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GASTROINTESTINAL MICROFLORA IN MAMMALIAN NUTRITION

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CONTENTS

INTRODUCTION

The Gastrointestinal Microflora

Even though representatives of only a relatively few species have been studied in detail, no doubt now exists that birds (97), adult mammals of all species, including *Homo sapiens* (45), and animals of many other species (106) have microbial floras indigenous to their alimentary canals. These microfloras develop soon after birth, consist of prokaryotic and eukaryotic microorganisms of many genera and species, and are essential for the health and welfare of their animal hosts. Mammals of many species (e.g. ungulates) and insects of certain

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species (e.g. termites) are known to derive as adults essential elements of their nutrition from products of their alimentary microfloras (12, 18, 128). Even when their essential nutrients need not be microbial products, animals of many and perhaps even all species interact physiologically in various ways with their microflora; indeed, their capacity to survive in nature depends upon the microbial cells comprising that flora (64, 65). Thus, mammals and animals of most if not all species can be regarded as creatures consisting of eukaryotic animal cells in symbiosis with eukaryotic and prokaryotic microbial cells (106). In other words, animals exist under natural conditions as complex interactive organic units consisting of both animal and microbial cells.

The microflora of the foreguts (rumens) of sheep, cattle, and ungulates of a few other species has been defined in taxonomic terms (12, 22, 128). Likewise, the microflora of the foregut (crop or stomach), midgut (small intestines), and hindgut (cecum, large intestine) is now understood in broad taxonomic terms for chickens, man, and mammals of a few other species (4, 34, 50, 77, 81, 92, 97, 106). The microbial communities in those floras are composed of many species of numerous genera of bacteria; most of the species are strictly anaerobic; some are present in the communities at population levels exceeding 1×10^{10} bacterial cells per gram dry (or wet) weight of material. Indeed, in the rumen and the foreguts (stomachs) of mammals of certain species (ruminants and pseudoruminants), and in the large intestines of birds and mammals of all species examined, the bacterial populations in aggregate can exceed 1×10^{11} cells per gram dry weight of material. Such enormous microbial populations composed of prokaryotic and eukaryotic cells of many taxonomic classes can perform biochemical reactions of a large variety of types (see next section).

Microbial communities in gastrointestinal tracts can be found in the lumen (often in association with particles of digesta), on epithelial surfaces, and even deep in the crypts of Lieberkuhn (106–108). Depending upon the animal species under study, such communities can be found in those sites in each major region of tract (stomach, small intestine, large intestine) and even in subareas of the major regions. In a given area or subarea, the community in the lumen, and especially on surfaces of particles of digesta, can differ from that on the epithelial surface in the relative proportions of the populations of the various species. Likewise, those communities can differ in composition from cryptal communities. Indigenous lumenal communities exist in major areas where the rate of passage of digesta does not exceed the rates at which microorganisms can multiply (e.g. the large intestine). Epithelial communities can exist in any area. Cryptal communities have been reported so far to be present only in the lower small intestine and cecums and colons of animals of certain species (107). The microbial populations in the various communities can be specialized in the biochemical functions they perform (4).

Biochemical Activities of the Microflora

Biochemical reactions catalyzed by microbial enzymes in the gastrointestinal canal can be categorized generally into two groups, one including reactions essential to the survival of the microbial cell, and one including reactions not obviously essential for cellular survival (109). Reactions essential to survival are those involved in hydrolysis, binding, and transport into the cell of nutrients; energy transfer and macromolecular synthesis and function; and motility and chemotaxis (when appropriate). Nonessential reactions are those the products of which are not obviously needed in some way in the functions of the microbial cells, for example deconjugation and transformation of steroids (16, 56).

Gastrointestinal microorganisms can perform many reactions that can be classified into one or the other of those broad categories (86). In general, however, any reaction of which such microorganisms are capable is one that can take place in the environment of the tract, which is virtually free of oxygen and has a quite low oxidation-reduction potential (86, 106). For that reason, few (if any) of the essential and none of the nonessential metabolic reactions involve oxygen. Moreover, many of the microbial enzymes involved can function well in experimental systems only in reaction mixtures poised at low oxidation-reduction potentials (86). Thus, the biochemical functions of the microflora reflect, as does its microbial composition, the anaerobic environment of the tract.

The enzymes catalyzing some essential reactions and even a few nonessential ones may be constitutive. However, the enzymes catalyzing some reactions are proving to be inducible (37, 68, 69, 72, 98). The latter reactions may or may not take place depending upon whether an appropriate inducer is present in the local environment. As amplified below, these properties have important implications for the nutritional role of the microflora.

Limitations and Goals

In this review, I assess the current knowledge about how the gastrointestinal microflora influences the nutrition of the animal tissues of adult mammals of certain species. The discussion is limited principally to findings made in studies with adult monogastric animals not regarded as pseudoruminants (animals not classified as ungulates but having a complex rumen-like fermentation in their foreguts). Information on the nutritional function of the complex microflora of the foregut in pseudoruminants and ruminants has frequently been the subject of reviews (12, 128). Therefore, any such information discussed is presented only for perspective. The overall goal of the analysis is to evaluate the potential for functions of the microflora in adult human nutrition.

THE MICROFLORA AND MONOGASTRIC NUTRITION

Intestinal Absorption

The indigenous microflora is well known to influence various physiological functions of the gastrointestinal tract. Evidence for such influences has come primarily from research with germfree animals, or with animals, including humans, suffering certain intestinal abnormalities resulting in disturbances of the microflora. Findings gained from experiments with germfree animals are described and analyzed in recently published books and reviews (23, 130). Likewise, intestinal diseases resulting from disturbances of the flora, especially those of man, have been a subject of intense research in recent years and, as a consequence, of numerous books and reviews (41, 61). Certain of the intestinal functions affected by the microflora, those that influence absorptive processes, are pertinent to the major goal of this review and are examined in the following paragraphs.

The indigenous microflora is known to influence transit time in the tracts of animals raised under ordinary conditions (conventional animals). Digesta passes more rapidly through the tracts of conventional animals than through those of germfree animals (90, 130). The mechanism by which this happens remains obscure, but may involve dietary components and microbial metabolic end-products acting synergistically on neurotransmittance to smooth muscles in the tract (133, 135). Rapid passage of lumenal content through the small intestine could be expected somewhat to limit digestion and absorption of nutrients.

The indigenous microflora also influences the turnover of and enzymatic activities in enterocytes (absorptive epithelial cells) in the small intestine. Enterocytes turn over in the gastrointestinal canal by migrating from their mitosis in the crypts of Lieberkuhn to their extrusion at the tips of the villi and destruction in the lumen (110). In the small bowels of conventional laboratory rodents, this process operates at an apparent rate almost twice that in the intestine of germfree animals (110). The apical (lumenal) membranes of enterocytes are organized into microvilli, and contain glycoprotein enzymes involved in digestive absorption (e.g. disaccharidases, peptidases). As assessed by methods involving the entire bowel mucosa (i.e. not isolated epithelial cells) the specific activities of those enzymes are two to five times higher in germfree rodents than in those with a flora (60, 125).

The activities of enzymes in the microvillous membranes are a function of the position of the enterocytes on the villi; cells nearest the extrusion zones at the villous tips have higher activities of disaccharidases, peptidases, and other microvillous enzymes than those nearest the crypts. The activities develop as the cells glide (migrate) along the villi from the crypt to the extrusion zone at the tip (111). Thus, they could be higher in the guts of germfree rodents because

they have longer to develop in cells migrating on the villi at a slower rate than they do in animals with a flora. Alternatively, the synthesis of glycoproteins associated with the microvillous membranes may differ in some way, rendering the enzymes less active in conventional than in germfree animals (119, 120). Neither of these possibilities explains entirely, however, the differences detected in the enzymatic activities in germfree and conventional mice.

In procedures by which epithelial cells can be isolated from intestinal mucosa, germfree mice yield from their upper small intestines one and one-half to two times more enterocytes than animals with a flora (111). In other words, by comparison with conventional mice, germfree mice have more enterocytes populating the mucosal surface of their small bowels. When assayed in such cellular preparations, the total activities of the enzymes in the microvillous membranes (i.e. the activity per total mass of cells) reflect those detected in assays of whole mucosa; the activities are two to three times higher in the preparations from germfree mice than in those from conventional animals (111). When assessed in relationship to the total amount of protein in the cell mass, however (that is, when specific activity is determined), the activities are essentially identical for cells from both germfree and conventional animals, except for a small fraction of cells nearest the villous tip (111). Cells nearest the tip have highest activities in germfree mice.

Since the specific activity of the enzymes per enterocyte is about the same in most cells from the germfree and conventional animals, the specific activities in the whole mucosa must be higher in the former than the latter because more enterocytes are present in the former than the latter (111). Thus, in mice at least, the microflora in conventional animals acts to maintain the activities of digestive-absorptive enzymes in the microvillous membranes below those found in germfree animals primarily by maintaining the population of enterocytes at levels below those found in the latter animals. The mechanisms of this microbial activity remain obscure.

Whatever those mechanisms and the mechanisms involved in the microbial influences on the transit time in the tract, conventional rats and mice could be expected to absorb carbohydrates, peptides, and other such nutrients less efficiently than do germfree animals. Evidence is mixed on this point (130), but derives from studies accomplished a number of years ago. The work deserves repeating in light of recent findings that fluids secreted by enterocytes near the villous crypts are absorbed by such cells near the villous tips (112). Such a process could facilitate intestinal absorption, and may have been disturbed by the methods used in the reported research.

The indigenous microflora influences water and electrolyte absorption in the rodent cecum, and undoubtedly that in other species. The water content of the digesta in the lumen of the cecum and colon in conventional rodents is lower than that in the cecum of germfree animals, while the content of Cl^- and

HCO_3^- is higher (40). In conventional animals, strains of bacterial species produce enzymes that catalyze hydrolysis of macromolecules passing into the lumen of the large bowel (87). In germfree animals, such macromolecules are not hydrolyzed, and thus accumulate in the lumen of the tract, leading to the high osmotic pressure and the content of water, Cl^-, and HCO_3^- (40, 87). This process could influence the absorption of bacterial metabolic products (see below) used as carbon and energy sources by the animal tissues. Such molecules undoubtedly absorb into the blood by processes coupled to electrolyte absorption (5).

Bile steroids may also influence absorption in the colon. Such compounds are known to be altered in structure by reactions catalyzed by microbial enzymes. The gastrointestinal microflora influences the blood levels, chemical classes, and function of bile steroids, including cholesterol (56, 130), bile acids, (56, 130), and certain hormones (127). The compounds can be deconjugated (56, 130), desulfurated (56), and transformed (16, 32, 48, 56) by enzymes produced by bacterial strains in such floras. The chemistry of these processes is well studied, and was recently reviewed (56). Suprisingly little is known, however, about the nutritional consequences for the normal animal of such microbial chemistry. One such consequence may be an influence on electrolyte absorption in the large intestine (49), and thus the absorption of bacterial metabolites in that area of the tract (40).

The macromolecules hydrolyzed by microbial enzymes in the large intestine of monogastric animals derive from exogenous and endogenous sources (86). The former derive from the animals' ingesta. These are mostly fibrous components of food that cannot be digested and absorbed by the enzymes produced by the host's animal cells (98). The latter derive from the host's animal cells themselves, and are materials such as intestinal mucus (51), the macromolecular components of sluffed epithelial cells (110), immunoglobulins secreted into the tract (106), and pancreatic and other enzymes (84, 87). The nutritional influences of such hydrolyses are the subject of the section following.

Nutrients Provided by the Microflora

PRODUCTS OF MICROBIAL ENERGY-YIELDING METABOLISM Lactic acid and ethanol are produced as metabolic end-products by bacteria and yeasts, respectively, living in epithelial communities in the stomachs of monogastric mammals of some species (6, 12, 63, 126). Such compounds could be absorbed and utilized as carbon and energy sources by the animal tissues. The population levels of the bacteria and yeasts in the gastric communities are low, however, rarely exceeding 1×10^9 organisms per gram of whole stomach with content. Therefore, except under conditions where the diet contains large amounts of readily fermentable monosaccharides [e.g. glucose (126)], the bacterial products are undoubtedly produced normally in amounts too small to contribute in

more than negligible ways to the carbon requirements of the animal cells. Whether the hosts derive any other "benefit" from having an ethanol "factory" in their stomachs remains at best a subject of speculation.

Short-chain volatile organic acids, principally acetic, propionic, and butyric acids, and the gases hydrogen, methane, and carbon dioxide are end-products of microbial fermentations in the foreguts of ruminants and pseudoruminants (12, 128). The volatile acids are well known to be important sources of carbon and energy for the animal tissues of such hosts (12, 128). Indeed, adult ruminants depend solely upon such microbial processes for their carbon and energy. The organic acids are also important sources of carbon and energy for monogastric mammals with extensive microbial fermentations in their hindguts (9, 31, 71, 88, 134). As mentioned above, the microbial populations in cecums and colons are enormous, resembling ruminant populations in their size and complexity. These microbial populations produce the short-chain acids as end-products of fermentations of carbon compounds such as monosaccharides and amino acids (86). Those compounds derive principally from hydrolyses catalyzed by microbial enzymes of macromolecules entering the large intestine (as mentioned above) from exogenous (dietary) and endogenous sources.

To be available to animal tissues, the organic acids produced in the lumen of the cecum and colon must be absorbed into the blood through the mucosas of those regions, or passed into the stomach and small intestine by coprophagy. The compounds undoubtedly absorb through the mucosa in all animals with a cecum (71, 88, 121). However, some such animals also practice coprophagy on a regular basis. This latter process is raised to its highest practice in lagomorphs (rabbits, hares) where special fecal pellets bound with a membrane and referred to as cecal pellets (or soft feces) are preferentially consumed (58). These animals can receive substantial proportions of their organic carbon as volatile acids from their cecal fermentations (71).

Monogastric animals of species lacking a blind cecum (e.g. man) may also derive nutritional benefit from the short-chain acids produced by microbial processes in their large bowels. The case is less clear, however, than it is for animals with a blind cecum, and is discussed in the section on microflora and human nutrition.

PRODUCTS FROM LYSIS OR DIGESTION OF MICROBIAL CELLS Bacterial cells can lyse in the gastrointestinal tracts of mammals, and thereby release into the lumen molecular substances from their cytoplasm, membranes, and walls. Some such lysis is undoubtedly due to enzymes endogenous to the bacterial cells; the enzymes lyse the cells from within as natural concomitants of cellular senescence. Most is due, however, to enzymes produced by the animal tissues of the host (e.g. pancreatic enzymes), including enzymes able to catalyze hydrolysis of peptidoglycan found in the cell walls of bacteria. These "mura-

lytic" enzymes (e.g. lysozyme) are present in many secretions of mammalian tissues (7).

Nutrients from digested bacterial cells may be of greatest benefit to a mammalian host when they are made available in the stomach or small intestine. Much of the microbial population in the rumens of adult ungulate mammals is digested in a gastric pouch of the animals, yielding precursors of macromolecules that are absorbed and incorporated into the macromolecules of the animal tissues (12). Microbial communities in the stomachs of monogastric animals or microbial populations from the hindgut passed into the stomach by coprophagy may also be sources of such compounds for their mammalian hosts. An interesting case in point is the recent discovery that queuine (the base of queuosine found in the first or "wobble" position of the anticodons of certain tRNAs) derives in mammals from the diet and intestinal microflora (33, 89). Such findings reveal that microbe-mammal symbioses extend to functions at the molecular and genetic level.

In addition to products of hydrolysis of the macromolecular constituents of their cells, lysed microbial cells are also sources of vitamins and other cofactors (1, 130). Germfree rats are known to require vitamin K in their diets, while conventional rats do not (130). Likewise, germfree rats and animals of certain other species require in their diets certain B vitamins (e.g. B_{12}, biotin, folic acid, and pantothenate) in concentrations higher than those required by their conventional counterparts (130). In these cases, the substances may derive predominantly from organisms residing in the cecums and colons, but may come as well from microbial cells growing on epithelial surfaces in the fore- and midguts.

Monogastric mammals can derive through coprophagy much of the nutritional benefit from the microbial cells in the hindgut flora (71). Noncoprophagic animals, e.g. adult humans, may derive little benefit unless molecules produced by or released from lysing microbial cells are absorbed into the blood in the cecum and colon. Evidence for that possibility is discussed in the section on the microflora and human nutrition.

Competition for Nutrients

IN NORMAL HOSTS As noted earlier, monogastric mammals without extensive foregut fermentations may still have microbial communities associated with epithelial surfaces in their stomachs and small intestines (106, 107). Those microbial layers are able to take advantage of nutrients in the host's diet, and thus to compete with the animal cells for those nutrients. The mass of animal cells far outweighs that of the microbial populations, of course, and thus, under normal circumstances, should have considerable advantage over the microbial cells in absorption processes. Still, the microbial cells thrive in their communi-

ties, and must therefore have mechanisms for competing with the animal cells. They must consequently deprive the latter of some share of the food ingested.

Also as noted earlier, the animal cells probably derive some of their nutrition from the macromolecular components of lysed microbial cells and the products of the energy-yielding metabolism of such cells. For the animal cells to benefit fully from nutrients consumed in the diet, however, all of the microbial macromolecules and products would have to be utilized by the animal cells. Such is not the case; bacteria and yeasts from gastric communities can be found in the feces of animals (106). Therefore, because of competitive activities of the microflora, not all of the nutrients ingested are available to the host's animal tissues. As emphasized earlier, however, the amounts of dietary constituents involved must be trivial; the microbial populations in epithelial communities in the fore- and midguts are less than 1% the size of the communities in the hindgut (106).

The extensive microbial communities in the hindguts (colons and cecums) of monogastric animals may normally compete little with the host's animal tissues for components of the ingesta. As discussed earlier, microorganisms in such communities thrive primarily by hydrolyzing and fermenting the substituents of endogenous polymers or exogenous ones in the diet that cannot be digested by enzymes made by the animals cells (86). Moreover, they obtain at least some of their nitrogen as ammonia by hydrolyzing urea, a waste product of the host's animal tissues passing into the bowel (36, 46, 78). Thus, the hindgut communities are principally contributors to the nutrition of the animal tissues.

IN HOSTS WITH ABNORMALITIES Anatomical abnormalities have been introduced into the gastrointestinal tracts of monogastric mammals in order to study the effects of microfloras developing in areas of the tract where they are not normally found. A typical case is a "blind loop" or pouch created in the small bowels of laboratory rodents or lagomorphs (118). Such abnormalities are introduced surgically, and involve isolating from the normal flow of digesta a loop or pouch in intestine. The loops or pouches usually fill with digesta and fluids that become stagnant in the region and allow for microfloras to develop in them. These floras can be quite complex in composition and biochemical activity and may resemble, at least superficially, the microfloras in the cecum and colon (118). A similar situation may develop especially on the epithelial surface in the small intestines of rats fed certain lectins (10, 11).

These microfloras can profoundly affect the physiology and even microanatomy of the areas in which they develop. For example, the mucosa may lose its villous architecture and most of the absorptive surface (41). The microorganisms can deconjugate and transform bile acids in the area leading to fat malabsorption; they can also compete with epithelial cells for vitamins (e.g.

B_{12}) and other essential nutrients (38, 41). Thus, abnormalities allowing complex floras to develop in the midgut can lead to generalized malabsorption and manifold nutritional deficiencies in the animal tissues of mammalian hosts.

Nutritional deficiencies may develop also when animals are given therapeutic amounts of antibacterial drugs. As is widely recognized, such drugs given at sub-therapeutic levels may potentiate growth and improve the efficiency at which food is utilized in animals of commercial value (24). Given at therapeutic levels, however, the drugs may disrupt the microflora, disturbing its normal functions (21, 44). Such disturbances may be subtle in terms of the microbial composition of the flora, but dramatic in terms of its biochemical activity and nutritional influences (21), and may have important implications for the long-term health and welfare of the host. Knowledge in this area is primitive and needs enriching with evidence from research.

Even when operating in their normal habitats, microbial floras may contribute to diseases in their hosts by producing harmful compounds from endogenous and exogenous chemicals. Bile acids and other compounds altered by bacterial enzymes in the gut are believed to be involved in inducing cancer in man (42, 47, 59, 79, 80, 93). Likewise, products of bacterial changes in drugs and xenobiotics (39) consumed by animals may damage certain of their tissues. Studies in these areas are in an early stage and should be given impetus. Likewise, studies should be encouraged of the biochemistry and genetics of the mechanisms by which intestinal organisms degrade toxic molecules such as dietary oxalate (2, 3), convert certain amino acids into toxic metabolites (117), and alter certain glycosides into mutagens (67, 116).

Overall Impact of the Microflora on Host Nutrition

FORE- AND MIDGUT FLORAS Indigenous microorganisms, principally strains of lactic acid bacteria and yeasts of certain genera, form epithelial communities in the stomachs and small intestines of monogastric mammals of some species. Microorganisms in those communities may influence the nutrition of the animal tissues of their host. For example, they may provide small amounts of certain vitamins, carbon energy, and nitrogen in the form of metabolic end-products and macromolecular precursors. The microbial cells may also compete for available dietary nutrients with the host's animal cells. However, the microbial populations involved are small by comparison with populations in the hindgut. Therefore, for these microbial communities, the contributive and competitive nutritional activities are probably of little overall significance to the nutrition of the animal tissues.

HINDGUT FLORAS Indigenous microorganisms, principally strains of strictly anaerobic bacteria, also form communities on epithelial surfaces and in the lumens of the cecums and colons of monogastric mammals (107). By compari-

son with those in the fore- and midguts, however, these communities contain enormous cellular populations (over 100-fold larger) and thus form substantial amounts of vitamins, metabolic end-products, and other compounds; they constitute a significant microbial mass. The metabolic products can be absorbed by the hindgut mucosa and utilized as carbon and energy sources by the animal tissues. In addition, such products, vitamins, and the constituents of the macromolecules in the microbial cells themselves may be utilized by the host's animal tissues, especially in animals that regularly practice coprophagy. All of these processes function in balance with the normal processes of the animal tissues as long as the microbial communities are not disturbed or displaced in some way. When the communities are disturbed, however, as through the actions of antibacterial drugs, animal tissues can suffer nutritional deprivation.

THE MICROFLORA AND HUMAN NUTRITION

The Microflora and Normalcy in Man

The student of the indigenous gastrointestinal microflora can view normalcy in humans from two perspectives, normalcy in people in developed countries and normalcy in at least some individuals native to certain developing countries. In both developed and developing countries, virtually all adults and children within a year or so after birth have in their large intestines fully developed communities of mixed microbial species (19, 34, 66, 81, 95). Individuals may differ substantially from each other in whether they have a flora in the small intestine, however, depending upon whether they are natives of a developed or a developing country. Normal adults in developed countries probably have no stable communities of microorganisms colonizing the epithelial surfaces of their small intestines (108). By contrast, adults in certain developing countries, functioning according to the norms of their populations, may possess communities of mixed bacterial species colonizing at substantial population levels the epithelium of at least the mid- and distal portions of their small bowels (14, 15, 20).

So far, no obvious and accepted explanation for these differences has emerged. Moreover, the experiments yielding the data from which the conclusions were derived, especially those concerning the small bowel floras in developing countries, should be reproduced and expanded. Nevertheless, the findings are sufficiently well grounded to pose interesting issues for a discussion of the role of the microflora in the nutrition of humans.

Nutrition in Developed Countries

The general points made above concerning the overall impact of the microflora on the nutrition of monogastric animals cannot be applied in their entirety to

ostensibly normal humans living in developed countries. Animals such as man, lacking stable microfloras in their stomachs and small bowels and not practicing coprophagy, would presumably experience few or none of the nutritional influences described for monogastric mammals possessing such floras and practicing coprophagy. Thus, for humans living in developed countries, the influences of interest are largely those of the microflora of the large bowel (53, 55). The past 10 years or so have seen active interest in research on those influences. Findings from the work are summarized in numerous recently published reviews (26, 73, 85, 114) and books (43, 132).

The research has focused on four general issues. The first of these is the biochemistry involved in the processes by which particular strains of species of indigenous bacteria hydrolyze endogenous and exogenous molecular polymers and utilize the end-products of the hydrolysis in their energy-yielding metabolism. The second concerns the organic end-products of those metabolic processes and whether those products are absorbed and utilized as carbon and energy sources by the host's animal tissues. (A corollary of this issue concerns gases produced as end-products in the microbial metabolic processes, their absorption into the blood, and excretion in air expired from the lungs.) The third research area concerns compounds produced by the microflora from exogenous or endogenous materials that may induce cancer or other diseases in the host. The fourth concerns efforts to manipulate the microbial metabolism, presumably to the nutritional advantage and health of the animal tissues.

The first of these issues has been explored most intensively with strains of species of two genera of bacteria isolated from human feces, *Bacteroides* and *Bifidobacterium*. The microflora of the adult human large bowel is similar in many respects to that in the cecums and colons of monogastric mammals of other species (22). Indeed, it even bears similarities to that of the rumen (128). It is composed principally of strains of many species of numerous genera of anaerobic bacteria. Strains representing over 400 species of at least 40 genera have been isolated from human feces. The populations of most of the strains are quite large, exceeding 10 billion cells per gram dry weight of fecal material (34). The aggregate population is so large, it may constitute over 50% of the mass of feces (115) and exceed the level of the total population of animal cells in an adult human (106). The greatest mass of that enormous population, however, consists of cells of strains of species of *Bacteroides* (34). Because their populations predominate in the tract, those strains have become tools of choice in studies of the biochemistry of polymer hydrolysis and energy-yielding metabolism (98). *Bifidobacterium* strains are present at high but not predominating population levels in the adult colon (34). They are of great interest, however, because they predominate in the feces of infants fed at the breast (19, 95).

Strains of species of both *Bacteroides* and *Bifidobacterium* can hydrolyze

and grow on the end-products of the hydrolysis of exogenous polymers of several chemical classes found in the human diet (Table 1). In addition, some strains can hydrolyze and grow on the end-products of the endogenous polymers gastric mucus, hyaluronic acid, and other tissue components (Table 1). The biochemistry of those processes was recently reviewed (98). Of interest to the purposes of this review is the discovery that enzymes involved in transport and hydrolysis of such polymeric carbon and energy sources are often not secreted by the cells and are invariably inducible (37, 72, 98, 102, 103). Under such circumstances, a particular compound or similar ones able to induce the enzymatic systems must be present in the colon for the enzymes to be synthesized by microbial cells with the genetic capacity to make them. Moreover, the systems may be strongly repressed by feedback inhibition when supplies of fermentable substrates (e.g. glucose) are adequate for microbial growth in the tract. Finally, because the hydrolytic enzymes are not secreted, the microbial cells must be in intimate contact with the target polymers in order to hydrolyze them. These discoveries have important implications for the design of experiments on all aspects of the role of the flora in nutrition.

Humans introduce into their gastrointestinal tracts dietary polymers of a large variety of chemical classes (98). Peptides, nucleic acids, and certain polysaccharides such as starch entering with the ingesta can be hydrolyzed by enzymes produced by animal tissues (132). Some such compounds in the diet undoubtedly make their way into the colon, the amounts depending upon how much is consumed and the materials with which they are associated in the food (17). Nevertheless, dietary polymers passing into the cecal portion of the large intestine and becoming available to the microflora are principally polysaccharides of chemical structures that cannot be hydrolyzed by enzymes produced by the animal tissues (Table 1). As noted earlier, endogenous macromolecular polymers, some of chemical classes other than polysaccharides, also enter the large bowel, principally intestinal mucus, digestive enzymes, secreted immunoglobulins, and the components of sluffing epithelial cells. Most research on the bacteria able to hydrolyze polymers has been accomplished, however, with complex dietary polysaccharides (98). Dietary polysaccharides are receiving most attention, in part because they predominate in the cecal content but also because of interest in dietary fiber and its impact on human nutrition (113).

The metabolic processes by which the human microflora gains carbon and energy from the components of the dietary and endogenous polymers produce end-products that are generally similar in chemical class to those produced in ruminant and cecal fermentations in mammals other than man (128). As discussed above, those products are principally short-chain organic acids and the gases H_2, CH_4, and CO_2. Acetic, propionic, and *n*-butyric acids predominate (19, 25, 50, 55). According to one study (55), the acids are present in feces at aggregate total concentrations ranging from 28 to 188 mM/kg (with an

Table 1 Some dietary and endogenous macromolecular polymers that may be hydrolyzed and utilized as carbon and energy sources by members of the indigenous gastrointestinal microflora of man[a]

Polymer	Microorganism	Reference
	ENDOGENOUS	
Chondroitin sulfate	*Bacteroides thetaiotaomicron*	98, 102, 103, 105
Mucin (gastric)	Microflora; *Bacteroides fragilis; Bifidobacterium* spp.	13, 50, 51, 76, 91, 98, 105
Glycoproteins	Microflora	82
Hyaluronate	*Bacteroides* spp.	105
	DIETARY	
Heparin	*Bacteroides* spp.	98, 105
Pectin	*Bacteroides* spp.; microflora	13, 27, 57, 98, 105
Ovomucoid	*Bacteroides* spp.	105
Amylose, amylopectin	*Bacteroides* spp.	98, 105
Dextran	*Bacteroides* spp.	105
Gum tragacanth	*Bacteroides* spp.; *Bifidobacterium* spp.	105
Gum guar	*Bacteroides* spp.; *Bifidobacterium* spp.; *Bacteroides ovatus*	8, 13, 37, 98, 105
Larch arabinoglactan	*Bacteroides* spp. *Bifidobacterium* spp.	13, 98, 99, 105
Alginate	*Bacteroides* spp.	98, 105
Laminarin	*Bacteroides* spp.	98, 104, 105
Psyllorium hydrocolloid	*Bacteroides* spp.	98, 101
Xylan	*Bacteroides* spp.	13, 98, 100
Polygalacturonate	*Bacteroides* spp.; *Bifidobacterium* spp.; *Bacteroides thetaiotaomicron*	72
Gum arabic	*Bifidobacterium* spp.	98
Cellulose	Microflora	98, 129

[a]This listing is not a complete record of all of the polymers known to be used for growth of intestinal bacteria (see 98). It is intended to indicate the diversity of the capacities of the bacteria.

average of 77 mM/kg, Table 2) in individuals consuming an ordinary diet. The concentrations may vary up to ±50% when samples are taken at different times during the day or on different days in the same individual (55). According to some reports, when adult humans are consuming defined diets containing single polysaccharides, the acids may be detected in feces at concentrations that vary depending upon the polysaccharide present (Table 2). Because the values can vary so widely from individual to individual, however, and even from day to day and time to time during the day (55), such findings are difficult to evaluate. Nevertheless, they do suggest that different dietary polysaccharides can influence, at least to some extent, the amounts of the metabolic products produced by the microflora.

Table 2 Short-chain volatile fatty acids (VFA) in human feces following consumption of diets containing defined polysaccharides[a]

VFA	"Ordinary Norwegian"	Course bran	Fine bran	Cellulose	Cabbage	"Basal"	Low E	Cellulose	Xylan	Pectin	Corn bran
Acetate	37[b]	69[c]	51[c]	53[c]	78[c]	38[d]	33[d]	35[d]	42[d]	34[d]	51[d]
Propionate	13	29	22	22	28	26	23	27	29	27	18
Isobutyrate	2	6	7	5	12	5	6	5	4	5	4
Butyrate	12	27	25	16	25	16	22	18	14	19	14
Isovalerate	3	7	8	6	15	9	11	9	7	9	8
Valerate	2	6	4	4	8	5	6	6	6	7	5
Caproic	1	-	-	-	—	-	—	—	—	—	-
Subjects (No.)	20	12	12	12	12	5	5	5	5	5	5
Reference	55	30	30	30	30	35	35	35	35	35	35

[a]Data from three recent studies are given (30, 35, 55). Values are given as reported by authors, but are rounded to nearest whole number.
[b]mM/kg feces.
[c]mM/5 ml "fecal inoculum."
[d]Percentage of total amount of VFA excreted.

Materials excreted in the feces are lost to the body as potential energy sources. To serve as energy sources to the host, therefore, volatile fatty acids must be absorbed by the large bowel mucosa, and must be produced in the cecum and colon at concentrations above those detected in the feces. Estimates have been made of the amounts of the acids produced in the tract; such estimates are based upon the amounts of acids in feces and theoretical yields of the compounds from bacterial action on dietary polymers. They indicate that greater amounts of the acids are produced than are excreted (73, 128). As noted, however, estimates of the amounts of the organic acids in feces are subject to a substantial variation. Moreover, the methods for estimating theoretical yields cannot be accepted as inviolate. Therefore, any speculations about nutrition based upon such estimates should probably be made with caution.

The estimates are supported, however, by findings from experimental tests. The acids disappear from dialysis bags placed in rectums (73) and from fluids perfused into colons (96). In addition, they can be absorbed and utilized by colonic epithelial cells maintained in culture (94). Butyric acid may be used preferentially by such cells (94).

The estimates are also supported by findings from experiments involving assays of absorbed fermentation gases. As noted earlier, in addition to the volatile fatty acids, the gases hydrogen and methane are produced in the fermentation processes (128). Hydrogen is produced in all individuals, while methane is produced in about 50% of persons (128). Both gases can be detected in the expired air and flatus (54, 70, 83). Calculations based upon measurements of the amounts of such expired gases and some assumptions about the amounts of organic acids and gases produced by the bacteria per unit amount of polymer hydrolyzed indicate that some organic acids must be absorbed by the host (54). Thus, humans may well gain carbon and energy from the products of the energy-yielding metabolism of microorganisms of their indigenous flora. The amount gained has been estimated to be about 10% of daily energy needs for persons in developed countries (73). That estimate should be evaluated with care, however; as noted, the amounts of acids produced probably vary considerably among individuals (55).

In spite of weaknesses in baseline data due principally to that and other sources of variation, investigators have begun to attempt to manipulate fermentation processes in the colon by feeding to humans diets containing various macromolecular sources (30, 35, 122). Most such research is motivated primarily by views that dietary fiber alters conditions in the bowel that lead to chronic diseases such as colonic cancer (122) and other serious conditions (123, 124). Some such research may be motivated, however, by a desire to find diets that increase the amounts of short-chain organic acids produced by the flora, and thus the amount available for use as carbon and energy sources by the host's

animal tissues. For example, experimental systems have been established in which individuals are fed diets differing in fiber source, and then their feces are assayed for volatile acids (30, 35) or their breath and flatus are assayed for hydrogen and methane (70).

The acids may vary in relative proportions and concentrations in the feces, depending upon the fiber source in the diets of the subjects (Table 2). Moreover, the amounts of fermentation gases may vary depending upon the dietary polysaccharide (70). The amounts of the short-chain acids in human feces vary so widely from sample to sample (55), however, that at this time such findings give little encouragement that the fermentations in situ can be manipulated in controlled ways.

Colonic fermentations are also being studied with fecal microflora cultured in continuous and discontinuous fermentation systems (68, 75). Such efforts also are fraught with problems. Fecal flora may differ in composition from that in the cecum and colon, and certainly differs in spatial organization; lumenal, epithelial, and cryptal communities cannot form in fermentation vessels (108). In addition, the nutritional and environmental milieu in the culture vessels may differ substantially from that in the cecum and colon; endogenous materials present in the colon are missing from the systems (68). Moreover, the amounts of acids produced vary over time (75), and with pH (29) in the fermentation vessels. Nevertheless, findings from such systems tend to confirm that the amounts of short-chain acids made per unit amount of content depend upon the polysaccharide present. They are not yet supportive of the hypothesis, however, that the biochemistry of the human colonic microflora is subject to controlled manipulation.

Viewed as an ecological system, the large bowel flora in situ is probably well buffered against changes induced by dietary manipulation. The flora is most complex in microbial composition and biochemical activity. It is not much affected in its species composition by dietary change (28). As has been noted, dietary polymers are not the only macromolecules available to the flora in a living host. Endogenous polymers may be significant proportions of the microbial diet in the large bowel (52), while urea may be an important nitrogen source as it is in the ceca of mammals of other species (131). Some such nondietary substrates support an anaerobic microflora containing methanogens in a sigmoid colon isolated from the normal fecal stream (74). The endogenous polymers may well buffer against change induced by exogenous materials not only the species composition of the flora but also its biochemical activity. During hydrolysis, those polymers may yield ample amounts of fermentable substrates that inhibit enzymatic systems involved in hydrolyzing exogenous polymers. Until evidence on these biochemical and genetic issues becomes stronger, efforts to manipulate the composition and chemistry of the flora by manipulating the diet might be considered premature.

Nutrition in Developing Countries

The microfloras indigenous to the large bowels of individuals living in developing countries are similar in composition and biochemical activities to those in the cecums and colons of persons living in developed lands (34). Thus, all comments about humans made above probably apply in both general and specific ways to the human populations in developing countries. However, in some cases at least, ostensibly normal persons living in developing countries have microfloras associated intimately with the epithelium of their small intestines (14, 15, 20). Therefore, the nutrition of such individuals could be influenced by the flora in ways similar to those discussed earlier for monogastric mammals with fore- and midgut floras. On the one hand, some such influences could be viewed as favoring the hosts. For example, the persons may be gaining some vitamins, energy, and carbon sources and some macromolecular precursors from the microflora. On the other hand, such individuals could be said to be suffering chronic malabsorption syndrome (41), a disease seen in developed countries only in persons with a microflora in their small intestines similar in composition to that in their cecums (41).

As discussed earlier, in certain abnormal circumstances a complex microflora may develop in areas of the small bowel, and cause malabsorption by hindering nutrient absorption. Individuals in developed countries may develop such conditions because of diseases (usually chronic and often of unknown etiology) that relax the factors regulating small bowel colonization (41, 106). Such persons may become acutely malnourished (41).

In some cases, persons in certain developing countries do suffer malabsorption in a condition called "tropical sprue" (62). This disease is said to be induced by some proteins ("toxins") synthesized and excreted by bacteria of certain species closely associated with the small bowel epithelium (62). If so, then the malabsorption is due to changes in absorptive cells similar to those occurring in acute bacterial diarrheas in both developed and developing countries (109), and is the abnormal result of the activities of microorganisms not normally found on the small bowel epithelium. Not all individuals with floras associated with their small bowel epithelia suffer from tropical sprue. Indeed, most of them are functioning physiologically according to the norms of their populations (14, 15, 20). Thus, they may be experiencing no harm from their small bowel microflora and may even be gaining some nutritional benefit. However, no direct evidence supporting such a concept is yet available.

SUMMARY

A mammal is a complex organism consisting of eukaryotic animal cells and eukaryotic and prokaryotic microbial cells. Most of the microorganisms reside in communities in the gastrointestinal tract. These gastrointestinal microfloras are known to serve nutritional functions in ruminants, pseudoruminants, and

monogastric mammals with only modest or no foregut fermentations but with extensive hindgut fermentations in blind cecal pouches. In adult animals, the microflora hydrolyzes exogenous (dietary) and endogenous polymers, and provides the adult with all or at least a significant proportion of its carbon, energy, vitamins, and macromolecular building blocks. The flora also functions as a conservator of nitrogen that would otherwise be excreted as urea. In exchange, the flora competes directly with the host tissues for nutrients ingested in the diet, and also competes indirectly by somewhat repressing the absorptive capacities of the animal tissues. When the synergism is in balance, the animal tissues and the microflora operate in harmony for the health and nutritional welfare of the host as a whole. The system may be unbalanced by antibacterial drugs that destroy the microflora and by diseases of the animal tissues that destroy the controls regulating where indigenous communities localize in the tract, their microbial composition, and their biochemical activities. At such times, the nutrition of the animal tissues can be adversely affected to the extreme.

Humans living in developed and developing countries have extensive microfloras in their hindguts. Humans living in developing countries may also have extensive microfloras in their small bowels. Those floras may function in nutrition of the animal tissues of man much the same as do floras in similar locations in the gastrointestinal tracts of mammals other than man. However, animals of some species other than human gain much of the nutritional benefit from their microflora through the practice of coprophagy. Since adult humans do not normally practice coprophagy, any nutritional benefit from the microflora depends upon the capacity of the bowel mucosa, principally that of the large bowel, to absorb bacterial products, e.g. short-chain volatile fatty acids. Such absorption undoubtedly occurs, but is surely not a major source of carbon and energy for the animal tissues of man.

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