

BACTERIAL FLORA ASSOCIATED WITH THE HUMAN GASTROINTESTINAL MUCOSA

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Thirty-four biopsies from various sites of human gastrointestinal tract were studied, using quantitative bacteriological procedures and special histological methods which permitted visualization of bacteria. Tissue homogenates of unaffected gastric and duodenal mucosa did not contain organisms at the lowest dilutions tested; homogenates of diseased gastric and duodenal mucosa usually contained bacteria. In 4 of 7 homogenates of unaffected jejunum, and in all of appendix and colon, microorganisms were cultured. Anaerobic streptococci were found more frequently and in higher concentration than any other group of bacteria. Histologically, bacteria were visualized in the investing mucus of the mucosa of jejunum, appendix, and colon, but concentration was greatest in the colon. The most common types of bacteria seen in the intestinal mucus, in order of frequency, were: gram-positive bacilli, gram-positive cocci, and gram-negative bacilli.

In recent years, interest has been renewed in the study of the bacterial flora of the gastrointestinal tract.¹⁻⁴ The demon-

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stration by Dubos and co-workers,^{5, 6} of a flora in mice intimately applied to the gastrointestinal epithelium and present in enormous numbers, suggests the possibility of a similar phenomenon in humans. Small numbers of bacteria or bacteria-like structures in human jejunal tissue have been found by Plaut et al.,⁷ Keusch et al.,⁸ and Sprinz (*personal communication*). The present work was undertaken to identify and enumerate bacteria associated with the gastric and intestinal mucosa of man, by quantitative bacteriological procedures and special histological techniques. These included the investigation of fastidious anaerobes and other intestinal bacteria and the preservation of tissue with the intact investing mucus.

Materials and Methods

Tissue samples for bacteriological examination. Thirty-four specimens of organ wall were removed from different levels of the digestive tract of 27 Guatemalan patients undergoing surgery in hospitals of Guatemala City. Twenty-three specimens were considered "uninvolved," because they were removed from nondiseased areas, although most came from patients suf-

fering from diverse diseases of the gastrointestinal tract. Eleven specimens were considered "involved," and were obtained from the site of the lesion that had led to surgery. All specimens were about 0.5 cm². They were suspended in "charcoal water" (CW),⁹ and were homogenized in Ten Broeck grinders within 1 hr of excision.

Tissue specimens were not washed before homogenizing because preliminary studies with mice in this laboratory indicated that washing was without detectable effect on final numbers of culturable organisms. Similarly, Dubos et al.,⁵ and Plaut et al.,⁷ found that, while bacteria could be removed from mice intestinal wall and human jejunum, respectively, in the washing medium, this action did not decrease significantly the number of bacteria cultured from the tissues.

Bacteriological techniques. Seven 10-fold dilutions of the homogenates were prepared and 0.01 ml of dilutions 10⁻¹, 10⁻³, 10⁻⁵, and 10⁻⁷ was plated on quadrants of eight kinds of media. The media employed and cultural procedures were those of Schaedler et al.,⁹ modified in this laboratory.¹⁰ Anaerobiosis was obtained and monitored with Gaspak (Baltimore Biological Laboratory) disposable generators.¹¹ Criteria to identify and enumerate intestinal facultative and anaerobic bacteria are described in detail elsewhere.¹⁰ The number of viable organisms was estimated for each tissue fragment by multiplying the colony count of the highest dilution by a factor of 100 (the inoculating loop dilution) and by a factor of 10 or 100 to correct for the mass of the specimen. Results were expressed as number of viable organisms per gram of tissue.

Histological techniques. Pieces of tissue about 0.5 cm² were frozen in a solution of 2% methylcellulose, 15 centipoise in 0.15 M NaCl (Savage et al.¹¹) and were kept at -30 C. Sections 6 to 8 μ thick were cut in a cryostat at -30 C and were stained with hematoxylin and eosin, Giemsa, and periodic acid-Schiff reagents (counterstained with hematoxylin), as well as with the following stains for bacteria in tissues: Brown and Brenn; Gram-Glynn stain,¹² modified to use 0.1% aqueous basic fuchsin solution instead of 0.5%; and Hucker stain.¹³ All slides were examined to determine the histological state of the tissue, the presence of mucus overlying it, and the presence of bacteria embedded in the mucus or mucosa. Bacteria were rarely visualized in hematoxylin and eosin-stained sections; Giemsa, Brown-Brenn, Gram-Glynn, and Hucker stains were better for identifying the bacteria. The latter was the

best for observing bacteria in the mucus. When bacteria were found, they were characterized morphologically and their numbers were arbitrarily graded on a 0 to 4+ scale. If less than five bacteria were found in a tissue section, it was graded "0." Sometimes foci or bacteria were seen to contain up to 100 organisms. If only one or two of these microcolonies were observed in the section, it was graded as 1+; if more than two were seen, it was graded as 2+. If a narrow or sparse band of bacteria was seen covering the mucosa, the section was graded as 3+ and a dense or wide band was rated 4+. In 7 cases, histological preparations were inadequate for study; therefore, microscopic findings are reported for only 27 of the 34 specimens.

Results

Uninvolved Tissue

Stomach and duodenum. Only one sample of uninvolved gastric tissue was available for study. There were no bacteria seen in sections, and no bacteria were isolated from the homogenates at the lowest dilution tested. The two specimens of unaffected duodenal mucosa were also devoid of bacteria in the histological preparations. No microorganisms were recovered from the homogenate at the lowest dilution tested (table 1).

Jejunum. All jejunal samples were derived from healthy tissues during gastric or intestinal resections and were histologically normal. Four of the seven biopsies studied had bacteria in concentrations of 10⁴ to 10⁶ per g of tissue (table 1). The predominant bacteria were anaerobic streptococci, which appeared in three of the four positive specimens (table 1). The remaining three did not contain organisms at the lowest dilution tested (less than 10⁴ per g of tissue).

In 3 of 5 cases, for which the histological preparation was adequate, bacteria were found embedded in the investing mucus in moderate numbers (1 to 2+); mixed microcolonies of gram-positive cocci in chains and small gram-negative rods were seen in all cases, while gram-positive rods and yeasts were seen in 1 case (fig. 1).

Appendix. The seven appendiceal specimens were obtained during elective appendectomies and all were grossly normal.

TABLE 1. *Bacteria isolated from homogenates of uninvolved and involved tissues*

Bacterial group	Uninvolved tissue and no. of biopsies				
	Stomach, 1	Duodenum, 2	Jejunum, 7	Appendix, 7	Colon, 6
Anaerobic					
Lactobacilli.....			41 ^b	7.4 (<4-9) 5	8.5 (<4-10) 2
Streptococci.....			4.3 (<4-5) 3	7.7 (<4-9) 6	7.4 (6-10) 6
Bifidobacteria.....			51	8.6 (<4-9) 3	8 (<4-9) 4
Bacteroides.....			61	8.4 (<4-11) 5	7.8 (<4-10) 5
Clostridia.....			51	7.5 (<4-9) 2	6.4 (<4-7) 5
Veillonellae.....				62	6.5 (<4-7) 2
Total anaerobic.....			4	7	6
Aerobic					
Enterococci.....				6 (<4-8) 4	7 (<4-8) 4
Enterobacteriaceae.....			51	7.1 (<4-9) 6	7.3 (5-9) 6
Staphylococci.....			51		81
Micrococci.....				6.3 (<4-7) 3	5.5 (<4-6) 2
Bacilli (gram +).....				51	81
Total Bacteria.....			4	7	6

Bacterial group	Involved tissue and no. of biopsies		
	Stomach, 7	Duodenum, 2	Colon, 2
Anaerobic			
Lactobacilli.....	61 ^a		71
Streptococci.....	5 (<4-6) 2	61	6.5 (6-7) 2
Bifidobacteria.....			61
Bacteroides.....			81
Clostridia.....	51		
Veillonellae.....			61
Total anaerobic.....	2	1	2
Aerobic			
Enterococci.....	51	51	41
Enterobacteriaceae.....	6.5 (<4-7) 2	71	62
Staphylococci.....	51	41	
Micrococci.....			41
Bacilli (gram +).....			41
Total bacteria.....	3	1	2

^a Blank spaces indicate no organisms in lowest dilution tested.

^b Numbers are arithmetic average concentration (log₁₀) of viable organisms per gram of tissue. Numbers inside parentheses are ranges of concentration (log₁₀) of viable organisms per gram of tissue. **Boldface figures** are numbers of cases in which cultures were positive, in total examined.

At least 10⁶ bacteria per g of tissue were cultured from all homogenates. The most common organisms were anaerobic streptococci and Enterobacteriaceae, which appeared in all but 1 of the cases. Lactobacilli and bacteroides were found in 5 of 7 cases (table 1). Bacteroides and bifidobacteria were the most numerous.

Histologically, bacteria in microcolonies were found primarily in the mucous tags emanating from the epithelial surface between crypts in all cases examined (fig.

1). The average score was 1.6+. Most bacteria were gram-positive rods and cocci and small gram-negative rods; long, gram-positive elements were seen in 1 case and yeasts in another.

Colon. Normal colonic specimens were removed from healthy portions of the colon during intestinal resections or were biopsied per rectum at the time of anal surgery or proctoscopy. All tissue homogenates contained at least 10^7 anaerobic bacteria and 10^6 aerobic bacteria per g of tissue (table 1). The most prevalent organisms were anaerobic and microaerophilic streptococci and Enterobacteriaceae, which were found in all specimens. Bacteroides and clostridia occurred in 5 of 6 cases and bifidobacteria and enterococci in 4 of 6 cases. All of the six colonic samples examined microscopically contained bacteria in the mucous lining, especially in the tags of mucus which were attached to the surface epithelium between the crypt orifices. Gram-positive rods and cocci were seen in the mucus most frequently, and gram-negative rods were next frequent. The scores ranged from 1 to 4+, averaging 2+ (fig. 2).

Involved Tissue

Stomach and duodenum. Five of the seven specimens were from patients with gastric carcinoma. Bacteria were isolated from three of these. Two had only *Escherichia coli* and gram-positive cocci, and one had only anaerobic bacteria (lactobacilli, clostridia, and streptococci) in concentrations of 10^5 to 10^6 per g of tissue (table 1). The remaining specimens did not contain bacteria at the lowest dilution tested. There were two samples of duodenal mucosa from patients with gastric carcinoma, one of which contained aerobic bacteria (table 1). In no instance were bacteria observed histologically in the mucous lining of the gastric and duodenal epithelium.

Colon. Colonic mucosa from 2 patients with Hirschsprung's disease was studied. Aerobic and anaerobic streptococci and *E. coli* were isolated from both cases in average concentrations of 10^4 to 10^8 per g of tissue, respectively (table 1). Gram-

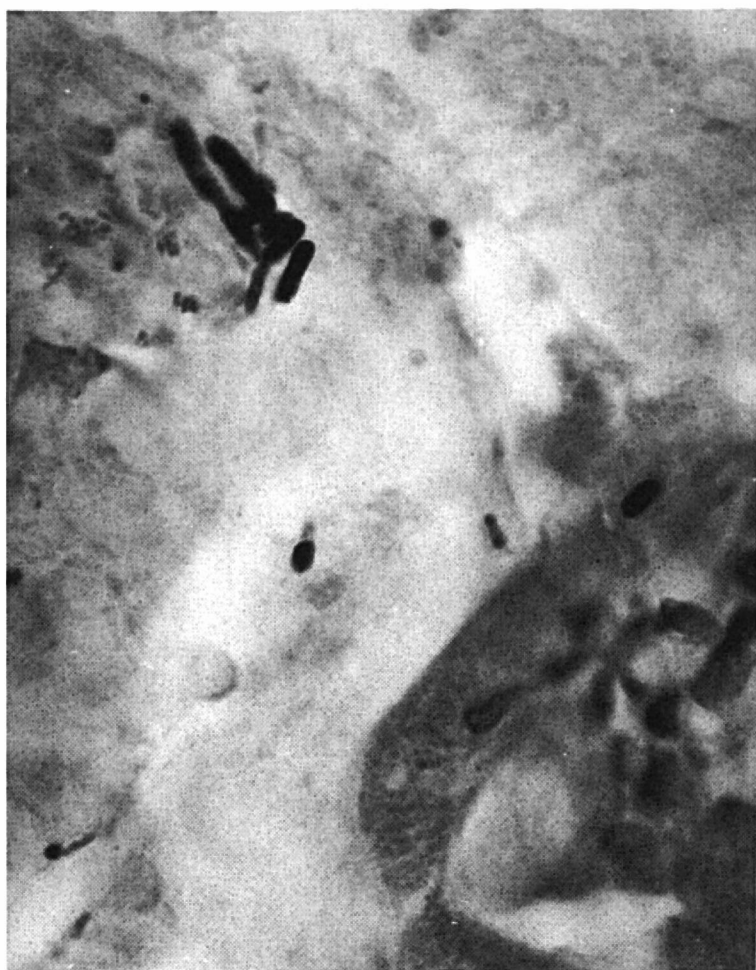


FIG. 1. Micrograph of section of normal jejunum, showing cocci, rods, and blastospores in the investing mucus (Hucker, $\times 950$).

positive rods and cocci and gram-negative rods and yeasts were observed in the mucous layer in 1 case (score 2+), while the second preparation was inadequate for study.

Discussion

Dubos et al.⁵ and Savage et al.⁶ demonstrated enormous numbers of bacteria in the mucous layer of the digestive epithelium of specially handled Swiss mice. Gram-positive rods and cocci were observed in layers on the epithelium of esophagus and stomach; lactobacilli and anaerobic streptococci were isolated regularly from the walls of these organs. Lactobacilli and anaerobic streptococci were isolated from the small intestine at all levels. In the mucous layer of the cecum and colon, gram-variable, fusiform bacteria were observed; these bacteria outnumbered all other species, but could not be cultured at that time.

In the present study, the nature and numbers of bacteria present in the human gastrointestinal mucosa were investigated bacteriologically and histologically

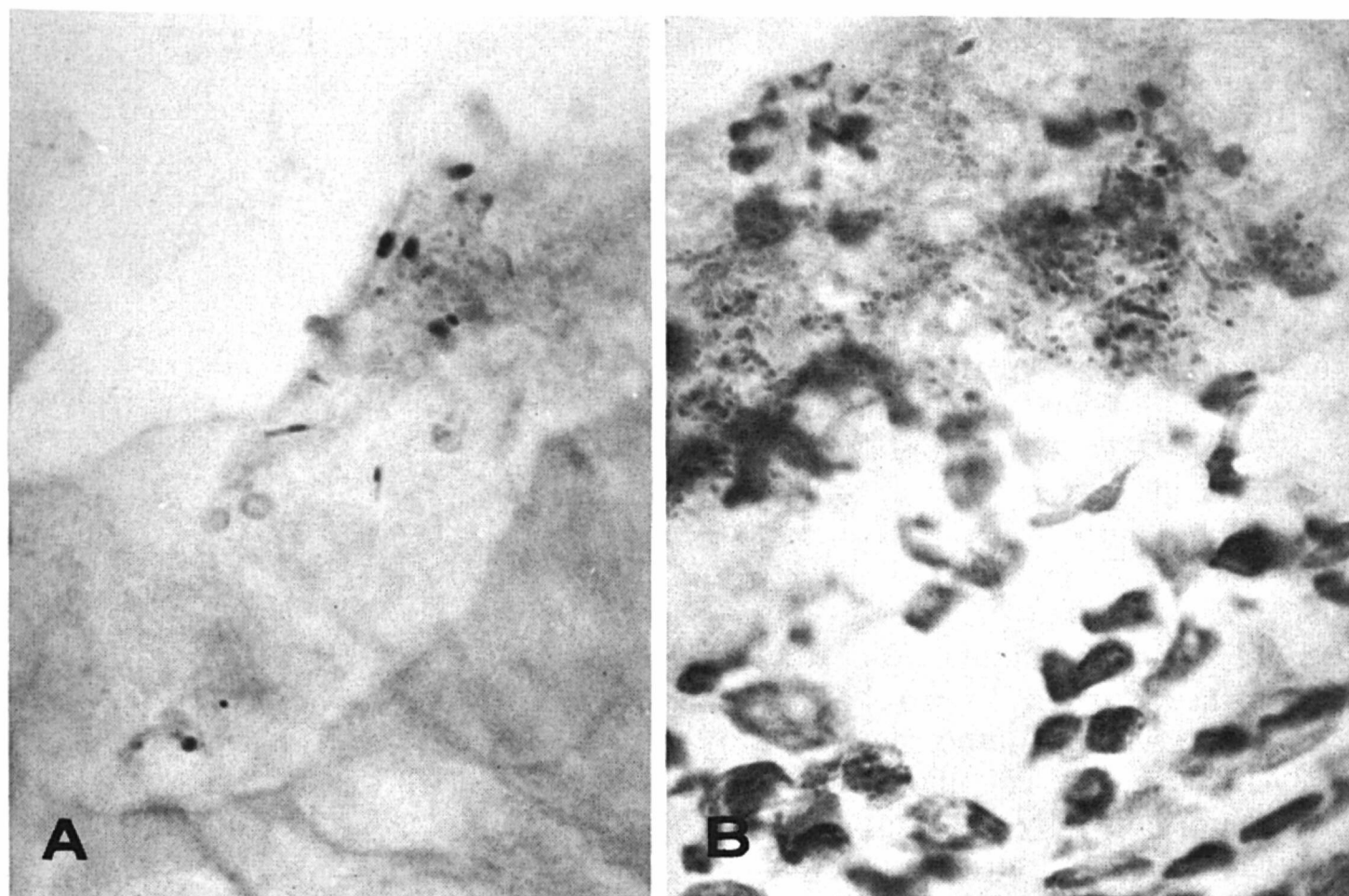


FIG. 2. Micrographs of sections of healthy colon. *A*, Section shows spindle-shaped rods, cocci, and blastospores embedded in the mucous layer (Gram-Glynn, $\times 950$). *B*, Section shows numerous cocci and rods embedded in the mucous layer and close to the epithelial cells (Hucker, $\times 950$).

with methods similar to those of Savage et al.⁶ In contrast with the mucosae found in mice, the unaffected gastric and duodenal mucosa of humans was devoid of bacteria by histological and bacteriological examinations. In diseased human stomach and duodenum, however, bacteria were cultured, sometimes in significant numbers. Gastric tissues containing bacteria were derived from patients with gastric carcinoma. Since this disease is often accompanied by a decrease in gastric acidity, it is possible that the proliferation of bacteria in this instance is a reflection of alterations in pH, as suggested by Franklin and Skoryna.¹⁴

Bacteria were found in the lower small intestine of humans, but they were not visualized in thick layers or plaques in the epithelial mucus as in mice. Instead, bacteria were embedded in tags of mucus attached to the mucosa, especially at the tips of folds or villi. Since this is the site for shedding of epithelial cells,¹⁵ three possibilities are suggested: (*a*) that the bacteria participate directly in stimulating the shedding; (*b*) that the pabulum

provided by desquamated cells and mucus is a particularly suitable habitat for these organisms; or (*c*) that the pumping action of the villi brings about the accumulation of the mucus and accompanying bacteria in these sites.

Appendiceal and colonic homogenates contained the greatest numbers of bacteria by bacteriological and histological techniques. Qualitatively, this tissue-associated flora resembles that found in feces of Guatemalans.¹⁰ Bacteria in feces, however, are more abundant. With the exception of bacteroides, which appeared more frequently in tissues, the order of prevalence of bacterial groups (by rank coefficient) in feces and tissues is very similar ($P < 0.01$). The finding of gram-positive cocci more often than gram-negative rods in the investing mucus of tissue section is described here; these bacteria were usually found in the mucous tags.

The frequency with which bacteria were found in concentrations of 10^5 to 10^6 per g of jejunum of Guatemalans contrasts with the results of Plaut et al.,⁷ who did not find such concentrations of

bacteria in jejunal biopsies of Bostonians. The composition of the flora also differed between the two groups. While it is possible that differences in technique may explain these observations, other explanations, including the nature of the diet, type of intestinal flora, different previous experiences with enteric pathogens, and physiological differences between Guatemalans and Americans in Boston, must be considered. With techniques similar to those reported by Savage et al.,⁶ the fusiform, gram-variable bacteria found in mice were not observed in human specimens. The predominant bacterial group in the human specimens studied was the anaerobic streptococci. Techniques different from those of Plaut et al.⁷ showed more bacteria in Guatemalan specimens than in those from Boston.

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