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Demonstration of Mucosa-Associated Microbial Populations in the Colons of Mice

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Gram-stained sections prepared in a microtome-cryostat and examined by light microscopy confirmed the observation with scanning electron microscopy made by other workers that microbes inhabit a zone adjacent to the mucosal surface of the proximal colon of mice. Microbes in the midcolon, in contrast, are restricted to the fecal pellets occupying the intestinal lumen.

A recent paper by Bollard et al. (1) presented evidence gathered by scanning electron microscopy that indigenous microbial populations are not associated with the mucosal surface of the midcolon of the rat. These workers stated in their article that, without the use of mucus-stabilizing antibodies in preparative methods for electron microscopy, artifacts were produced in intestinal specimens. These artifacts, it was stated, create the impression that microbes are closely associated with the mucosal epithelial surface. The investigators considered that their observations threw doubt on the general concept that some members of the normal microflora of the rodent intestinal tract associate with mucosal surfaces (4).

Methods used in preparing specimens for electron microscopy, it is readily admitted, may produce artifacts. Another procedure is available, however, for making visible the distribution of microbes in intestinal tract specimens. This method involves the preparation of frozen sections, which are subsequently stained and examined by light microscopy. During the past 14 years I have prepared and examined hundreds of colon specimens from mice by this method.

Mice were killed by carbon dioxide anesthesia and cervical dislocation, and proximal and midcolon specimens were removed. The intact lengths of colon specimens were immediately placed in a microtome-cryostat (International Equipment Co., Div. Damon Corp., Needham Heights, Mass.), maintained at −20°C, and embedded in a 2% solution of methylcellulose (Fisher Scientific Co., Pittsburgh, Pa.) in 0.85% saline. Four-micrometer-thick sections were cut from the frozen samples, affixed to glass slides with methanol (30 s), and stained by using a Gram stain method for histological specimens (1% aqueous crystal violet for 1 min, three washes in water, Gram's iodine for 1 min, one wash in water, decolorization with ether-acetone [1:3, vol/vol], saturated basic fuchsin for 2 min, one wash in water, one acetone wash, 0.1% picric acid in acetone to remove excess fuchsin, one acetone wash, one wash with acetone-xylene [1:1, vol/vol], three washes in xylene). Complete transverse sections of colon specimens with undisturbed contents were obtained by this procedure. The possibility of artifactual redistribution of microbes is minimized with this method.

Photographs prepared by using low-objective-power magnification with the light microscope (Fig. 1) and composite photographs prepared from high-objective-power light microscope micrographs (Fig. 2) show microbial cells inhabiting a zone adjacent to the mucosal surface of the proximal colon. A relatively sharp demarcation is seen between this mucosa-associated zone, which contains mainly gram-negative bacteria, and the lumen contents. The lumen contains both gram-positive and gram-negative bacteria, as well as plant structural fragments. In the midcolon, in contrast, microbes and undegraded plant materials are visible in the fecal pellets but not in the zone (presumably composed of mucus) adjacent to the mucosal surface. The specific distribution of microbial types in colon specimens is particularly striking when viewed in color but can be seen satisfactorily in black-and-white photographs.

Bollard and colleagues were correct in that microbes do not occur in a clearly demarcated zone adjacent to the mucosa of the midcolons of rodents. Extrapolating their observations on the mid-colon to other regions of the intestinal tract and to the general concept of the microbial colonization of intestinal surfaces was incorrect. A zone heavily colonized by microbes is readily observed adjacent to the mucosal surface of the proximal colon of rodents. Demonstration of this mucosa-associated layer of microbes by the preparation of stained sections prepared in a microtome-cryostat confirms, together with the isolation of numerous anaerobic bacteria from washed samples of large bowel mucosa and previously described light microscopy examinations, the electon microscopy observations reported by other workers over many years (2–6).

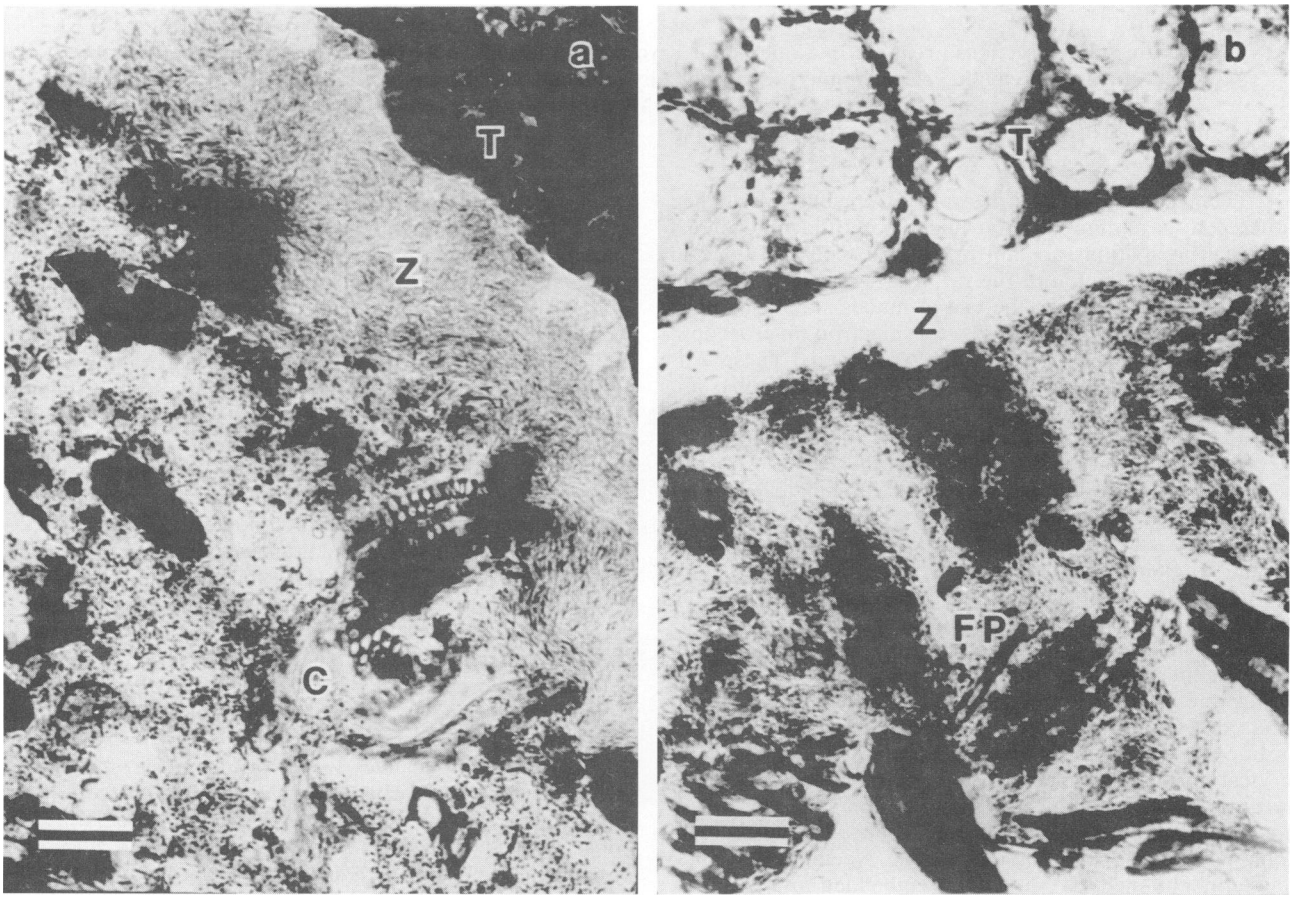
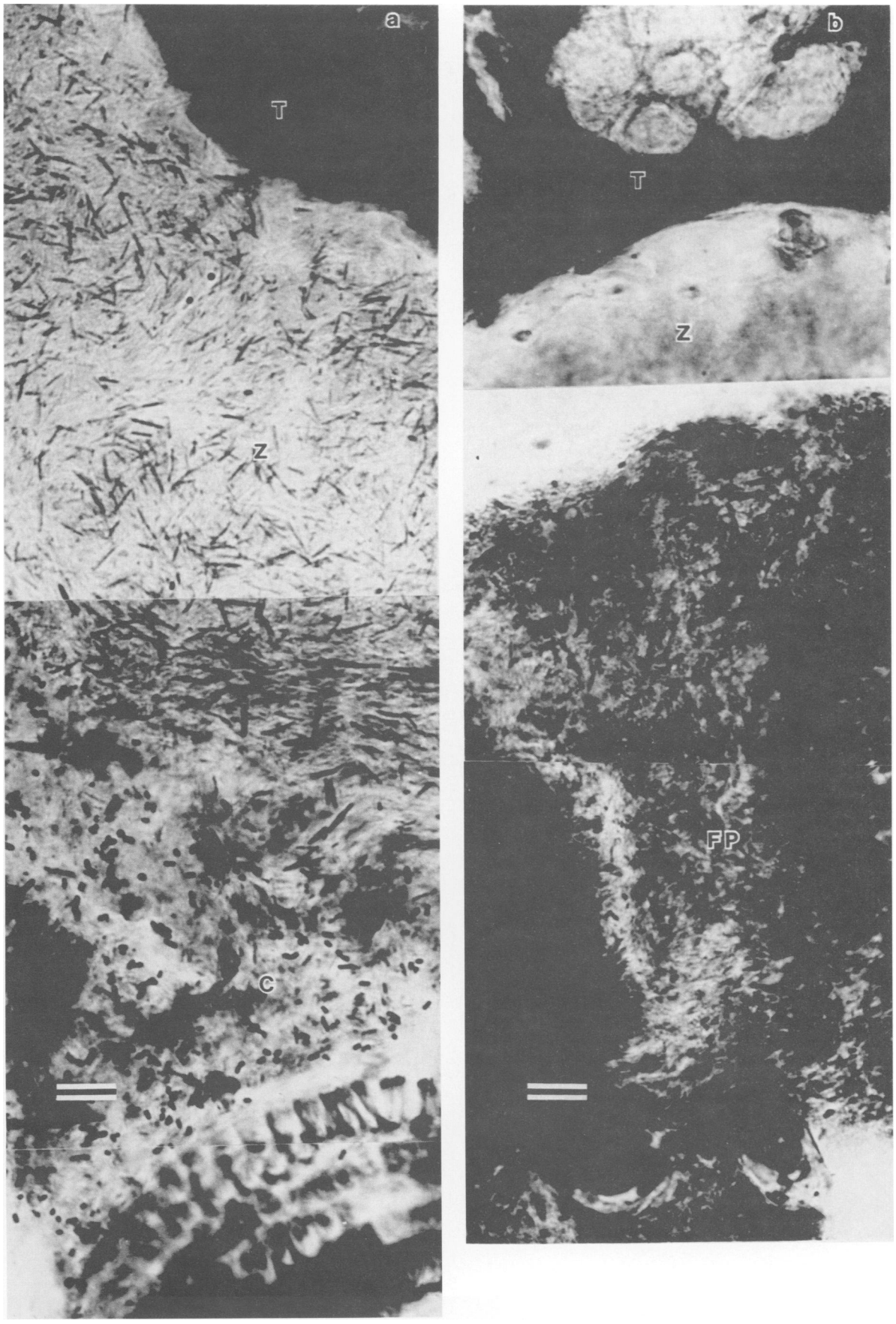


FIG. 1. Light micrographs of gram-stained sections of mouse colon observed with a low-power ojective lens. (a) Proximal colon. Abbreviatons: T, tissue; Z, zone inhabited by predominantly gram-negative bacteria; C, lumen contents containing gram-positive and gram-negative bacteria and plant structural fragments. Bar = 20 μm. (b) Midcolon. Abbreviations: T, tissue; Z, zone (free of bacterial cells) adjacent to the mucosal surface; FP, fecal pellet. Bar = 20 μm.

FIG. 2. Composite light microscopy micrographs of gram-stained setions of mouse colon observed with a high-power objective lens. (a) Proximal colon. Abbreviations: T, tissue; Z, zone inhabited by predominantly gram-negative bacteria; C, lumen contents containing gram-positive and gram-negative bacteria and plant structural fragments. Bar = 5 μm. (b) Midcolon. Abbreviations: T, tissue; Z, zone (free of bacterial cells) adjacent to the mucosal surface; FP, fecal pellet. Bar = 5 μm.


a
T
Z
C
b
T
Z
FP

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LITERATURE CITED

1. **Bollard, J. E., M. A. Vanderwee, G. W. Smith, C. Tasman-Jones, J. B. Gavin, and S. P. Lee.** 1986. Location of bacteria in the mid-colon of the rat. Appl. Environ. Microbiol. **51:**604–608.
2. **Dubos, R. J., D. C. Savage, and R. W. Schaedler.** 1967. The indigenous flora of the gastrointestinal tract. Dis. Colon Rectum **10:**23–34.
3. **Lee, A., and M. Phillips.** 1978. Isolation and cultivation of spirochaetes and other spiral-shaped bacteria associated with the cecal mucosa of rats and mice. Appl. Environ. Microbiol. **35:**610–613.
4. **Savage, D. C., and R. V. H. Blumershine.** 1974. Surface-surface associations in microbial communities populating epithelial habitats in the murine gastrointestinal ecosystem: scanning electron microscopy. Infect. Immun. **10:**240–250.
5. **Savage, D. C., R. Dubos, and R. W. Schaedler.** 1968. The gastrointestinal epithelium and its autochthonous bacterial flora. J. Exp. Med. **127:**67–76.
6. **Savage, D. C., J. S. McAllister, and C. P. Davis.** 1971. Anaerobic bacteria on the mucosal epithelium of the murine large bowel. Infect. Immun. **4:**492–502.