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Influence of Dietary Fiber on the Intestinal Environment[1] (42198)

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The human colon contains one of the most complex bacterial ecosystems known. At least 400 distinct species of bacteria are represented, although 15 major species account for nearly two-thirds of the isolates (1). The concentration of bacteria in the colon is very high, about 10^{10} per gram wet weight. Approximately one-third of colon contents is bacteria (1). The bacterial flora of the colon is presumably in equilibrium with its human host. We assume that this equilibrium is a dynamic one because the composition of the host's diet and the physiological state of the host can change. We also assume that the predominant species of colon bacteria are uniquely adapted to survive and flourish in the colonic environment. However, little is known either about the specific features of the interaction between host and flora or about the particular characteristics that are most important for the survival of organisms in the human.

To understand how colonic bacteria interact with their human host, we need to be able to answer three types of questions: First, which species of bacteria are found in the colon; i.e., what is the species composition of the colonic flora? Second, what are the bacteria in the colon actually doing; i.e., what are their metabolic activities? Third, where are the bacteria located; i.e., what is their environmental niche? To date, most research has focused on the first of these questions, despite the fact that answers to the last two questions are actually more important for understanding the interaction between the microbial flora and its host. The purpose of this review is to summarize recent research on the composition and activities of the colonic flora and to assess what progress has been made toward being able to answer all of the questions outlined above. Special emphasis will be given to one particular aspect of the interaction between the colonic flora and its host, namely, the breakdown of dietary fiber by colonic bacteria. Breakdown of dietary fiber is a major activity of the colonic microflora. Results of nutritional studies in humans demonstrate that at least half of the cellulose and hemicelluloses that are ingested in the diet are degraded during passage through the colon (2). Not only does dietary fiber appear to be an important source of carbon and energy for colon bacteria but products of this bacterial fermentation (e.g., volatile fatty acids) are reabsorbed by the host (3). Thus the catabolism of dietary fiber provides a good example of one of the many possible interactions between host and microflora and illustrates how bacterial metabolism can affect the colonic environment.

Species Composition of the Colonic Flora. Knowing which species of bacteria are found in the human colon is important because it gives us some idea of the metabolic potential of the colonic flora. A considerable amount of effort has been expended on the isolation and identification of the numerically predominant species of colon bacteria, and as a result we now have a reasonably complete picture of the types of bacteria that are found in the colon. Over 90% of these bacteria are obligate anaerobes. The numerically predominant genera are *Bacteroides* (ca. 25% of isolates), *Fusobacterium* (ca. 8% of isolates), *Eubacterium* (ca. 25% of isolates), *Peptostreptococcus* (ca. 9% of isolates), and *Bifidobacterium* (ca. 12% of isolates). Among the numerically predominant species are at least five species of *Bacteroides,* one species of *Fusobacterium,* at least four species of *Eubacterium,* one species of *Peptostreptococcus,* and four species of *Bifidobacterium* (1, 4). The species composition of the colonic flora of different individuals is similar but not identical (1, 4).

The species composition of the fecal flora is similar to the species composition of the flora in the contents of the ascending, transverse, and descending colon (1). This is important because virtually all studies of the human colonic flora use feces rather than colon contents. The statement that the fecal flora is

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essentially the same as the colonic flora is somewhat misleading because it does not take into account bacteria that adhere to the mucosal surface. However, it is probably accurate to say that the species composition of the flora in the lumen of the colon is similar to the species composition of feces.

Moore and Holdeman, in their classic studies of the colonic flora (4), were able to account for about 90% of the fecal organisms that could be seen on gram-stained slides. Since, however, some types of bacteria such as spirochetes are not easily visualized by such a procedure, there may still be some major groups of colon bacteria that have not yet been identified. Most of the predominant species of colon bacteria that were identified by Moore and Holdeman (4, 5) ferment carbohydrates and produce carbon dioxide, volatile fatty acids (mainly acetate, butyrate, and propionate), and lactate and succinate. When amino sugars are fermented, ammonia is a product of fermentation. When pectin is fermented, methanol is a by-product.

Carbohydrate-fermenting colon bacteria have to obtain their carbon and energy from complex carbohydrates because simple sugars and disaccharides are absorbed higher up in the gastrointestinal tract and do not reach the colon. Plant polysaccharides from the host's diet and glycoproteins from host secretions are two potential sources of carbohydrate for colon bacteria. Surveys of fecal isolates have shown that many strains of colon bacteria can ferment polysaccharides (6, 7). Most of these strains belong to one of several *Bacteroides* species: *Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides ovatus, Bacteroides vulgatus, Bacteroides distasonis,* and *Bacteroides "3452,"* an unnamed DNA homology group. Many of these strains can degrade several types of polysaccharides. For example, strains of *B. ovatus* can ferment galactomannans, polygalacturonic acid, starch, mucopolysaccharides, noncellulosic β-glucans, arabinogalactan as well as oligomers such as raffinose and stachyose (6, 8). Digestion of microcrystalline cellulose is uncommon among colon bacteria (9) but no studies have been done to determine whether colon bacteria can digest the hydrated cellulose that is found in plant cell walls.

A number of the colon bacteria that require a fermentable carbohydrate for growth appear not to be able to utilize polysaccharides (7). It is not clear what are the natural carbon sources for these bacteria, some of which are present in the colon in high concentrations. Possibly these organisms are able to use polysaccharides other than those which have been used in published surveys. Because previous surveys of polysaccharide utilization by colon bacteria were limited to substrates that were commercially available, these surveys should not be considered exhaustive. Further studies are needed to detect and identify colon bacteria that can degrade components of plant cell walls, particularly those components which have not been tested.

It is also possible that those colon bacteria that appear not to be able to utilize polysaccharides may be able to utilize oligomers that are by-products of polysaccharide breakdown by other bacteria. Such cross-feeding is known to occur among rumen bacteria but has not been demonstrated with colon bacteria. Because it is convenient to use pure cultures of bacteria for studies of bacterial metabolism, virtually all of our information about bacteria comes from studies done with single cultures. The possibility that mixed cultures can break down a substrate that pure cultures cannot should always be kept in mind.

Colon bacteria can carry out a wide variety of reactions that cannot yet be placed within the metabolic activities that are involved in obtaining carbon and energy. These include metabolism of steroids (10) and bile acids (11). Whatever their function, these reactions take place in the colon itself, and the species that carry out at least some of these activities have been identified (10, 11). A particularly interesting activity that appears to be carried out by colonic *Bacteroides* is the production of a substance that is mutagenic in the Ames test (12). The structure of the mutagen is known but the precursor has not been identified. It has been suggested that production of mutagens might be associated with colon cancer (12).

Diet and Species Composition of the Colonic Flora. It is not unreasonable to expect that changes in the host's diet may affect the colonic flora. An early hypothesis was that changes in the host's diet lead to changes in the species composition of the colonic microflora. A number of attempts were made to test

this hypothesis (13, 14). From the results of these studies it appears that the composition of the colonic flora does not change drastically with diet. However, this conclusion should be viewed as tentative at best. In the majority of these studies, no attempt was made to determine the species composition of the fecal flora. Rather, only major groups of bacteria such as "anaerobes" or "facultative anaerobes" were enumerated. The reason for this is that the amount of time and effort required to determine the relative concentrations of even the 10–15 major species in one fecal specimen, not to mention repeated specimens from a number of different subjects, is far beyond the capacity and budget of most of the groups that have undertaken these surveys.

Determining Species Composition. To determine the species composition of the microbial flora in a fecal specimen, one must first dilute the feces and plate the dilutions to obtain isolated bacterial colonies (1). The growth medium and growth conditions that are used must be capable of sustaining growth of all of the bacteria that are likely to be found. Fecal specimens must be processed as quickly as possible, and with minimal exposure to oxygen, so that the number of viable organisms is as high as possible. Freezing the specimen is not desirable because this may kill some organisms. Once the bacteria are isolated, a representative number (ca. 50) must be identified. To determine genus and species of most colon anaerobes, it is necessary to carry out a number of biochemical and fermentation tests and to use gas–liquid chromatography to identify the major fermentation products (5). To make matters worse, it is difficult in some cases to make species assignments on the basis of fermentation and biochemical tests.

Recently, we have been testing a new approach for determining the species composition of complex mixtures of bacteria such as those found in feces.[2] Briefly, we have cloned fragments of *Bacteroides* DNA that are species specific, i.e., that hybridize only with DNA from strains of a particular *Bacteroides* species. These cloned fragments are labeled with ^{32}P by nick translation and used as DNA probes to simultaneously identify and enumerate the bacteria. A fecal specimen is diluted and centrifuged to obtain the bacteria. Bacteria are then lysed and the DNA is partially freed from protein and other contaminants by further centrifugation and by extraction with phenol–chloroform. The DNA is trapped on a nitrocellulose filter, which is then incubated with the ^{32}P-labeled species-specific DNA probe. The amount of radioactivity that is bound to the filter is proportional to the concentration of that particular species in the fecal specimen. A standard curve is generated by adding known amounts of bacteria to fecal specimens that are processed in parallel.

This method has some advantages over the conventional procedures. First, it is not necessary to grow bacteria or to isolate individual species; therefore no special equipment is needed to cultivate anaerobes. For the same reason, the procedure takes much less time than standard identification procedures. Second, identification is done at the level of DNA–DNA hybridization. This is not only faster but also more precise than the fermentation tests that are normally used for identification. Third, bacteria need not be viable to be detected, and the fecal specimens can be stored by freezing prior to analysis.

There are also some drawbacks to this procedure. The most serious is its lack of sensitivity. Nitrocellulose nonselectively binds the DNA from all species in the sample. Thus, unless an organism's DNA is at least 2–5% of all the DNA present in the sample, it will probably not be detectable at high enough levels for quantitation to be reliable. Second, the method requires the use of radioisotopes. Not only does this pose safety and disposal problems but ^{32}P-labeled probes have only a very short shelf life. However, new methods for labeling DNA that do not require the use of radioisotopes are now becoming commercially available. Finally, species-specific DNA probes are currently available for only several of the colonic species, although it is certainly feasible to construct probes specific for other species.

Further work is needed to make this method reliable enough and simple enough for routine use. We think that, despite its shortcomings, this type of approach has considerable promise and may make it feasible in the future to determine the effects of diet on the concentrations of the major species of colon bacteria.

[2] Kuritza and Salyers, submitted for publication.

Metabolic Activities. The fact that some species of colon bacteria can degrade polysaccharides under laboratory conditions, i.e., when pure cultures of the bacteria are grown in medium that contains high concentrations of the polysaccharide, does not prove that these bacteria are the ones that degrade polysaccharides in the colon. For one thing, bacterial growth rates in the colon are probably much lower than those attainable in laboratory medium. Even in the ascending colon where fermentable substrates are found in highest concentrations, competition by other bacteria and limitations on essential nutrients probably keep growth rates to levels much slower than the rates of 2–3 hr per generation that pertain in many laboratory experiments. In the lower segments of the colon, growth rates may be very slow because only the less digestible substrates such as plant cell walls remain. Although we do not know what the real growth rates of colon bacteria are, we can guess that they fall in the range of 8–35 hr per generation. The mass of bacteria in the colon can double only once every 24–36 hr (the average transit time through the colon) so faster growth rates, e.g., in the ascending colon, must be compensated for by slower growth rates later on. We have tested the effect of very slow growth rates on the ability of *Bacteroides* to utilize polysaccharides and we have found that efficient utilization occurs at generation times as long as 25–30 hr (15, 16).

The polysaccharide-degrading systems of colon bacteria such as *Bacteroides* are quite complex. They involve not only polysaccharide-degrading enzymes but membrane proteins that appear to be involved in bringing the polysaccharides into contact with the enzymes, which are usually located inside the cell. For example, when *B. thetaiotaomicron* is growing on chondroitin sulfate, it produces two chondroitinases, seven or eight new outer membrane polypeptides, and several new inner membrane polypeptides, as well as enzymes for breaking the disaccharide product of the chondroitinases into monosaccharides (17–19).[3] A similarly complex system is used by this organism to degrade polygalacturonic acid (20). Each of these systems appears to be designed specifically for its substrate. That is, components of a system for degrading one polysaccharide are not used in degrading a different polysaccharide. We do not know why the polysaccharide-degrading systems are so complex. Possibly they were evolved to maximize the organisms' ability to bind and take up polysaccharides while minimizing the loss of the products of the initial steps of catabolism (i.e., oligomers, monosaccharides) to competing organisms. The outer membrane polypeptides may also have something to do with binding to plant cell walls or mucosal cells, although there is as yet no evidence to support this hypothesis. Another possibility is that specialized outer membrane polypeptides are needed to allow large molecules to move through the outer membrane without making the bacteria more susceptible to the many membrane-active substances such as bile salts that are present in the colonic environment.

The proteins that make up the polysaccharide-degrading systems of *Bacteroides* appear in general to be inducible (8, 20, 21). That is, synthesis of the proteins is enhanced by 10- to 50-fold when the bacteria are grown on the appropriate polysaccharide. Uninduced bacteria can utilize the polysaccharide but do so much more slowly than induced bacteria. It is tempting to speculate that the ability to induce different polysaccharide-degrading systems gives colon bacteria great flexibility. An organism which has the genetic capacity to utilize a variety of polysaccharides need only synthesize a particular set of proteins at enhanced levels when the appropriate polysaccharide is available. Thus it does not waste energy and carbon synthesizing high levels of unnecessary protein.

The observation that *Bacteroides* polysaccharidases are produced at high levels only when the inducing polysaccharide is being degraded suggested to us a way of determining which, if any, *Bacteroides* were using a particular polysaccharide in the colon.[4] We surveyed polygalacturonate lyases from five *Bacteroides* species, which are believed to be among the main polygalacturonic acid utilizers in the colon, and found that the enzymes could be distinguished from one another on the basis of

[3] Kotarski *et al.*, submitted for publication.

[4] McCarthy and Salyers, submitted for publication.

isoelectric point. The p*I* values of the different *Bacteroides* enzymes ranged from 5.8 to 7.7. We found that a polygalacturonate lyase activity was easily detectable in a sonicated bacterial pellet from a fresh fecal sample. Upon partial purification of this fecal enzyme, we determined that it had a p*I* value of 4.4. Thus, it appeared that this fecal polygalacturonate lyase was not a *Bacteroides* enzyme. For all but one species, *Bacteroides* produce polygalacturonate lyases of sufficiently high specific activity that, given the number of *Bacteroides* in feces, we should have been able to detect a *Bacteroides* lyase if it were being produced at induced levels. Since we did not detect any polygalacturonate lyase activity with a p*I* value between 5.8 and 7.7, we can tentatively conclude that none of the *Bacteroides* species in feces were utilizing polygalacturonic acid. Our findings do not rule out the possibility that one or more of these bacteria were utilizing polygalacturonic acid higher up in the colon but switched to another substrate as they moved through the colon. The fecal polygalacturonate lyase (p*I* 4.4) could have been produced by another colon bacterium. It is also possible that it was an enzyme that was present endogenously in undegraded plant particles and was released during the sonication step used to disrupt the bacteria.

Despite its limitations, this approach to determining what bacteria are actually doing in the colon may be very useful in cases where enough is known about the metabolic activities of the organisms to provide a means of differentiating enzyme activities in a complex mixture. It should be emphasized, however, that as long as feces are used rather than colon contents, findings apply at best to the state of bacteria in the descending colon and cannot be extrapolated to deduce the state of bacteria in the ascending or transverse colon.

Location of Colon Bacteria. The colon contains many sites for bacterial colonization (22). For example, bacteria may adhere directly to mucosal cells. This phenomenon is observed in the small intestine and colon of rodents and other animals (23). The question of whether this occurs in humans is still somewhat controversial, but it is unlikely that humans differ from other animals in this respect. Bacteria may also be trapped in the mucin layer that lines the colon. This mucin layer is probably a dynamic structure of varying depth rather than a single unbroken layer. Virtually nothing is known about bacterial colonization of the mucosa or the mucin layer, if indeed these can be considered to be separate niches. Although we know little about them, these bacteria may be of greater importance to the host than bacteria that dwell in the lumen of the colon because of their proximity to the mucosa.

Because the flow rate through the colon is so slow, bacteria can survive and grow to high concentrations in the lumen of the colon. However, it is probably not accurate to think of this population as a single undifferentiated mass. Some of these bacteria may adhere preferentially to particulate matter whereas others may be free-living. It is possible that many of the apparently free-living bacteria are bacteria that can adhere to surfaces but cannot find any unoccupied attachment sites.

At present, the only way to establish the location of bacteria is by examining the morphology of bacteria that can be seen by electron microscopy in cross sections of the intestine or of fecal material. It may be possible to use DNA or antibody probes to facilitate this analysis. However, obtaining such sections from the colons of normal healthy humans is not feasible at present, and studies of this type are necessarily limited to fecal specimens. Studies involving laboratory rodents may provide valuable insights into the localization of bacteria in the colon.

Effect of Bacterial Digestion of Fiber on the Host. At present, we can only advance a few tentative hypotheses about the possible significance of bacterial digestion of polysaccharides for the host. First, since some bacteria can digest either host products or plant polysaccharides, it is possible that these bacteria utilize host products such as mucins and mucopolysaccharides whenever there is not enough fermentable dietary polysaccharide. If this is the case, a high fiber diet could help to prevent destruction of the mucin layer that protects the mucosa. There is some evidence that a high fiber diet stimulates epithelial cell turnover (23). This could also have a protective effect. Two critical questions need to be answered: First, do colon bacteria actually degrade mucin in the colon? Second, are the mucin degraders found mainly in the mucin layer adjacent to

the mucosa or free-living in the lumen? Bacteria that are lodged in the mucin layer may not normally encounter dietary polysaccharides because the mucin itself prevents diffusion of large molecules. It would also be interesting to know whether fiber in the diet affects the location of some bacteria. If bacteria can attach either to mucosa or to plant cell wall surfaces, a high fiber diet could cause certain types of bacteria to be found preferentially in the lumen rather than attached to the mucosa.

The host absorbs volatile fatty acids that are products of carbohydrate fermentation by colon bacteria (3). Except for ammonia (from amino sugars) the products that result from fermenting mucins are essentially the same as those that result from fermenting plant polysaccharides. However, a high fiber diet, if it increases the amount of fermentable carbohydrate in the colon (especially hemicelluloses and pectins), could cause an increase in the amount of fermentation products made and thus in the amount absorbed.

Recently, it has been found that certain people excrete substances that are mutagenic on the Ames test (12). It is not clear why only some persons excrete the mutagens. All people have high concentrations of *Bacteroides,* the organisms that produce the mutagen, in their colons. Possibly some component of diet or some aspect of individual physiology is responsible. The effect of fiber in the diet on mutagen production and excretion is not known, although mutagen excretion is less common among populations who consume diets that are associated with a low incidence of colon cancer (12).

It is almost certainly the case that further investigations of the colonic microflora and its relationship to its host will uncover other possible effects of dietary fiber. However, new methodological approaches and noninvasive sampling procedures are needed if we are to make significant progress toward a deeper understanding of the dynamic equilibrium between bacteria and host.

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