Human Colonic Microbes: Ecology, Physiology and Metabolic Potential of Intestinal Bacteria

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Introduction

Humans live in close association with vast numbers of microorganisms that are present on the skin, in the mouth and in the genital and gastrointestinal tracts. In many cases, an intimate relationship exists between the host and its microflora that has developed as humans as a species have evolved. The best example of this is the complex assemblage of bacteria that constitutes the microbiota of the large intestine. Although fecal bacteria were first observed microscopically more than 300 years ago, the sheer scale of colonization of the lower intestinal tract by microorganisms was not appreciated until relatively recently. Savage [1] observed that while there are approximately $10^{14}$ cells associated with the human body, 90% of these are microorganisms, the majority of which reside in the colon. Viewed in another way, at any given time, 1 g of large intestinal contents contains about 150 times more bacteria than there are people on earth. Perhaps not surprisingly, therefore, the metabolic potential of the human colonic microflora is formidable, and because it plays an important role in digestion, and in many other aspects of host physiology and metabolism, it is tempting to view the microbiota as an organ in their own right.

The Upper Gut

In healthy individuals living in Western countries, viable bacterial counts progressively increase from the proximal to the distal small bowel. In the upper
small gut, viable bacterial counts are usually less than $10^4$/ml, and consist mainly of gram-positive facultative anaerobes and aerotolerant species such as streptococci, staphylococci and lactobacilli. Gastric acid is important in restricting entry of bacteria to the small intestine, while rapid peristaltic movements and transit of digesta through this part of the digestive tract (2–4 h) does not allow time for stable bacterial communities to establish [2]. Bacteria are also found in the biliary tract, particularly aerobes and facultative anaerobes. Although few bacteria can be recovered from the duodenum, jejunum and upper ileum, stasis of digestive material at the ileocecal valve results in a qualitative and quantitative increase in bacterial numbers [3, 4], and anaerobes increase, as pH and redox potential drop. Small bowel overgrowth can occur as a result of disorders in gut motility, achlorhydria, drugs, antibiotic treatment, radiation therapy, cirrhosis, strictures, diverticulae and small bowel resection.

**The Large Gut: Physiologic and Anatomical Characteristics**

In persons living in Western countries, the main area of permanent colonization of the gastrointestinal tract is the large bowel. The main reason for this is that the flow of material through the gastrointestinal tract slows markedly, providing time for stable bacterial populations to develop. Large intestinal transit times vary considerably between individuals, but usually range from about 20 to 120 h, with a mean of about 60 h [5].

The colon is an open system in the sense that food residues from the small bowel enter at one end, and feces are excreted at the other. The adult large gut is typically over 1 m in length, with an internal surface area in the region of 1,300 cm², and a total volume of about 500 ml. Fecal excretion in persons living in industrialized Western communities is approximately 120 g/day, but fecal output in Third World and other underdeveloped countries is considerably higher. Although feces are mainly water, bacteria are a major component, comprising 55% of fecal solids [6].

From anatomic, microbiological and environmental perspectives, the proximal bowel (cecum, ascending colon) and distal gut (descending colon, sigmoid/rectum) are quite different from each other. When digestive residues from the ileum enter the cecum, they encounter a pool of partially digested foodstuffs and bacteria, which rapidly begin to utilize simple carbon and nitrogen sources, and initiate the breakdown of complex carbohydrates and proteins. Due to production of fermentation acids, pH is reduced to about 5.5 or less in the proximal colon [7]. Due to high levels of substrate availability, the cecum and ascending colon are sites of the most intense microbial activity in the gut, but because of substrate utilization and water absorption, bacterial cell population densities increase distally through the colon [8].
Interactions with the Host

The microbiota play an important role in establishing and maintaining normal gut structure and function. Bacteria interact with the intestine in many ways, for example, bacterial cell mass stimulates peristaltic movement, thereby facilitating the passage of digestive residues through the bowel, while studies with germ-free animals indicate that small intestinal uptake of sugars, amino acids, minerals and vitamins is more efficient than in animals with a conventional microflora [9]. The role of bacterial metabolites in providing fuels for the colonic epithelium will be discussed later, but the influence of the microbiota on gut structure is seen in germ-free rodents, where the cecum is greatly distended, while lymphoid tissue and the lamina propria are atrophied. The cecal mucosa is also thinner than in normal animals, and gross alterations in cellular morphology are evident [10].

Host Defenses

While there is evidence that bacteria can translocate from the colon into portal blood and the liver, the invasion of body tissues by colonic organisms is prevented by a number of host and microbiological controls. The peristaltic action of the gut, pH of contents in the stomach and cecum, bacterial metabolites, bile salts, mucous layers and gastrointestinal epithelia, as well as host immune processes, all serve in one way or another to protect the host. In general, bacteria can only pass through the gut wall when its permeability is increased or its integrity compromised.

Mucins are high molecular mass glycoproteins secreted by cells lining the digestive tract, and they are amongst the most important host defenses. In the colon, mucus is secreted by goblet cells in the epithelium, forming a viscoelastic gel covering the gut surface. It acts as a physical barrier against luminal bacteria and many of their toxins and secretory products. Antigen–antibody complexes stimulate secretion of mucus, which anchors secreted antibody onto enterocytes, where antigens may be bound and subsequently degraded by adsorbed pancreatic and bacterial peptidases. This may be important in preventing internalization of antigens. Because many microorganisms in the large bowel degrade mucus, the colonic epithelium needs to produce glycoprotein at a faster rate than the bacteria can break it down, and a balance must therefore exist between mucin production and its destruction by the microbiota.

Host immune processes probably have little effect on ecologic events in the colonic lumen. However, interactions between intestinal bacteria and the immune system occur at the mucosal surface, and full structural and developmental expression of the intestinal immune system depends on continued exposure to bacterial antigens from the gut lumen.
Ecology

The vast majority of microorganisms in the healthy large intestine are bacteria. Yeasts are sometimes detected in low numbers, but protozoa are seldom found. While some gram-negative species occur in high numbers in the gut, the predominant organisms in the large bowel appear to be gram-positive anaerobic rods and cocci [11]. Several hundred different bacterial species and strains have been isolated from fecal material [12], but surprisingly little is known of the metabolic interactions that occur between different groups of microorganisms in the large gut, or of the ecology and multicellular organization of the microbiota as a whole. While culturing studies have shown that the ecosystem contains large numbers of phylogenetically and physiologically distinct microorganisms, molecular analysis of the microflora indicates that many intestinal bacteria are not being cultured [13]. Indeed, it is thought that only about 40% of the bacteria in the human large bowel are culturable [14].

Bacterial species diversity in the gut largely derives from the multiplicity of different carbon and energy sources available for growth, and the principal host factors regulating the microbiota in health are probably substrate availability and colonic transit time. Moreover, competition (e.g. for nutrients or space) and cooperative interactions (e.g. polymer breakdown) between individual groups of bacteria are also important in defining community structure in the microbiota.

The microbiota is a stable and immensely complex entity, which is to a large extent self-regulating. Although many microorganisms are able to infect the gastrointestinal tract, through competitive exclusion, indigenous species afford a degree of protection to the host by acting as a barrier to invading pathogens; however, the effectiveness of this process is frequently diminished during illness, or by antibiotic treatment [15].

The Gut Microbiota and Resistance to Disease

Many different types of microorganism including bacteria, viruses, fungi and protozoa are agents of disease in the gastrointestinal tract. Bacteria indigenous to the colon play an important role in preventing colonization of the gut by pathogenic organisms. Studies with germ-free or antibiotic-treated animals illustrate the protective effects of indigenous bacteria, since these animals are more susceptible to salmonella, campylobacter and shigella infections. Moreover, colonization of the gut by anaerobic pathogens such as Clostridium difficile, the primary etiologic agent of pseudomembranous colitis, and possibly, Clostridium botulinum, is prevented by normal gut microbiota. The barrier effects exerted by indigenous bacteria have also been demonstrated in human studies in which patients with pseudomembranous colitis and ulcerative colitis have been treated, to some effect, with rectal enemas containing slurries of feces from healthy donors [16].

The significance of colonization resistance to pathogens is sometimes seen in patients undergoing antibiotic therapy, which can have serious side effects.
on the gut microbiota, and the inadvertent removal of protective species may allow invaders to establish, as in pseudomembranous colitis [17, 18]. Clindamycin, tetracycline, chloramphenicol, and orally administered ampicillin have been associated with the onset of this disease [19]. Other pathogens may also establish during antibiotic treatment, including enterotoxigenic *Clostridium perfringens*, while overgrowth of facultative anaerobes such as yeasts, enterobacteria and pseudomonads may also be seen.

**Biofilms in the Large Gut**

Intestinal microorganisms occupy many different microhabitats and metabolic niches on the mucosa, in the mucus layer and on the surfaces of food residues in the colonic lumen [20]. These microcosms are continuously in a dynamic state of change, as resources are consumed or recycled. Intestinal bacteria are unlikely to exist as individuals in the gut, and probably occur in microcolonies, in complex associations with other species. Particle-associated and mucosal bacterial populations are likely to be components of highly evolved assemblages, analogous to those in oral biofilm communities [21]. Close spatial relationships between bacterial cells growing in gut biofilms may be important in relation to metabolic communication between microorganisms in the microbiota. Their ecological significance is that they reduce potential growth-limiting effects on bacterial cross-feeding populations, such as those involving mass transfer resistance [22]. Another characteristic of bacterial biofilms in the large bowel is that species colonizing surfaces in the gut lumen are directly involved in the digestion of complex insoluble polymeric substances, imparting a significant competitive advantage in the ecosystem [23].

**Physiology**

**Growth Substrates and Nutrition of Intestinal Bacteria**

Large intestinal microorganisms exist by digesting dietary residues (mainly carbohydrates and proteins) and a range of other substrates that are produced by the body itself. The anaerobic breakdown of organic matter in the colon is termed fermentation. Bacteria gain energy, carbon and nitrogen for cell growth from fermentation, and produce a variety of waste products, that are absorbed from the gut, and which are often of physiological importance to the host.

**Carbohydrate Breakdown**

Pancreatic amylase is the only polysaccharide-degrading enzyme secreted into the digestive tract in humans, despite this, starch is the most important dietary carbohydrate to reach the colon [24]. Evidence that this polymer is incompletely digested in the small gut comes from several sources, including intubation, breath hydrogen and ileostomy studies. For a variety of reasons,
several types of starch are resistant to pancreatic amylase, but they are hydrolyzed by bacterial amylases in the large bowel. The other major group of complex carbohydrates fermented by colonic bacteria are the non-starch polysaccharides, or dietary fiber. They are structural components of plant cell walls, and include cellulose, hemicelluloses, pectins, inulin and various gums. They are not digested by mammalian enzymes, but intestinal bacteria produce an extensive range of polysaccharidases and glycosidases that degrade these polymers and make their constituent monomers available for fermentation [25]. Microbiologically, breakdown of complex polysaccharides in the colon is both a cooperative and competitive process, involving many different groups of organisms; however, bacteria belonging to the genera bacteroides and bifidobacterium seem to play a key role in depolymerization of these substrates [26–28]. Although dependent on diet and host intestinal transit time, fermentable carbohydrate is often limiting in the distal large bowel, due to its utilization by bacteria in the proximal gut. This has important consequences for the host, because the digestion of complex carbohydrates is a beneficial process in the large bowel that reduces the formation of putrefactive metabolites [29, 30].

Proteolysis

Unlike carbohydrate availability, there is no shortage of proteins and peptides in the distal large bowel [31]. It is estimated that between 3 and 25 g of these substances enter the colon every day [32–34], partly in the form of dietary residues (e.g. plant, sarcoplasmic and myofibrillar muscle proteins), although a significant proportion comes from the host’s upper gastrointestinal tract. The large intestine is also a source of proteins, such as bacterial secretions and lysis products, colonic mucins and desquamated mucosal cells [35]. However, in quantitative terms, hydrolytic enzymes elaborated by exocrine cells of the pancreas, including proteases (trypsin, chymotrypsin, elastase), lipases, amylase and nucleic acid hydrolases are amongst the most important sources of protein in the large bowel [36].

The human large intestine is one of the most proteolytic natural environments known. Measurements of protease activity in small intestinal contents and in material taken from the proximal and distal colons show that proteolysis progressively declines as digestive materials move through the gut [37]. This occurs because pancreatic proteins are broken down by bacteria in the large bowel, while host antiproteases inhibit pancreatic endopeptidases in the gut [38, 39]. The role of bacteria in degrading these enzymes is shown in animal studies, where pancreatic protease activities are considerably higher in the feces of germ-free rats compared with conventional animals [40]. In humans, fecal trypsin increased 100-fold in patients treated with antibiotics, although chymotrypsin and elastase activities were only 2–3 times higher [38]. Pancreatic endopeptidases probably undergo autodigestion in the colon, and there may be synergistic effects with bacterial proteases [37, 41].
**Fermentation**

Anaerobic chemoheterotrophic populations in the colon include organisms that carry out anaerobic respiration, as well as fermentative bacteria, that produce adenosine triphosphate (ATP) through substrate level phosphorylation reactions. Fermentative bacteria predominate in the gut. In fermentation, the electron acceptors are metabolic products derived from the original substrate, consequently fermentation reactions are self-balancing, with the redox differential between substrates and products determining the amount of energy that can be produced. Compared to oxidative metabolism, fermentations are energetically inefficient processes that give low ATP yields. Large amounts of substrate are therefore required for growth in fermentative bacteria, which results in large quantities of metabolic end-products being formed. Fermentations are governed by the need to maintain redox balance, mainly by the reduction and oxidation of ferredoxins, flavins and pyridine nucleotides. This affects the flow of carbon through bacteria, the energy yield obtained from the substrate, and the metabolic end-products. Short-chain fatty acids (SCFAs) are the main fermentation products produced in the large intestine, while formation of reduced substances such as hydrogen gas, lactate, succinate, butyrate and ethanol is used to effect redox balance [42].

**SCFAs**

Acetate, propionate and butyrate are the principal products of carbohydrate and protein fermentation in the large bowel [42]. The vast majority of SCFAs (>95%) formed by gut microorganisms are absorbed and metabolized by the host [43]. This allows salvage of energy from food that is not digested in the upper gastrointestinal tract, and can account for up to 9% of the hosts energy requirements [44]. SCFAs have a wide range of physiological functions in the body, including colonocyte metabolism [45], cell growth and differentiation [46], epithelial cell transport [47], metabolism of lipids and carbohydrates in the liver [48], intestinal motility [49], as well as energy generation in muscle, kidney, heart and brain [50]. SCFAs also inhibit phagocytic cell function [51, 52]. Butyrate is believed to be protective against colon cancer, and has been shown to arrest cell growth early in G1 and induce cell differentiation, while stimulating cytoskeletal organization and alterations in gene expression [53–56]. The arrest of cell growth by butyrate is associated with differentiation which occurs in many human cell lines. Butyrate modulates the expression of many different genes and differentiation in tumor cells, and is linked to changes in their cytoskeletal architecture and adhesion properties [57].

**Gas Production**

Gas is a major product of carbohydrate and protein fermentation in the large bowel. Gas formation is diet-related, but the total amount formed each day can amount to as much as 4 liters. Foodstuffs such as beans, brussels sprouts,
some fruit juices and some types of soluble dietary fiber can generate large amounts of fermentation gases, principally, hydrogen, carbon dioxide, and in some individuals, methane. Approximately 80% of fermentation gas produced in the large intestine is excreted as flatus, where the major gases are nitrogen (64%), hydrogen (19%) and carbon dioxide (14%) [58]. Nevertheless, considerable variation occurs, and between 10 and 20% of colonic gas is absorbed and excreted in the breath [24, 50]. A significant amount of hydrogen produced in the colon is sequestered by specialized groups of bacteria to produce either methane (methanogenesis), hydrogen sulfide (dissimilatory sulfate reduction) or acetate (acetogenesis). The organisms involved derive energy from these processes, and they can occur in high numbers in the large bowel [42]. By reducing the partial pressure of hydrogen in the gut, hydrogenotrophic species have important ecologic and physiologic roles in the microbiota [42, 59, 60].

**Metabolic Potential**

*Products of Putrefaction*

Not all of the metabolic activities of intestinal microorganisms are benign. The absorptive capacity of the large intestine is considerable, and many bacterial metabolites are toxic to host tissues, particularly those resulting from protein breakdown and amino acid fermentation, such as ammonia, amines, phenols and indoles [36]. Moreover, several sulfur-containing organic compounds are formed by anaerobic bacteria from the S-containing amino acids methionine and cysteine. Methanethiol and mercaptoacetate, in particular, are strong reducing agents that are toxic to isolated colonocytes in vitro [61]. Normally the products of protein digestion are detoxified in the mucosa and liver, by sulfate or glucuronide conjugation, but in some circumstances, their production in the large intestine exceeds the body’s abilities to effect their disposal.

*Ammonia*

Fecal ammonia concentrations range from about 3 to 44 mM [62], and in the large bowel, concentrations of this metabolite increase distally through the gut, but this is not as marked as with other products of amino acid fermentation, particularly phenols and branched chain fatty acids. It is still unclear how much ammonia results from urea hydrolysis; however, studies in which human volunteers were infused with $^{15}$N-labeled urea indicated that most ammonia in the gut results from deamination of amino acids [63]. The physiologic significance of ammonia formation is that low concentrations (<10 mM) can alter the morphology and intermediary metabolism of intestinal cells, while increasing DNA synthesis and reducing their lifespan [64].
Amines

Amines are major products of dissimilatory amino acid metabolism in the gut, and their excretion in urine is directly related to protein intake. Histamine, piperidine, pyrrolidine, cadaverine, putrescine, agmatine, tyramine, 5-hydroxytryptamine, methylamine, dimethylamine and propylamine are all produced by gut microorganisms, and are rapidly absorbed from the bowel, whence they are detoxified by mucosal and liver monoamine and diamine oxidases. However, hypertensive symptoms and migraine have been linked to amines produced in the large gut [36]. Many of these metabolites such as histamine, putrescine, tyramine and cadaverine are pharmacologically active, variously functioning as pressor or depressor substances, and as stimulators of gastric secretion or vasodilators [65]. Amine formation in the large bowel is therefore important because of the pervasive effects of these metabolites on different organ systems in the body.

Phenols and Indoles

The abilities of bacteria to produce phenolic and indolic metabolites is widespread in the colonic microbiota. These products of aromatic amino acid metabolism are formed in a series of deamination, transamination, decarboxylation and dehydrogenation reactions [29]. Levels of tyrosine breakdown products, such as phenol and p-cresol, increase markedly in the distal bowel, further showing that protein breakdown becomes more significant in this region of the gut. After absorption from the gut, phenols and indoles are detoxified and excreted from the body as sulfate or glucuronide conjugates. Little is known of the physiologic and environmental factors that control aromatic amino acid metabolism in the large intestine, but long colonic transit times result in reduced carbohydrate availability and more protein breakdown in the distal bowel. This was seen in feeding studies with human volunteers, where phenol excretion was related to both carbohydrate and protein intake, and where increasing the availability of fermentable carbohydrate reduced phenol formation due to saccharolytic bacteria sequestering tyrosine for biosynthetic purposes [66].

Phenols and indoles have been linked to cancer [67], but they also have other effects on host physiology. For example, the tryptophan metabolite skatole is associated with malabsorption, anemia and schizophrenia [68, 69]. In some animals, skatole causes tryptophan-induced acute bovine pulmonary emphysema, whereas in weanling pigs, p-cresol acts as a growth depressant [69, 70].

Hydrogen Sulfide

Dissimilatory sulfate reduction is an important route of hydrogen disposal in the large gut [71]. Sulfate-reducing bacteria (SRB) use sulfate as a terminal electron acceptor in metabolism, producing hydrogen sulfide, which is normally detoxified by the colonic mucosa. SRB can out-compete other
**Table 1.** Biochemical activities of gut microorganisms that affect host metabolism

<table>
<thead>
<tr>
<th>Activity</th>
<th>Examples</th>
<th>Effect on host</th>
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<tbody>
<tr>
<td>Metabolism of neutral steroids</td>
<td>Chemical modification of steroid hormones, cholesterol and plant sterols</td>
<td>Reduction of cholesterol to coprostanol and coprostanone</td>
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<td>Reabsorption and recycling of reduced corticosteroids, progesterone, estrogens</td>
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<td>Possible role in breast cancer</td>
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<tr>
<td>Bile acid metabolism</td>
<td>Deconjugation and dehydroxylation of bile acids, desulfation of bile acid sulfates</td>
<td>Absorption of secondary bile acids</td>
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<td>Detoxication of mutagens</td>
<td>N-Dehydroxylation of N-hydroxycetyl-aminofluorene</td>
<td>Possible promoting activity in colon cancer</td>
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<td></td>
<td>Reduction of nitropyrene to its less toxic derivative aminopyrene</td>
<td>Protective</td>
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<td></td>
<td>Breakdown of N-nitroso compounds such as diphenylnitrosamine, nitrosopyrroldine, dimethylnitrosamine</td>
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<td>Transformations of xenobiotics</td>
<td>Activation/inactivation of drugs</td>
<td>Prolonged enterohepatic circulation of foreign compounds</td>
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<td>Exemplified by conversion of the drug sulfasalazine to the active form, 5-aminosalicylic acid, which is used in treatment of inflammatory bowel disease</td>
<td>Direct toxic effects on body tissues</td>
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<td></td>
<td>Release of cyanide from amygdalin. Desulfation and deconjugation of drugs excreted in bile</td>
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<tr>
<td>Lignan and phytoestrogen metabolism</td>
<td>Substances occur naturally in some plants. Conversion to enterodiol, enterolactone and equol</td>
<td>These compounds have estrogenic and anti-estrogenic effects. May affect fertility and breast cancer</td>
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<tr>
<td>Toxic compound or precursor molecule</td>
<td>Metabolic significance</td>
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<tr>
<td>Fecapentaenes</td>
<td>Plasmalogens (polyunsaturated ether lipids) are converted to mutagenic fecapentaenes by some bacteroides</td>
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<tr>
<td>Diacylglycerol</td>
<td>Metabolism of phosphatidylcholine (dietary lipid) in conjunction with bile acids</td>
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<td>Azo compounds</td>
<td>Azo dyes used as food colorings. Other azo compounds used in pharmaceuticals and cosmetics. Mutagenic and possibly carcinogenic after chemical reduction to primary aromatic amines by intestinal anaerobes</td>
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<tr>
<td>Secondary bile acids</td>
<td>Toxic, co-mutagenic and co-carcinogenic activities. Formed by breakdown of glycine and taurine conjugates by bacterial hydrolases, 7 α-dehydroxylation and other reactions</td>
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<tr>
<td>Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs)</td>
<td>Toxic, mutagenic and carcinogenic in laboratory animals. Toxicity usually results from reduction of a nitro group on heterocyclic and aromatic nitrocompounds to an amine group by bacterial nitroreductase</td>
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<td>Cholesterol metabolites</td>
<td>Bind to DNA in vitro. Bacterial catalytic mechanisms unclear. Cholesterol α-epoxide is tumorigenic in animals</td>
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<td>Various plant glycosides</td>
<td>Toxic aglycones produced by bacterial glycosidase activity towards glycoside conjugates, e.g. hydrolysis of cycasin by β-glucosidase to release the carcinogen methylazoxymethanol Others include potentially mutagenic hydroxylated anthraquinones found in a number of plants, including rhubarb</td>
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<tr>
<td>Phenolic compounds</td>
<td>Possible co-carcinogens formed by metabolism of tyrosine</td>
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<tr>
<td>Indoles</td>
<td>Bladder co-carcinogen produced from tryptophan. Other metabolites of this amino acid reported to be genotoxic</td>
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<tr>
<td>Ammonia</td>
<td>Formed by amino acid fermentation. Affects DNA synthesis and reduces lifespan of colonic epithelial cells. More toxic to normal than transformed cells. May select for neoplastic growth</td>
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<tr>
<td>Amines</td>
<td>Mainly result from decarboxylation of amino acids and N-dealkylation of choline</td>
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<tr>
<td>N-Nitroso compounds</td>
<td>Produced by condensation of nitrite with a secondary amine (e.g. dimethylamine piperidine) or tertiary amine</td>
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</table>
hydrogenotrophic microorganisms in the colon for hydrogen, and people who have high numbers of SRB in their gut usually have low numbers of methanogenic archae [60]. Sulfate availability strongly affects the outcome of competition because most SRB have an obligate growth requirement for inorganic S-containing electron acceptors. SRB are not able to breakdown proteins and carbohydrates by themselves, and have an obligate dependence on other bacteria in the microbiota to produce substrates for their growth. However, hydrogen consumption by SRB can have profound effects on fermentation processes, by increasing acetate formation and lowering the production of electron sinks and other reduced metabolites [72].

Production of Genotoxic Substances

The gut microbiota displays considerable metabolic potential and diversity, in addition to its activities in relation to carbohydrate and protein metabolism (table 1). Bacteria in the colon deconjugate bile acids and modify steroids, which affects their enterohepatic circulation. In addition, intestinal microorganisms are able to chemically transform drugs and other xenobiotic compounds [73], as well as producing and detoxifying mutagenic and carcinogenic substances. There is also strong evidence for their involvement in the etiology of colon cancer (table 2). The colon is the second commonest site of cancer in humans, and this disease is a major source of morbidity and mortality in developed countries [74]. The Ames test indicates that feces from many individuals in Western populations are mutagenic [75], and there is a good correlation between the excretion of fecal mutagens and large bowel cancer. Although there is no general agreement concerning the etiology of colon cancer, a number of factors have been implicated, such as diet, the environment and genetics. The gut microbiota clearly has a role in the initiation of these diseases, possibly by converting nontoxic precursor compounds to substances with mutagenic or carcinogenic potential (table 2). A number of bacterial enzymes are thought to be involved in activating mutagens, including β-glucosidase, β-glucuronidase, azoreductase and nitroreductase [76]. They have been extensively studied in relation to diet, and the results have shown that high levels of meat and fat increase their synthesis by the bacteria. However, it is not clear whether this by itself leads to an increased cancer risk.

References


Human Colonic Microbes

Discussion

As the authors of this paper were unable to join the workshop, the presentation and the discussion were conducted by Florence Rochat, Switzerland.

Dr. Guesry: First of all Dr. Rochat I would like to congratulate you on this magnificent presentation, knowing that you had less than 24 h to prepare, but I have also two questions. You pointed out the difference between breast-fed and bottle-fed infants, the first showing bifidus bacteria, the second bacteroide, as the predominant flora. But do you have any data on a baby who would be bottle-fed with the mother's own expressed milk, to try to see the difference between the mere composition of the milk and the important contact between the baby and the skin of the breast?

Dr. Rochat: I have no data on such an experiment and I am not aware of such data in the literature comparing the groups in the same study. It would be very interesting to have this approach, but all 3 models of feeding are needed to compare the results.

Dr. Guesry: Dr. Marini, maybe you have data like that?

Dr. Marini: We are not experts on this. But I would like to ask about the problem of pasteurized human milk which is used a lot in preterm units simply because there is a risk with human milk of cytomegalovirus (CMV) infection, there is reactivation during pregnancy and in very small preterm babies there could be a risk. We have seen cases of sepsis due to CMV infection acquired since birth through human milk. What is happening with pasteurized milk, because we know that fresh human milk is the best for protection?

Dr. Rochat: But certainly we lose an important part in this pasteurized milk because of the structure of carbohydrate, many important compounds will be changed during pasteurization, and this is the fourth group in the proposed experiments.

Dr. Guesry: It is not only pasteurization; the cells disappear when you put breast milk into a bottle. The cells stick to the glass.

Dr. Rochat: To the glass yes, and so the container is really important to consider.

Dr. Marini: I would like to make a comment about hydrolysate and colonization. We have done a study in guinea pigs from birth up to 20 days of age with different kinds of feeding, not extensively hydrolyzed protein, intact protein and mother's milk [1]. We have found that with hydrolyzed protein there is a pattern of biliary acid secretion similar to human milk, and this can also be a factor in good colonization because we know that the bile is not only for digestion but it also has some antibacterial effect, at least in the ileum and jejunum. It has also been demonstrated that oligosaccharides can influence biliary secretion.

Dr. Rochat: The guinea pig is a very interesting model for gut alteration. There is only one limitation, we know there is no perfect model but for us one of the limitations to be perfect or closer to the human is the intestinal microflora because it is quite different from the human, there is no bifidobacteria, and that is why there is some limitation to this approach.

Dr. Salminen: Thank you for your nice presentation, I think it very nicely covered all the different areas that are currently of interest. I would like to put one specific question to you. In your slides you pointed out the work that is done on bifidobacteria and prebiotics, and we saw how bifidobacteria are promoted by prebiotics. Do you have any information on the species composition? We have worked on that area and...
shown with Dr. Isolauri that there is very different species composition in allergic and nonallergic infants.

Dr. Rochat: We are finalizing the analyses from one of our studies. In a study done with different prebiotics, we have observed the increasing bifidobacteria, but we do not have the results right now.

Dr. Salminen: I think it is of crucial importance if you think about infants and prebiotics, because by defining what kind of microbiota he or she receives, more adult type or elderly type, compared to healthy infants, you really do have the question there.

Dr. Rochat: It is true, it is really important for infants and for the moment I think there are few data with prebiotics in infants. For the time being there are few data demonstrating the impact of the prebiotics on the gut intestinal microbiota in the infant.

Dr. Walker: Dr. Rochat, you did a wonderful job of covering this topic. Several studies have suggested that as formula has changed, the differences in colonization between breast-fed and formula-fed infants are becoming similar. One observation that you made is the increase in bifidobacteria with both. There have been some studies that suggest that peptides have a bifidogenic effect as well as oligosaccharides [2]. I wonder if anyone has looked at the breakdown of the protein content of formula in the context of the peptides to discover if they have a bifidogenic stimulatory effect? Do you know of any studies?

Dr. Rochat: I know that this is one possible explanation for the change that we observed in many studies, and we have more bifidobacteria. It is true that the appearance of bifidobacteria in bottle-fed babies is more important, but there are still some other changes that remain: the diversity, the difference in the pH. So this is not very close for the moment. But the peptide and also some changes in the production of formula may influence the promotion of bifidobacteria.

Dr. Papageorgiou: Was there any difference in the degree of hydrolysis in your rat experiments in terms of effect, which has some relevance on the size of peptides?

Dr. Rochat: This was quite extensively hydrolyzed protein.

Dr. Marini: I think it was about 700 if I read correctly.

Dr. Bedford-Russell: Just the question you asked about the raw breast milk. Virtually all the long-term epidemiological studies on this have been done on either a mixture of formula and breast milk or breast milk, and some of that breast milk was pasteurized and some of it was frozen. So it is very interesting whether raw milk has all the immunological factors. What I can say about CMV though is that there was an article published in the Lancet some years ago in which babies were given raw breast milk in a neonatal unit and the CMV incidence was found to be about 25%. We looked at our own babies and published a study a couple of years ago [3]. Our practice often is to freeze the breast milk and our own rate was significantly reduced by that because of course freezing gets rid of about 98% of CMV, but we don’t know what effect it had on getting rid of the other goodies in the breast milk.

Dr. Rochat: It is interesting for me also to see that, depending on the country, there are different procedures. A few months ago it was quite new to me that there is not a single procedure for the treatment of breast milk: in some countries it is pasteurized, and in others it is frozen.

Dr. Bindslev-Jensen: I am not a neonatologist or a pediatrician so I was rather astonished to learn that the fetal pH is 1.6 units. The neuroimmunidades and the sialyl transferases in contrast to the fucosyl transferases don’t work at a pH above 6.5. So there might be a connection to what is going on if there is such a big difference in the fetal pH. Is it present already at birth? What is the pH in the fetus at birth and what constitutes this pH acidity?

Dr. Rochat: The pH is not so low at birth but it changes when the bacteria appear. There are a lot of changes when the bacteria colonize the gut, and this is also part of
the important changes which will determine which bacteria will stay after that in the digestive tract.

Dr. Hill: What is known about bacterial flora in totally breast-fed infants who present with atopic dermatitis in the first month of life compared to nonatopic dermatitis breast-fed infants? Is there a difference in the bacterial flora?

Dr. Rochat: A difference was described.

Dr. Vandenplas: I have two questions, the first one regards the floral development in formula-fed infants. The liquid formula is sterile, powdered infant formula is not sterile, mineral water is also not sterile. Do you know if there is a different floral development whether liquid formula or powdered formula is used?

Dr. Rochat: I don’t know. I have no data.

Dr. Vandenplas: The second question is the role of gastric pH because gastric acid is probably important. A lot of infants who have gastroesophageal reflux are treated with antacids or acid-blocking drugs. Does anyone know about the role of having no gastric acid on floral development or the flora that you have?

Dr. Rochat: We know that the pH is really important because we can easily correlate the pH and certain bacterial compositions. But there is a difference between the pH in the upper part of the digestive tract and the fecal pH. We know that when there is a less acidic condition we have a chance to have more bacterial growth in the upper part of the digestive tract.

Dr. Neijens: By studying the microbes from the gut so intensely, did you take the opportunity to analyze the interaction between specific bacteria and the way they might more specifically influence the spectrum of mediators, and secondly is there interaction between the various strains?

Dr. Rochat: The exchange of genes was studied mainly by people working on antibiotic treatment to see if there is a possible transfer from antibiotic resistance from one bacteria to another. As to the first part of your question on the interaction between the bacteria, some studies have been done, and gnotobiotic animals are really a useful model for this because we can place in competition two different types of bacteria, two different species, and see the interaction. This is the case in the work done by Corthier et al. in which they demonstrate the interaction between the bifidobacteria and the Clostridium difficile. But here again a lot of work needs to be done on this because there are so many species, and firstly we were working with quite well-known bacteria, quite easy to cultivate. I am sure that we have to be more deeply interested in bacteria such as peptostreptococci or eubacteria which are really the main components in the digestive tract. So these will be really important bacteria to study. That is why I also mentioned that in the future some people will start to work with mono-associated mice with specific bacteria to see what the effects are on the genome. We have to do the same with pluri-associated mice, but this is a big job. In my opinion there is a lack of knowledge at present.

Dr. Neijens: Gene interaction will be very important because it might have several effects: it might have a beneficial effect but also a risk because there is a risk that they change antibiotic-resistant strains between them, so that should be carefully studied.

Dr. Rochat: That is true, and there is interaction between the bacteria and the host also.

Dr. Sorensen: If I understood you correctly you said that only 40% of the flora can be cultivated. So is that 40% of all the species or of the total bacterial load? If 60% is not detected then are you looking at the right bacteria?

Dr. Rochat: This is a good question. This is 40% of the total bacteria that we can easily enumerate. But the other part is also really interesting and we have a poor knowledge today of these bacteria, we know that they play a role in colonization resistance. Here we are talking about strict anaerobe bacteria because they are very
complicated to cultivate, as the peptostreptococci or eubacterium for example, and it will be very interesting to investigate the role of these bacteria further. That is why when I showed one of the first graphs prepared by Gibson demonstrating the dichotomy between healthy bacteria and harmful bacteria, I said that probably in the future we will also have some changes because we will understand the interrelation between these types of bacterial species better.

Dr. Marini: It is about the problem of gastric pH. Ten years ago we did a study comparing gastric pH and total acid output in the stomach of near-term babies who were divided into 3 groups: one was fed mother's milk; one was fed not extensively hydrolyzed protein, and the third one was fed intact protein. We also had another group with a low chloride content in the formula. We found that the babies fed the hydrolyzed protein behave the same as those fed mother's milk, and the babies who received the formula with low chloride content have less gastric acid output [4]. One of the problems is why the babies fed mother's milk do not have a very low gastric pH during the whole feeding time. This is important to me because you can save IgA, IgM and IgG from the mother's milk for the protection of the gut.

Dr. Rochat: And this also gives the infant the chance to be in sufficient contact with exogenous bacteria to make its 'own choice' for the final colonization of its digestive tract.

Dr. Marini: Of course in mother's milk there are two things: there is a high gastric pH and also a defense system like IgA, IgM, IgG. When artificial feeding is given, the pH level is perhaps the same as that of the hydrolysate, but protection is less because there is no IgA, IgM and so on.

Dr. Guesry: In your study with baby rats bottle-fed with extensive hydrolysate or intact protein, in which part of the intestine did you measure the bacterial overgrowth, and did you also measure the nitrogen residue at this same level? I ask this because the very small peptides of the extensive hydrolysate should have been totally absorbed in the jejunum, whereas for the intact protein you probably have a nitrogen residue coming down to the colon. So I think it is very important to correlate the two.

Dr. Rochat: Unfortunately I don't have the results on the nitrogen residue but the effect was observed mainly in the upper part of the digestive tract, in the jejunum and ileum. On the cecum level there was no difference between the 3 groups.

Dr. Vandenplas: I just wanted to react to the hypothesis of the gastric acid and breast-feeding and formula-feeding. When you do gastric pH monitoring it is clear that mother's milk has a very rapid gastric emptying and 30–45 min after having fed mother's milk or hydrolysate you again have a pH of about 1–1.5 in the stomach, and when you give a casein-predominant formula it neutralizes the gastric acid for 2 h or even more. So I am not convinced that with breast-feeding you have less gastric acid in your stomach than with formula-feeding.

Dr. Marini: I am talking about the neonates. In the neonate you have very frequent feeding. Probably in your case you don't have frequent feedings, but we have intervals between feeding of about 2 h.

Dr. Vandenplas: We did that in premature newborns who were fed 8 times a day. Much more gastric acid was clearly present in those breast-fed than those formula-fed, certainly if it is a casein-predominant formula.

Dr. Marini: Did you also measure total gastric acid output?

Dr. Vandenplas: No, I said gastric pH monitoring.

Dr. Guesry: On this point, one of the very important differences between breast milk and formula for the pH of the intestinal content is the level of phosphate. Phosphate is a very important buffer which we studied a long time ago. When we give a low-phosphate formula we decrease the pH of the stool, whereas when we give a casein-predominant high-phosphate level, the pH remains neutral.
Dr. Schiffrin: We heard today that some of you have given prebiotics to preterm babies that generate a fermentation in short-chain fatty acids, and at the same time we had some concerns about butyrate and necrotizing enterocolitis or butyrate and the stimulation of inflammatory cytokines in enterocytes. Is there any concern about the administration of prebiotics in preterm babies?

Dr. Marini: We have experience with preterm babies and prebiotics, and they tolerate them very well. There was no problem. Actually indirectly we were able to show that they have a better calcium absorption from the gut.

Dr. Walker: What I was referring to is the observation that butyrate can activate or stimulate the inflammatory response in the small intestine. Therefore theoretically there is a concern because these may be increased levels of the fermented oligosaccharides in the small intestine based on bacteria, but I can only talk about this as a theoretical possibility.

Dr. Salminen: I am a little bit concerned about prebiotics in the infant right now because to my knowledge we don’t really have very specific prebiotics yet, and so we don’t know the long-term consequences in the gut microbiota development, especially thinking about species composition and bifidobacteria. So I may be wrong, but I am still a bit concerned.

References