California Medicine

Intestinal Bacteria

The Role They Play in Normal Physiology, Pathologic Physiology, and Infection

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■ *Anaerobic bacteria predominate in the normal human fecal flora, outnumbering aerobes at least 100 to one. The two most prevalent organisms are* Bacteroides fragilis *and* Bifidobacterium. *Ileostomy flora is, on the other hand, chiefly aerobic and the total count is lower (10^8 per ml of fluid, compared to 10^{10} per gram for feces). In normal people, small bowel bacterial counts are generally 10^5 per ml or less. The upper small bowel consists primarily of Gram-positive aerobes in small numbers. In the terminal ileum, counts are higher and aerobes and anaerobes are present in equal numbers. In the presence of acute obstruction and certain bowel stasis or other syndromes, the small bowel flora may become relatively profuse and fecal in type. The stomach normally has less than 10^3 organisms per ml but counts are higher in gastric samples with pH above 4.0.*

Intestinal bacteria are important in such processes as conversion of bilirubin to urobilinogen, supply of vitamin K to the host, defense against infection, bile acid deconjugation and conversion, infections related to the bowel, the malabsorption of blind loop and other bacterial overgrowth syndromes, and hepatic coma.

IT IS LIKELY that we are accounting for only 10 to 25 percent of the normal intestinal bacterial flora by the best of techniques currently being used systematically. Until recent years, most bacteriologic studies of bowel flora used only aerobic incubation, and the few studies which included anaerobic technique were quite inadequate. Indeed, most studies of the anaerobic intestinal flora are still inadequate. Limited studies utilizing the excellent anaerobic techniques developed by Hungate and modified by Moore have revealed the presence of organisms in human feces previously thought to be present only in ruminants—*Methanobacterium*[1] and *Butyrivibrio*.[2] Choice of media may also influence results significantly. Certain

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organisms have specific growth requirements; for example, some strains of *Bacteroides melaninogenicus* require menadione or similar compounds. The use of a battery of media, including selective and differential media, not only simplifies bacteriological analysis but may lead to greater recovery of certain organisms. Incubation at room temperature may be necessary to recover the intestinal pathogen, *Yersinia enterocolitica,* but is not known to be necessary for any normal component of the intestinal tract.

Enteric Bacteria in Normal Adults

The normal human fecal flora of adults is predominantly anaerobic, aerobes accounting for only 0.1 to 1.0 percent of the cultivable flora.[3] The anaerobe which is most prevalent and probably invariably present normally is *Bacteroides fragilis;* the average count of this species is 10^{10} to 10^{11} per gram of wet feces. Other Gram-negative anaerobic bacilli are found less frequently. *Sphaerophorus* is found in over half of humans studied, with a mean count of 10^9 per gram when present. *B. melaninogenicus* is present in somewhat fewer than half the population and is found in small numbers (10^4 per gram). Fusiform bacilli are found only occasionally, but counts may be relatively high. *Bacteroides oralis* is probably not part of the resident flora of the bowel.

The second most prevalent organism is *Bifidobacterium,* a Gram-positive, non-sporulating anaerobic bacillus also known by such names as *Lactobacillus bifidus* and *Actinomyces bifidus.* It is found in two-thirds of fecal samples, with average counts of 10^9 per gram.

Other Gram-positive non-sporeforming anaerobic bacilli belonging to the tribe *Lactobacilleae* are found in similar numbers in at least one-third of people. The classification of these organisms may be very difficult; most belong to the genera *Eubacterium* and *Ramibacterium.* The more aerotolerant members of this tribe belong to the genus *Lactobacillus* and are really aerobic or microaerophilic. These organisms are found in 50 percent of people, with mean counts (when present) of 10^7 per gram.

Other anaerobes, found in feces of over one-third of people, include *Clostridium* and anaerobic cocci of various types—both showing counts of 10^8 per gram when present.

The major elements of the aerobic fecal flora are coliforms and streptococci. *Escherichia coli* is the predominant coliform, present in virtually all people in a count of 10^7 per gram of feces. Enterococci of various types, chiefly *Streptococcus faecalis,* are found in similar numbers in most people. Streptococci of the viridans type are found less frequently.

The flora of ileostomy effluent, by contrast, is chiefly aerobic and the overall count is lower (10^8 per ml, compared with 10^{10} grams for feces.[4,5] Mean counts of coliforms, aerobic streptococci and *Lactobacillus* are similar in ileostomy effluent and feces, but enterococci are found in a smaller percentage of ileostomy patients. Staphylococci or micrococci and yeast *(Candida)* are found in moderate numbers ($10^{5\text{-}6}$ per ml) in about half of ileostomy patients. *B. fragilis* was absent from ileostomy effluent of all five patients studied in our laboratory, but several other anaerobes were found (each in one patient only); the mean count of anaerobes was $<10^6$ per ml.

In normal persons, the small bowel flora is relatively simple and large numbers of organisms are not found.[6,7] Total counts are generally 10^5 or less per ml, except for the distal ileum, where counts usually are about 10^6 per ml. The flora increases in number and type as one progresses from the duodenum to the ileum. The upper small bowel consists primarily of Gram-positive aerobes, with no *Bacteroides* and rare anaerobes of other types. In the terminal ileum, there are approximately equal numbers of aerobes and anaerobes; here, coliforms are the most prevalent aerobes and *Bacteroides* and bifid bacilli the most common anaerobes. Gram-positive cocci and rods, similar to organisms found in the intestinal juice, have been found in small numbers associated with the mucous layer of small intestinal biopsy specimens.[8] In the presence of acute obstruction of the small intestine, the small bowel flora may become relatively profuse and fecal in type.[9]

The stomach normally has very small numbers ($<10^3$ per ml) of viridans streptococci, lactobacilli and fungi.[6,10] Counts tend to be distinctly higher in gastric samples which have higher pH, particularly above 4.0.[10]

Role of Normal Intestinal Bacteria

There is much to be learned concerning the role the normal intestinal flora plays in various physiologic processes of man. Conversion of bilirubin to urobilinogens takes place in the intestine through the activity of the intestinal flora. This conversion

may be decreased by administration of chlortetracycline (and undoubtedly by other antibiotics as well), but the component(s) of the flora responsible for the changes are unknown. The vitamin K needs of the host are supplied by intestinal bacteria; both coliforms and *Bacteroides* are known to be sources of this vitamin, but since *Bacteroides fragilis* normally is present to the extent of 100 to 1,000 times that of *E. coli* the anaerobe is much more important in this regard.[11] The decrease of serum cholesterol and the "intermediate" pool of cholesterol resulting from administration of oral neomycin[12] is probably not a result of changes in the intestinal flora.

The gut flora is undoubtedly important to the host in other aspects of nutrition but studies in man, in particular, are very inadequate. For additional information, the excellent review by Donaldson[13] should be read.

The intestinal microflora may be an important component of our defense against infection but this has not been adequately studied in man. Mice are rendered much more susceptible to experimental *Salmonella* infection after treatment with oral streptomycin reduces the *Bacteroides* count in the colonic flora.[14] On the other hand, *Endamoeba histolytica* cannot infect the germfree guinea pig unless one of several aerobic bacteria is also present.

Conjugated bile acids are split by the action of the normal large bowel flora[15]; usually only free bile acids are found in the feces.[16] Although enterococci are very active in this deconjugation process, the anaerobes *B. fragilis, Sphaerophorus,* and *Bifidobacterium* are also very active and are much more prevalent in the colonic flora.[17] Although it had been thought that bile acids were absorbed or reabsorbed from the lower small intestine, recent work indicates that there is significant absorption of bile acids from the human large bowel.[18] Cholic acid is converted to deoxycholic acid in the colon and both of these free bile acids are absorbed well from this site.[16,18] Various Gram-positive nonsporulating anaerobes are known to be effective in this conversion[19]; the Gram-negative anaerobes have not been studied adequately.

Enteric Bacteria and Infection

Knowledge of the normal bowel flora is also important in proper management of a variety of infections in which they may become involved—such infections as appendiceal abscess, peritonitis, subphrenic and subhepatic abscess, pyogenic liver abscess, pylephlebitis, diverticulitis, peri-rectal abscess, and postoperative infections following surgical operations on the bowel or elsewhere in the abdomen.[20-22] Gram-negative anaerobic bacilli, anaerobic cocci and, to a lesser extent, coliforms, enterococci and clostridia play very important roles in such infections. The Gram-positive non-spore-forming rods are distinctly less pathogenic and lactobacilli are virtually non-pathogenic.

Disturbances of the normal flora secondary to administration of antibacterial compounds may lead to several types of problems, most poorly defined and inadequately studied. Thus, overwhelming and fatal enteric infection or endotoxemia or both have been reported in guinea pigs and hamsters receiving penicillin G, erythromycin, tetracycline, or lincomycin.[23] The most commonly recognized enteric complication in man has been staphylococcal enterocolitis,[24] reported after use of a variety of antibiotics ineffective against staphylococci. Less well established is the possibility of *Salmonella* enteritis following antibiotic therapy.[25] It is likely that other pathogens may also be implicated in antibiotic-induced diarrhea. Still less well investigated is the prospect that changes in normal flora may account for diarrhea.[26]

There is also evidence to indicate that the intestinal flora may be involved in protection from systemic infection and the effects of endotoxin.[27] Preoperative bowel preparation with antibiotics before operation for cancerous lesions may predispose to subsequent recurrence of the cancer at the suture line.[28]

Enteric Bacteria in Non-Infective Diseases

It is also known that bowel flora, normal and abnormal (sometimes abnormal only in location and numbers), plays a very important part in certain pathophysiologic states but, again, our knowledge of these processes and the organisms involved is still fragmentary.

Overgrowth of bacteria in the small bowel is responsible for the malabsorption seen in such conditions as blind loop syndrome, intestinal stricture, gastrocolic fistula, enteroenterostomy, diverticula, and poorly functioning gastrojejunostomy.[13] It is less well established, but similar bacterial overgrowth is very likely responsible for the malabsorption which may be seen in intestinal scleroderma[29] and diabetic neuropathy[30] and which will

respond to therapy with agents such as tetracycline. It may also be involved in some cases of sprue-like illness in patients with dysgammaglobulinemia[15]; the diarrhea in some of these patients responds to tetracycline. The steatorrhea seen in these syndromes is thought to be due to bacterial deconjugation of bile acids in the upper small intestine where these acids are normally found in the conjugated form. Conjugated bile acids are necessary for normal micelle formation, in turn necessary for normal fat absorption. The relative abilities of various bacteria to deconjugate bile acids has already been noted. For this and other reasons (foul odor of upper intestinal contents in some cases, ineffectiveness of neomycin, and effectiveness of both tetracycline and lincomycin in bacterial overgrowth syndromes), it is reasonable to feel that anaerobes play a crucial role in the majority of patients with this type of malabsorption.[31] The mechanism of vitamin B_{12} malabsorption is less well understood, but many feel it is due to bacterial binding of the vitamin.

Increased activity of intestinal bacteria in patients with malabsorption is also reflected in increased urinary excretion of bacterial metabolites of tryptophan such as indoleacetic acid.[13] Elevated urinary levels of indican and indoleacetic acid may also be seen in patients with Hartnup disease due to poor intestinal absorption of tryptophan and bacterial action on this compound. Bacterial conversion of dibasic amino acids poorly absorbed from the intestine may be responsible for high excretion of heterocyclic amines in patients with cystinuria.

The facts that patients with tropical sprue respond to antibacterial agents[32] and that antibacterial agents administered prophylactically (in a controlled study) were moderately effective in preventing diarrhea in tourists[33] indicate the likelihood that intestinal bacteria play significant roles in these situations as well. To date, there has been little direct support for this; eventually, appropriate techniques should establish the role of bacteria in these diseases.

Whipple's disease is more likely a specific infection than a syndrome resulting from derangement of normal bowel flora. Although tetracycline therapy is effective, there is much disagreement as to the specific identity of the bacillary bodies seen in the gut on electron microscopy.

Finally, hepatic coma is recognized as a state in which bacterial activity in the bowel contributes significantly to the clinical manifestations. Oral neomycin or kanamycin have been used extensively for prevention and treatment of this condition, but other drugs such as chlortetracycline are known to be effective. Bacterial production of ammonia from nitrogenous substrates is one of the basic problems in this syndrome. The studies of Martini and coworkers[34] demonstrated that, although normal persons had a negligible bacterial flora in the small bowel, cirrhotic patients had many coliforms and enterococci in the ileum and some had these organisms in the jejunum and duodenum as well. Other important factors that may contribute to hepatic coma are inability of the liver to detoxify, and absorption from the gut of ammonium that has by-passed the liver through portal collateral vessels.

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COLOVESICAL FISTULA FROM DIVERTICULITIS

"In our series of 17 patients with colovesical fistula from diverticulitis, the 100 percent symptoms—symptoms found in all of the patients—included pneumaturia and gross pyuria. The 50 percent symptoms were fever, feces in the urine, and urinary cystitis symptoms; and way down the line were hematuria and melena.

"The history was far and away the most helpful and most important aid in diagnosis. I think that perhaps in this day and age we don't pay enough attention to the history. We tried to figure out how long the patients had had the process going on before a fistula became overt; and it was about two years. So I think in a patient with diverticulitis with symptoms of bladder irritation, perhaps we might consider this possibility a little earlier. Cystoscopy ordinarily is just suggestive. It's confirmatory; but you don't just look in and see a nice big hole that you can put a catheter in or something of that sort. Urography was quite helpful in a number of cases."

—HARRY M. SPENCE, M.D., Dallas

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