileal epithelial cells showed the same characteristic modifications when microbes were attached to them in both mice and rats. Consequently, the following descriptions will pertain to the microbes and epithelial cells found in both animal types.

Each filament was made up of segments, and had a modified segment that was involved in attachment of the filament to an epithelial cell (Fig. 3). The segments had the ultrastructure of procaryotic cells. Some segments contained intrasegmental bodies that also had procaryotic cell structure (Fig. 4 and 7). These bodies did not have the ultrastructure of bacterial endospores.

The first segment of each filament was intimately associated with the murine epithelial cell plasma membrane. This cell had a nipple-like appendage that projected the bacterial cell surface further into the epithelial cell (Fig. 3 and 5). The outermost homogeneous layer of the cell wall on the first segment showed different electron densities. If the wall was located next to the epithelial cell, it appeared to be less electron dense than the portion of the wall that was not adjacent to the epithelial cell (Fig. 3 and 6). The diameter of the first segment was approximately 0.7 μm, whereas segments further along the filament were about 1 to 1.3 μm in diameter (Fig. 3 and 4). This first segment probably did not penetrate the plasma membrane of the epithelial cell; some sections showed the membrane intact near the nipple-like appendage (Fig. 5).

Although the first segment might not penetrate, its intimate association with the cell resulted in localized ultrastructural modifications of the microvilli, plasma membrane, and apical cytoplasm of the epithelial cell. The microvilli were either displaced, removed, or modified anywhere a segment was located adjacent to them (Fig. 3 and 6). The area beneath and up to the plasma membrane became elec-

TABLE 3. *Colonization of suckling and adult mice by segmented, filamentous microbes*[a]

Mouse strain	Age of mice (days)[b]										
	7	13	14	17	19	21	22	25	28	35	50
COBS CD-1 (ICR)BR	0/10	ND	0/10	0/9	3/10	3/9	8/10	10/10	10/10	10/10	10/10
ARS Ha(ICR)f ...	0/10	0/10	0/10	0/10	0/4	3/16	3/9	10/10	10/10	10/10	10/10

[a] Segmented, filamentous microbes were detected in Gram-stained frozen sections.

[b] Ratio represents the number of mice in which the bacterial type was detected to the number of animals examined. ND, Not done.

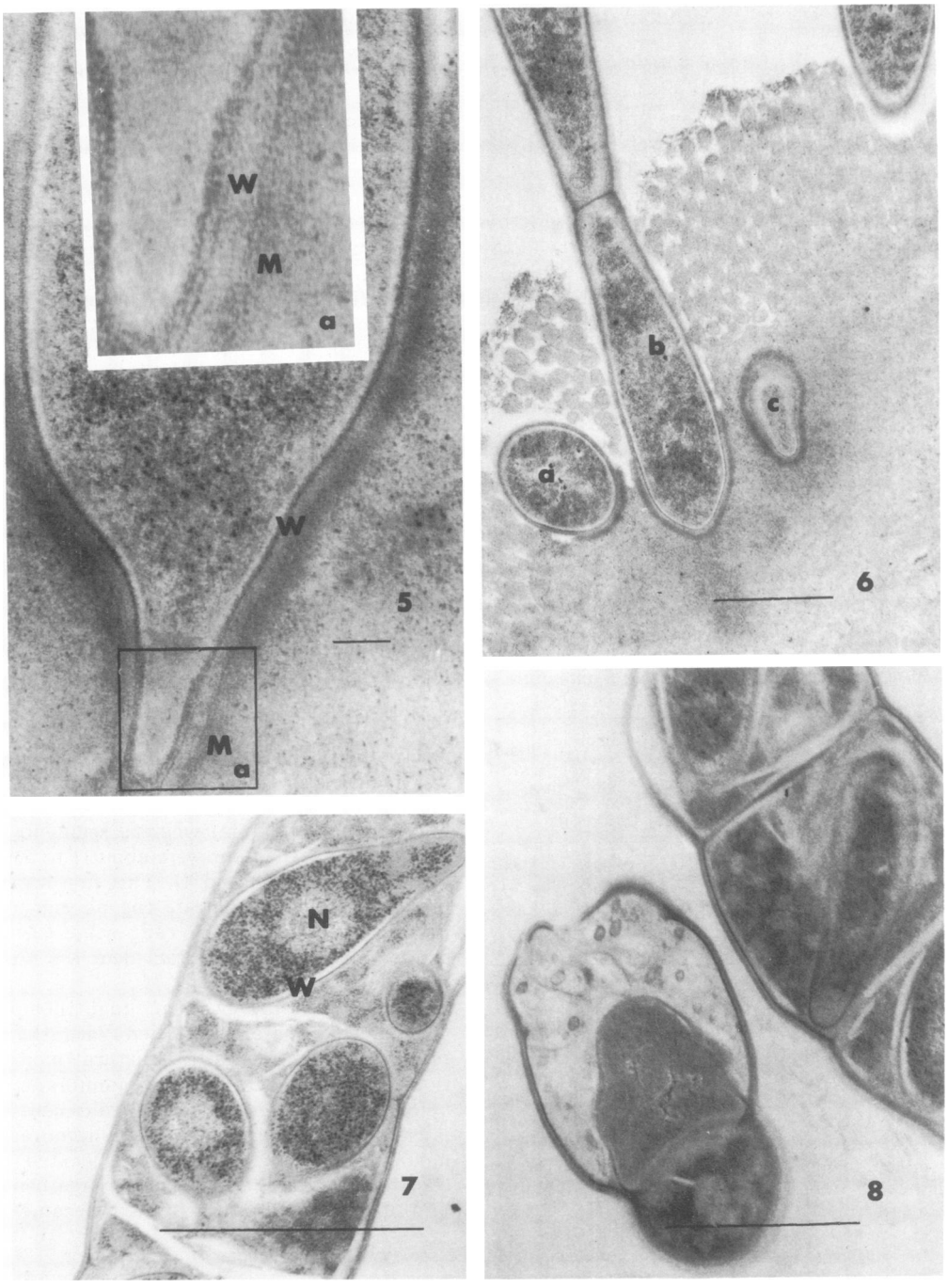

FIG. 5. *This electron micrograph of a section through a nipple-like appendage indicates that the membrane (M) of the epithelial cell can be found next to the outermost layer of the cell wall (W). Bar represents 0.1 μm. ×88,400. (a) ×182,500.*

FIG. 6. *Three microbes are shown attached to one epithelial cell in this electron micrograph. The organism on the far left (a) is shown in cross-section, the microbe (b) is sectioned longitudinally, and microbe (c) is a transversely sectioned part of a nipple-like appendage. The microbe in the upper right corner has displaced the epithelial cell microvilli. Bar represents 1 μm. ×13,500.*

FIG. 7. *This electron micrograph shows the intrasegmental bodies that have a cell wall (W) and nuclear areas (N). Note the absence of septa. Bar represents 1 μm. ×29,900.*

FIG. 8. *As in Fig. 7, this section shows the lack of septa. This section shows also the cytoplasm exposed to the lumenal environment. Bar represents 1 μm. ×24,300.*

tron dense wherever a segment touched it (Fig. 3, 5, and 6). The segment could be either the first segment or other segments that came into contact with the membrane. The plasma membrane was electron dense next to the cell wall of a segment cut in cross-section (Fig. 6). Likewise, the apical cytoplasm of the epithelial cell, directly underneath the densely staining membrane area, was slightly more electron dense than the remaining apical cytoplasm and contained no cellular organelles (Fig. 3, 6, and 11). More than one microbe may be attached to an individual epithelial cell (Fig. 6).

The filament was divided by septa; each segment then formed may or may not have had one or two intrasegmental bodies within it (Fig. 3 and 4). Segments had a wide range of lengths, extending from 3.5 μm to less than 0.6 μm. Their diameters ranged from 0.7 to 1.3 μm (Fig. 3 and 4). In some sections, the septa between some segments appeared to be missing (Fig. 7 and 8).

The morphology of the individual segments vaired from segments with homogeneous cytoplasm and nuclear areas to segments that showed one or two intrasegmental bodies with cell walls, cytoplasm, and nuclear areas distinct from that of the surrounding cell (Fig. 4 and 7). Some of these intrasegmental bodies appeared to be dividing (Fig. 4). Some segments did not have septa, but still had the bodies within the filament. Other segments had neither septa nor intrasegmental bodies (Fig. 7 and 8). The cytoplasm was exposed to the lumenal environment by a discontinuation in the cell wall (Fig. 8). Occasionally, a single segment that resembled an intrasegmental body was observed attached to an epithelial cell (Fig. 9).

Exfoliated cells could be seen with organisms attached (Fig. 10). Also, exfoliating epithelial cells could be observed occasionally that possessed an empty attachment site (Fig. 11). Both of these cell types appeared only infrequently in preparations from the normal murine host.

The microbes did not elicit inflammation; no reddening or swelling of the small bowel was observed. No inflammatory cells could be seen infiltrating the mucosa in the area of the microbes. No microbes were observed phagocytized by either epithelial cells or by macrophages.

DISCUSSION

Several authors have reported that microbes associate closely with epithelial cells in the small intestines of laboratory rodents (11, 13, 14). Hampton and Rosario (11) observed organisms attached to distal ileal epithelial cells in mice. Some of the organisms they observed possessed nipple-like appendages. They stated that the organisms were culturable *Streptobacillus moniliformis*. We have attempted repeatedly to culture the segmented, filamentous organisms from both mice and rats, but have been unable to do so. Hampton and Rosario also reported that they saw no exfoliated epithelial cells with organisms attached. We have observed several exfoliated cells with organisms attached.

Reimann (13) found organisms in distal rat ilea that he described as either invaders or as microbes pushed into cells. Also, he observed round bodies and spore-like structures within the microbial segments. He suggested that the round bodies represent stages in the life cycle of the organism (13). Other investigators (S. Erlandsen, D. Chase, G. Wendelschafer, and J. Rolston, Abstr. Annu. Meet. Amer. Soc. Microbiol., 1973, p. 57) used scanning and transmission electron microscopy to describe ultrastructural evidence for a life cycle of a segmented, filamentous microorganism attached to rat epithelial cells. They suggested that the round bodies within the segments are released from the filaments and then attach to epithelial cells. They also reported seeing structures resembling bacterial spores.

Our findings support the hypothesis of Reimann (13) and Erlandsen et al. (Abstr. Annu. Meet. Amer. Soc. Microbiol., 1973, p. 57) that in rats the intrasegmental bodies are reproductive units of the segmented, filamentous microbe. In addition, our results indicate that mice possess similar microbes with such a life cycle. The cycle, as suggested by Erlandsen et al. (Abstr. Annu. Meet. Amer. Soc. Microbiol., 1973, p. 57), would consist of the attachment of a single segment to an epithelial cell, development of the segment into a segmented, filamentous organism with intrasegmental bodies, release of the bodies, and then attachment of the bodies to epithelial cells to complete the cycle. The sequence supporting this life cycle is illustrated in Fig. 3, 4, 6, 7, 8, and 9. However, the life cycle hypothesis is supported by only descriptive data and cannot be proven until the microbe is cultured.

We have not observed in normal rats and mice any intrasegmental bodies resembling spores. S. Erlandsen (personal communication) indicates that such structures are observed only rarely in rats. They may be as rare in normal mice. We have observed structures resembling spores, however, in segmented, filamentous microbes in the small bowels of rats treated with penicillin (unpublished observations).

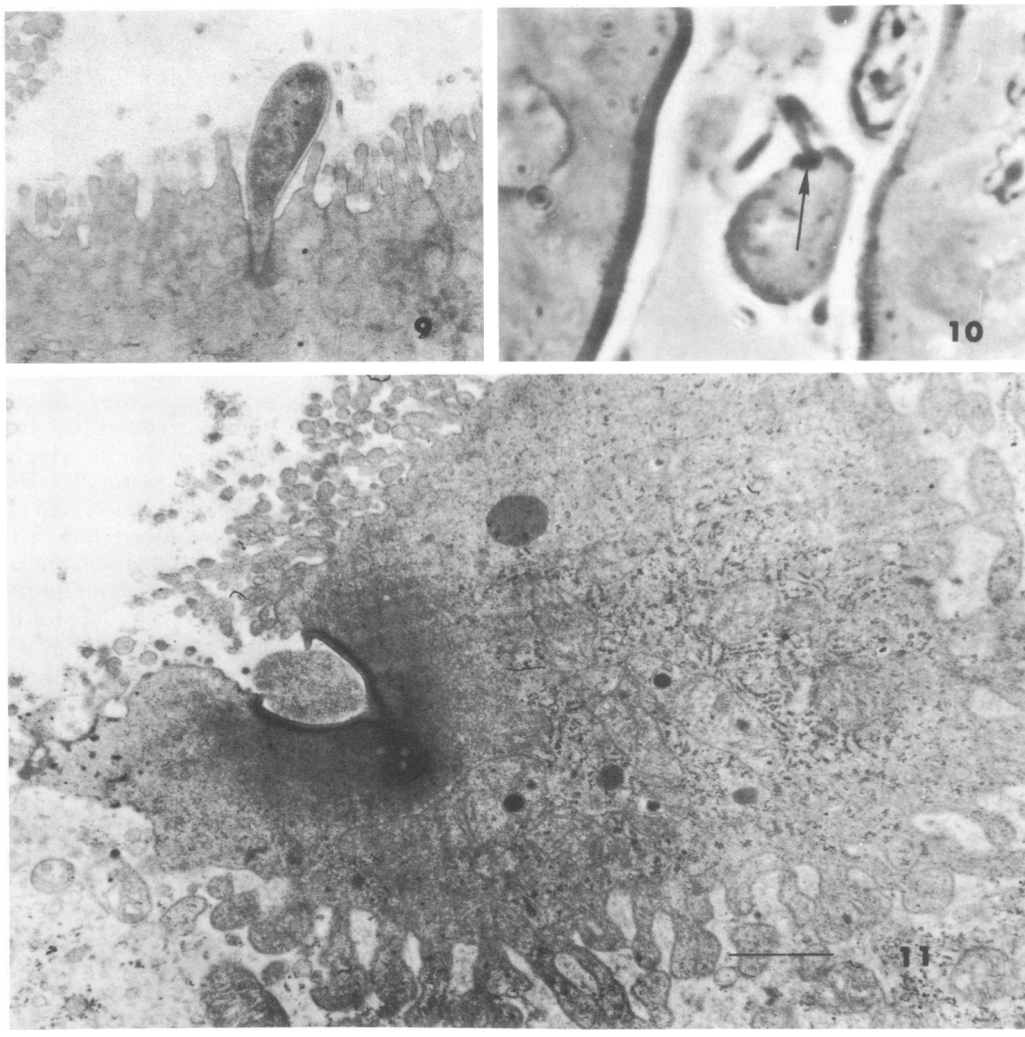

FIG. 9. *This electron micrograph shows a single segment that closely resembles the intrasegmental bodies. It is attached to an ileal epithelial cell.* ×*12,800.*

FIG. 10. *This light micrograph shows an exfoliated ileal epithelial cell with an organism attached at an attachment site (arrow).* ×*4,370.*

FIG. 11. *An attachment site, with only bacterial debris within it, is shown on an exfoliating epithelial cell in this electron micrograph. Note the absence of cellular organelles in the electron dense area adjacent to the attachment site. Bar represents 1 μm.* ×*11,800.*

Long chains of bacteria observed on the surface of rat ileal villi begin to colonize the epithelium only after the animals are weaned (14). Our results with the segmented, filamentous microbes in mice parallel the results with rats. Consequently, rats and mice are colonized by strikingly similar organisms at about the same developmental stage in their lifespan.

Our data show that one- to three-quarters of the length of the mouse small bowel can be populated by these microbes. However, the data indicate that the small bowels of CD-1 females are colonized to a lesser extent than that of CD-1 males, whereas the small bowels of Ha(ICR) females are colonized to a greater extent than that of the males. This finding suggests that the colonization of the small bowel by segmented, filamentous microbes is influenced in some complex way by the animal's sex. Furthermore, because the differences were reversed between the males and females in the two strains while the males from both strains showed the microbes present in about the same length of small bowel, the findings may indicate

that the colonization is influenced in females by the strain of the animal. We know of no other data indicating that the length of the gastrointestinal tract habitat colonized by microbes is related to the sex of the animal. The reasons for such differences in the habitat are unclear. Either microbial or host physiological factors or both may play a significant role in altering gastrointestinal habitats and, thus, could be partially or fully responsible for the differences we observed in the female mice.

Reimann suggested that the epithelial cells might capture and phagocytize the microbes (13). Our observations suggest a different role for the epithelial cell in this host-microbe association. We view the epithelial cell role as one of cooperation. It cooperates with the microbe in forming an attachment site on the surface of its lumenal plasma membrane. It may also cooperate by providing the microbe with some nutritional factors. In mice, epithelial cells in the small intestine are renewed about every 2 days (1). Thus, they could serve as a constant supply of cells to which new microbes, the intrasegmental bodies, could attach. This would imply a 2-day cycle in the association of the segmented, filamentous microbes and the animal cells.

These microbes were found in all rats examined and in almost all mice except those of one strain, C57 BL/6St Crl BR, obtained from Charles River Inc. One explanation for this finding is that the cells of those mice are genetically incapable of interacting with the microbes to form attachment sites. Another possible reason could be that the animals simply had not been exposed to the microbes. Recent results (unpublished observations) favor the latter hypothesis.

We have tried to culture these organisms in vitro by many methods. We have used aerobic, anaerobic, and microaerophilic incubation and numerous different media, but have never cultivated them in recognizable form. This problem has hampered the investigation of the animal-microbe association and has prevented us from identifying the organism with certainty. The organism shares, however, many of the characteristics of the family *Arthromitis* in the order *Caryophanales*. Members of *Arthromitis* are trichomes that have been found attached to the intestinal walls of insects and tadpoles (2).

Many bacterial and mycoplasmal species attach to mammalian mucosal membranes and cause disease (15, 20). Only a few microbial types are known to attach, however, and cause no overt disease (9, 16). Of these, only the segmented, filamentous microbe is known to interact with the epithelial cell to form an attachment site. No significant host response is engendered by this attachment. In addition, the organism possesses a segment well adapted for attachment to murine epithelial cells. Therefore, we believe that the animal and microbe evolved together to form their present association.

This unusual microbe apparently can differentiate into several morphological forms, such as segments with a nipple-like appendage, segments with intracellular bodies, or as the intracellular body itself. Thus, the microbes may have a life cycle more complicated than usually ascribed to bacteria. This possibility is worth more study. In addition, the microbe and epithelial cell of the animal seem to cooperate to form an attachment site. The formation of this attachment site by the bacteria and epithelial cells could serve as a model system for the investigations of host-parasite relationships, membrane dynamics, and cell to cell interactions.

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