Developmental microbial ecology of the neonatal gastrointestinal tract1,2

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ABSTRACT  The gastrointestinal tract of a normal fetus is sterile. During the birth process and rapidly thereafter, microbes from the mother and surrounding environment colonize the gastrointestinal tract of the infant until a dense, complex microbiota develops. The succession of microbes colonizing the intestinal tract is most marked in early development, during which the feeding mode shifts from breast-feeding to formula feeding to weaning to the introduction of solid food. Dynamic balances exist between the gastrointestinal microbiota, host physiology, and diet that directly influence the initial acquisition, developmental succession, and eventual stability of the gut ecosystem. In this review, the development of the intestinal microbiota is discussed in terms of initial acquisition and subsequent succession of bacteria in human infants. Intrinsic and extrinsic factors influencing succession and their health significance are discussed. The advantages of modern molecular ecology techniques that provide sensitive and specific, culture-independent evaluation of the gastrointestinal ecosystem are introduced and discussed briefly. Further advances in our understanding of developmental microbial ecology in the neonatal gastrointestinal tract are dependent on the application of these modern molecular techniques. Am J Clin Nutr 1999;69(suppl):1035S–45S.

KEY WORDS  Intestinal microbiota, acquisition, succession, breast-feeding, formula feeding, lactobacilli, bifidobacteria, obligate anaerobes

INTRODUCTION

The microbial community inhabiting the gastrointestinal tract is characterized by its high population density, wide diversity, and complexity of interactions. All major groups of microbes are present in the gut. Bacteria are predominant but a variety of protozoans are commonly found (1–3). Anaerobic fungi are widely distributed in the gastrointestinal tract of herbivores (4) as are yeasts (5) and bacteriophages (6). It has been estimated that the human colon contains > 10^{11} bacterial cells/g contents belonging to as many as 400 different species (7–9). Note that these numbers were derived from fecal samples and may not accurately represent the intestinal microbiota, especially in terms of species abundance and their relative importance. The prominent role played by anaerobic bacteria in this dynamic ecosystem is evident from the finding that > 99% of the bacteria isolated from human fecal specimens will not grow in the presence of atmospheric oxygen (9). Importantly, bacterial cells outnumber animal (host) cells by a factor of 10 and have a profound influence on immunologic, nutritional, physiologic, and protective processes in the host animal (10, 11). In fact, the gastrointestinal microbiota can be considered a metabolically adaptable and rapidly renewable organ of the body.

The gastrointestinal tract is a specialized tube divided into various well-defined anatomical regions extending from the lips to the anus. For the purposes of this and most papers on gut microbiology, discussion is restricted to the stomach, small intestine, and large intestine as well as fecal material, because it is more readily obtained. Indigenous bacteria are not distributed randomly throughout the gastrointestinal tract but instead are found at population levels and in species distributions that are characteristic of specific regions of the tract. The stomach and proximal small intestine contain relatively low numbers of microbes (10^{2}–10^{3} bacteria/g or mL content) because of low pH and rapid flow in this region. Acid-tolerant lactobacilli and streptococci predominate in the upper small intestine. The distal small intestine (ileum) maintains a more diverse microbiota and higher bacterial numbers (10^{6}/g or mL content) than the upper bowel and is considered a transition zone preceding the large intestine. The large intestine (colon) is the primary site of microbial colonization because of slow turnover and is characterized by large numbers of bacteria (10^{10}–10^{12}/g or mL content), low redox potential, and relatively high short-chain fatty acid concentrations. In addition to an increasing gradient of indigenous microbes from the stomach to the colon, there are also characteristic spatial distributions of organisms within each gut compartment. At least 4 microhabitats have been described: the intestinal lumen, the unstirred mucus layer or gel that covers the epithelium of the entire tract, the deep mucus layer found in intestinal crypts, and the surface of mucosal epithelial cells (8, 10).

The distinction between indigenous (autochthonous) and non-indigenous (allochthonous) microbes in studies of the acquisi-

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tion and development of gastrointestinal microbiota is critical to an ecological understanding of colonization, succession, and mechanisms of interaction between intestinal microbes and their host. This distinction is difficult especially in infants, in whom bacteria are acquired transiently during and immediately after the birth process as well as from the surrounding environment during this important developmental phase. In general, the terms autochthonous and indigenous are considered as synonyms and imply that these microbes are ubiquitous in the gastrointestinal ecosystem and occupy all habitats and niches available (12, 13). On the other hand, allochthonous species found in a habitat would not be established but merely in passage being derived from food or water, from another habitat in the gastrointestinal ecosystem, or from elsewhere on the host (12, 13). Clearly, some pathogens are autochthonous to the gut ecosystem and can live in harmony with their hosts, becoming pathogenic only when the ecosystem is disturbed in some way. Also, a particular microbial species may be autochthonous in one habitat in the intestinal tract but allochthonous to another, through which it normally passes after it is shed from its native habitat (12, 13). The critical distinction to be made is that an autochthonous microbe colonizes the habitat natively, whereas an allochthonous one cannot colonize the same habitat except under abnormal circumstances. Colonization describes a bacterial population in the gastrointestinal tract that is stable in size, over time, without the need for periodic reintroduction of bacteria by repeated oral doses or other means. This implies that colonizing bacteria multiply in a particular intestinal niche at a rate that equals or exceeds their rate of washout or elimination at that site (14).

MOLECULAR ECOLOGY

Current knowledge of gut microbial ecology and diversity is almost exclusively based on the use of classic culture techniques. The 2 major problems faced by gut microbiologists are the inevitable bias introduced by culture-based enumeration and characterization techniques and the lack of a phylogenetically based classification scheme (15–19). Modern molecular ecology techniques based on sequence comparisons of nucleic acids (DNA or RNA) can be used to provide molecular characterization while at the same time providing a classification scheme that predicts natural evolutionary relations (Figure 1). In principle, nucleic acid probes can be designed to hybridize with a complementary target sequence and thus provide a complete description independent of the growth conditions and media used (15, 20, 22, 23). An example of the power of molecular ecology techniques is provided by the analysis of 16S ribosomal RNA sequences (average length of 1500 nucleotides). The highly conserved regions of the ribosomal RNA molecule can serve as primer binding sites for in vitro amplification by polymerase chain reaction (24, 25). The more conserved regions are also useful, serving as targets for universal probes that react with all living organisms or for discriminating between broad phylogenetic groups such as the domains Archaea, Bacteria, and Eucarya.
The more variable sequence regions are more appropriate for genus-, species-, and sometimes even strain-specific hybridization probes (21, 26, 27). The use of molecular probe-based techniques is likely to revolutionize our approach to microbial ecology in the gastrointestinal tract, leading to not simply a refinement or increased understanding, but possibly a complete description for the first time of the complex cascade of regulatory mechanisms and factors, both internal and external, that control the gastrointestinal ecosystem (17, 28). Despite the advantages of a phylogenetically based ecological analysis and the specificity and sensitivity of molecular ecology techniques, few studies of this kind have been carried out in neonatal humans during this critical phase of initial acquisition and subsequent succession of intestinal bacteria.

Thus, our current understanding of gut microbiology and ecology in infants is based almost entirely on classic enumeration and phenotypic classification techniques, the limitations of which must be taken into account when evaluating and reviewing this critical area of research in gut microbiology. In this article, development of the microbiota in the human infant intestine is discussed in terms of initial acquisition and subsequent succession of bacteria as revealed by conventional cultivation-based techniques. Intrinsic and extrinsic factors that influence succession, and their health effects, are discussed. With a thorough understanding of these factors, combined with novel insight from a molecular ecology approach, it may become possible to manipulate succession for the benefit of the host. This aspect is also considered briefly.

**ACQUISITION OF THE GUT MICROBIOTA BY NEONATES**

**Origin of human intestinal microbiota**

The processes involved in the establishment of microbial populations are complex, involving microbial succession as well as microbial and host interactions and eventually resulting in dense, stable populations inhabiting specific regions of the gut. Fetuses are sterile in utero. However, during and after the birth process, infants are exposed to microbes that originate from the mother and the surrounding environment. It is well established that the type of birth delivery has a significant effect on the development of the intestinal microbiota. With a vaginal delivery, the longer the birth process the more likely it is to be able to isolate viable microbes from the stomach and mouth of the infant (29, 30). The same Escherichia coli serotypes were found in both the mouths of babies immediately after birth and in their mothers’ feces, implying that during natural birth microbes from mothers’ feces contaminate infants (29, 30). The gastric content of 5–10-min-old babies was similar to that of their mothers’ cervix (30). Also, immediately after birth, the nasopharynxes of 62% of babies contained bacteria that were consistent with those of their mothers’ vaginas immediately before delivery (31). Infants born by cesarean delivery can also be exposed to their mothers’ microbiota, but initial exposure is most likely to environmental isolates from equipment, air, and other infants, with the nursing staff serving as vectors for transfer (29, 32, 33).

After birth, environmental, oral, and cutaneous microbes from the mother will be mechanically transferred to the newborn by several processes including sucking, kissing, and caressing. Thus, the proximity of the birth canal and the anus, as well as parental expression of neonatal care, are effective methods of ensuring transmission of microbes from one generation to the next (34).

The few studies that have been performed on infants in developing countries show a pattern characterized by heavy exposure to and acquisition of bacteria very early in life. For example, Indian infants from Guatemala passed bacteria-containing meconium samples 4–7 h after birth. Enterobacteria and streptococci were the first groups to establish, and all infants were colonized by E. coli within 48 h (35). A pronounced dominance of bifidobacteria was observed over the entire breast-feeding period, with a corresponding reduction in facultative bacteria. A comparison between Pakistani infants from poor areas and Swedish hospital-delivered infants showed that regardless of delivery method (vaginally or abdominally), the Pakistani infants were colonized significantly earlier (36). Similarly, very early colonization with enterobacteria has been described in Nigerian infants regardless of delivery mode (37). These results confirm pronounced exposure of infants to environmental bacteria very early in developing countries.

Thus, factors that regulate the fate of ingested microbes differ between newborns via milk are more likely to colonize than are those entering healthy adults with stable climax communities. Thus, factors that regulate the fate of ingested microbes differ between newborns and adults. Because the intestinal tract is a dynamic ecosystem that is influence by host, intrinsic, and environmental factors, opportunities may arise in which colonization by ingested microbes in infants is favored (42).

**Major groups and species of bacteria**

Up to 18 different groups of microbes have been found in the lower genital tract of pregnant women, and these are the first bacteria to which the neonate is exposed (Table 1). E. coli and streptococci are the most commonly isolated organisms from the upper digestive tract immediately after birth. Detection of lactic acid bacteria has been variable. Lactobacilli are numerous throughout the intestinal tract (62). The lactobacillus species usually found in infant feces, Lactobacillus acidophilus, L. salivarius, and L. fermentum, are also present in adults (48, 60). Presence of bifidobacteria has been reported to be both high and low (42), with Bifido-
Anaerobes or facultative anaerobes exposed to diverse microbial populations. However, not all components of these populations will colonize the digestive tract. Specific microbes become established in particular hosts during different phases of development in a process referred to as microbial succession.

**MICROBIAL SUCCESSION**

Microbial succession during the first few weeks of life in the alimentary tracts of humans, chicks, pigs, and calves is remarkably similar even though neonatal animals are exposed to greater numbers of fecal and environmental bacteria than are human neonates (38, 67, 68). Within a few days of birth, coliforms and streptococci dominate the microbiota in all the above species. Obligate anaerobes appear some time later. Clostridia and lactobacilli may also be present in most hosts within a short period of time. The external and host factors controlling which of the ingested bacteria will establish and the order of succession of the colonizing strains is of major importance. However, this is a difficult topic to both study and review because of extreme subject variability, great variation in rearing and feeding practices, and limitations in sampling and analyzing gut microbial communities. Despite these limitations and variations, an attempt has been made to summarize the trends that have emerged from human infant studies.

**General trends in humans**

The development of the intestinal microbiota in infants was divided into 4 separate phases by Cooperstock and Zedd (48). Phase 1 is the initial acquisition phase over the first 1–2 wk,
phase 2 is the remaining period of solely breast-feeding, phase 3 is the time between the beginning of supplementation and the cessation of breast-feeding, and phase 4 is the period of conversion to adult biota patterns beginning after completion of weaning. A consistent finding is that all infants are initially colonized by large numbers of E. coli and streptococci, often reaching 10^8–10^9/g feces. It has been proposed that these bacteria are responsible for the creation of a reduced environment favorable for the establishment of the anaerobic genera Bacteroides, Bifidobacterium, and Clostridium by day 4–7 (49, 69). In breast-fed infants reduction in numbers of E. coli and streptococci, as well as bacteroides and clostridia, can then decrease in relation to dominance of fecal microbiota by bifidobacteria. Once dietary supplementation begins, the bacterial profile of breast-fed infants resembles that of formula-fed infants in which bifidobacteria are no longer dominant. During phases 3 and 4, after introduction of solid food and weaning, respectively, differences between breast-fed and formula-fed infants are lost and the fecal microbiota resembles that of adults by about the second year of life (48, 49). Numbers of bacteroides and anaerobic Gram-positive cocci (peptococci and peptostreptococci) gradually increase during Phases III and IV (48). In addition, many other bacterial groups including eubacteria, veillonella, staphylococci, propionibacteria, bacilli, fusobacteria, and yeasts are present in adult feces and can also be detected in infants (42).

Generally, fluctuations in fecal microbial populations are greater for infants than adults with larger day-to-day and diet-induced variations. Relatively small amounts of formula supplementation of breast-fed infants will result in shifts from a breast-fed to a formula-fed pattern (42, 49, 70). There is also considerable variation in the detection of bifidobacteria. Some infants have no detectable bifidobacteria until solid food is introduced, with more formula-fed infants than breast-fed infants lacking bifidobacteria. Frequently, infants with no detectable bifidobacteria have high numbers of bacteroides, clostridia, and E. coli (49, 50, 71–73).

**Breast-feeding compared with formula feeding**

To resolve this controversial topic Tannock (34) proposed that, if differences do exist, then it should be possible to predict whether an infant is breast-fed or formula fed by the amounts of various groups of bacteria found in feces. He found that numbers of clostridia were always lower in breast-fed babies and that clostridia were the only group predictive of formula-fed babies when comparing several individual studies. Conway (42) made a systematic attempt to quantify these findings based on extensive analysis of numerous original studies cited. Results of the various studies were grouped as follows: development during the first week, bacterial numbers in 1-wk-old infants, and bacterial numbers in 1–19-wk-old infants. Also, studies before 1960 were excluded because milk-formula composition and microbiological techniques have improved since. During the first week of life, as measured by mean values for specific groups of bacteria, the initial colonizers were enterobacteria and streptococci in both formula- and breast-fed babies, reaching their highest numbers by age 3.3 days on average (42). The bifidobacteria and bacteroides appeared 1 day later and then reached their highest numbers on days 5–6. Interestingly, bifidobacteria and bacteroides took a day longer to colonize the breast-fed infants. Conway (42) speculated that by age 3 day breast milk has passed through the tract and that breast milk, but not formula milk, contains antibacterial factors. Another possible explanation is that breast milk, but not formula or cow milk, contains specific bifidobacterial growth factors (74), although it takes longer for the neonate to reach an adequate intake of these growth factors because lactation must first be established. This accounts for the longer time for colonization in breast-fed than in formula-fed infants. When viable counts from 1-wk-old infants were compared, bifidobacteria occurred in larger numbers in feces of breast-fed than in with formula-fed infants (52, 54). This was not the case for viable counts averaged for 1–19-wk-old infants in which fecal bifidobacterial counts of formula-fed infants increased and were comparable with those in breast-fed infants. The higher counts of bifidobacteria in breast-fed infants may be present up to age 5–6 wk. Bacteroides numbers were low in 1-wk-old breast- and formula-fed infants, and increased in formula-fed infants >1 wk of age. The mean value for clostridia in 1–19-wk-old formula-fed infants was log_{10}6.6 ± 0.8 colony forming units. Counts of enterobacteria and streptococci were slightly lower in breast-fed infants in both age groups. Variations between studies and between individuals and day-to-day sampling were more marked for breast-fed infants than for those receiving formula. This was most likely due to unrecorded additives to the diet of breast-fed infants.

Consistent with the finding that numbers of bifidobacteria in 1-wk-old infants were higher breast-fed infants than in those receiving formula, bifidobacteria were more frequently isolated from breast-fed (84%) than from formula-fed infants (62%). A high correlation was found between viable counts and frequency of detection. For example, clostridia counts were higher in formula-fed infants for both age groups, as was the frequency of detection (49).

Succession of the microbiota in the intestinal tract at the group level as determined by selective enumeration is complicated by the fact that there is also a succession of bacterial species and strains. Thus, although total numbers of specific bacterial groups may not vary with time or diet, the dominant species and strains are likely to vary (42). Unfortunately, few studies examined the fecal microbiome to the level of species and strain. However, in one example, the species of bifidobacteria most frequently isolated and occurring in highest number was B. breve in both breast-fed and formula-fed infants, with B. adolescentis, B. longum, and B. bifidum occurring less frequently and in lower numbers (51, 52). Others report that B. infantis was dominant in breast-fed infants with B. longum and B. bifidum being the next most common (53, 72, 75). In one European study, B. breve was most dominant in breast-fed infants, whereas B. longum and B. adolescentis were more frequent in formula-fed infants (54). Interestingly, B. longum and B. adolescentis are dominant in adults (76); this is consistent with the concept that supplementation of infants with formula initiates the development of a microbiota more closely resembling the adult profile (42).

On the basis of the expression of surface K1 antigens (virulence marker) and serum killing, it is thought that E. coli strains differ in breast-fed and formula-fed infants (77). In addition, enterobacteria (other than E. coli) were more common in formula-fed than in breast-fed infants (77, 78). Of interest is the finding that E. coli strains are continually changing in adults providing further evidence that acquisition of the intestinal microbiota is an ongoing process that is not restricted to neonates (42). Little is known of the bacterial factors that confer an ability to colonize and persist in the intestinal microbiota. Certain E. coli O-group serotypes are more frequently encountered than others in the intestinal population in European and North American.
FACTORS AFFECTING MICROBIAL SUCCESSION

External

Microbial succession in the gastrointestinal tract is influenced by numerous external and internal host-related factors. Extrinsic factors include the microbial load of the immediate environment, food and feeding habits, and composition of the maternal microbiota. In addition, dietary and temperature stress can influence succession of microbes (87, 88).

The most studied external factor that influences establishment of the intestinal microbiota is diet. The effects of specific dietary regimens on the composition of the intestinal microbiota in adults have been reported (56, 57). Specific dietary supplements, such as oligosaccharides, that affect bacterial species as well as strain composition and fermentation in the colon are receiving considerable research attention (89–91). Oligosaccharides have also been added to infant formulas to lower fecal pH (92). Nutritional modulation of intestinal microbial ecology is discussed in a related symposium paper by Collins and Gibson (93).

Most attention has focused on comparing the effects of breastmilk feeding with formula feeding. Introduction of formula supplements and solid foods correlates strongly with the development of obligate anaerobic populations. Midveldt and Midveldt (94, 95), by using various measures of microbial activity, showed that the effects of breast-feeding on the microbiota extend for a considerable time beyond cessation of exclusive breast-feeding, even though the culturable bacterial groups appeared relatively stable. For example, profiles of short-chain fatty acids for breast-fed and formula-fed infants did not overlap until ≥12 mo of age and propionic acid levels differed over the entire 2 y of study. Acetic acid levels were comparable in the 2 groups by 12 mo of age, which may reflect the decrease in bifidobacteria as the dominant population. It is worth noting that breast milk contains antimicrobial components that influence the microbiota as well as numerous growth factors that stimulate development and maturation of the intestinal mucosa, both of which promote stability and decrease susceptibility to intestinal disturbances (96, 97).

Internal

Intrinsic or host-related factors that influence succession include contributions from host physiology, endogenous nutrients, and the microbiota. Numerous factors influence the stability of the microbiota and shifts, or successional changes, in populations. These include intestinal pH and \( E_h \), microbial interactions, environmental temperature, physiologic factors, peristalsis, bile acids, host secretions, immune responses, drug therapy, and bacterial mucosal receptors (42). Two examples are presented that show the influence of these factors on microbial succession. Adhesion of a lactobacillus strain and E. coli K88 fimbriated cells to gastric and intestinal mucosa of piglets aged 5, 26, and 47 d were measured (98). The adhesion pattern of the lactobacillus strain was the reverse of that for E. coli with the former reaching a minimum immediately before weaning at 30 d and the latter reaching a maximum at this time. Receptors on the epithelial mucosa that are involved in bacterial colonization differ with age. Thus, successional changes in lactobacillus and E. coli populations in piglets during the weaning period can be related to receptors on the epithelial mucosa that are involved in bacterial colonization.

Antibiotic therapy will also have a major effect on succession of the microbiota (58, 99, 100). Three important points concerning microbial succession can be made in relation to antibiotic therapy. First, antimicrobial agents can have specific effects on individual components of the microbiota rather than a general nonspecific suppression of all microbes. Second, that the resultant microbial profile influences the populations that emerge after treatment has stopped. Third, that effects of antibiotic therapy can persist. Because antibiotics have specific modes of action and organisms exhibit differing sensitivities (as reflected by variations in minimal inhibitory concentrations), it is not surprising that particular antibiotics influence individual groups. A comprehensive review of changes in human intestinal microbiota in relation to administration of antimicrobial agents is available (101).

MICROBIOTA-ASSOCIATED CHARACTERISTICS IN HUMANS

It is always difficult and not always possible to determine whether a particular microbe is truly indigenous to a particular gut compartment or site in a host species. This limitation reflects the fact that criteria for determining whether an organism is indigenous are largely incomplete because of severe constraints in sampling, culturing, enumerating, and identifying intestinal
microbial species (11, 17). Rather than enumerating individual bacterial groups, there has been increasing interest in using microbiota-associated characteristics (MACs) that represent bacterial metabolites or compounds altered in concentration or composition because of bacterial activity (102). Several of the indicators measured routinely because of ease of sampling and analysis are listed in Table 2, together with the bacterial groups active in these biochemical transformations.

The establishment and development of MACs in feces of healthy infants over the first 2 yr of life was intensively studied by Midtvedt et al (93, 115–119) and has been summarized by Conway (42). Of interest is the finding that at age 2 yr some indicators remained undeveloped in relation to the adult, namely conversion of cholesterol to coprostanol, degradation of fecal tryptic activity, conversion of bilirubin to urobilinogen, and concentration of valeric acid. This reinforces the concept that whereas numbers of bacterial groups remain relatively unchanged in the developing infant after introduction of solid food, the dominant bacterial species and strains may shift, resulting in changes in metabolic activity. Other indicators changed continuously during the 2-y period. Acetic, propionic, n-butyric, i-butyric, and i-valeric acid reached adult levels at 1, 6, 9, 18, and 18 mo respectively. i-Butyrate and i-valerate were first detected after 1 mo of age whereas n-valerate was rarely found before 6 mo of age. Acetic, propionic and n-butyric acid accounted for 94% of the total short-chain fatty acids. Fecal tryptic activity increased to 80% of adult levels by 9 mo of age after which time it decreased to 30% of adult levels. Thus, development of a trypsin-inactivating microbiota fluctuates and is prolonged. These results are difficult to interpret because the net amount of fecal tryptic activity is the balance between pancreatic secretion, which increases in response to dietary stimulation, and degradation by microbial activity. Fecal urobilinogen concentration slowly increased to a maximum of 30% of adult concentrations by age 2 yr. The only bacterial species, isolated thus far, capable of carrying out this conversion is C. ramosum. Whereas conversion of cholesterol to coprostanol was first detected after 6 mo of age, it reached a maximum of 44% of adult levels at 15 mo of age. This is consistent with earlier findings that microbes capable of reducing cholesterol to coprostanol do not colonize the gut in significant numbers until after age 1 yr. To date, all isolates capable of this conversion have been Eubacterium lentum. In general, eubacteria are isolated frequently from adults and less often from infants.

Mucin degradation was detectable at 1 mo of age and progressively increased over the first 9 mo reaching adult levels by the end of the first year (119). Glycoproteins present in breast milk are similar to intestinal mucin glycoproteins and may result in substrate competition during the breast-feeding period (120). Several species can degrade mucin including peptostreptococci, B. bifidum, B. infantis, Ruminococcus AB, and R. torques (109, 110). These bacterial species are generally associated with the microbiota from older infants and adults with the exception of Bifidobacterium spp. The role of gastrointestinal carbon and energy sources for resident bacteria and their contribution to the stability and structure of the bacterial community remain largely unexplored and warrant closer research focus and effort.

### MANIPULATING THE DEVELOPING MICROBIOTA

It is widely reported that the intestinal microbiota plays an important role in the health and well-being of the host, although a thorough scientific description and evaluation of this interaction is still lacking. Because microbial composition is influenced by the acquisition and succession of microbes and the factors controlling these processes, the concept of manipulating the development and composition of the intestinal microbiota to improve the health of the host has been promoted. This can be achieved by controlling external factors that have a direct effect on the composition of microbiota characteristics such as diet and medication. The addition of oligosaccharides to infant formulas, which purportedly promotes growth of bifidobacteria in the colon and results in lowered fecal pH (92), is an example.

Another approach that has received much attention recently is the concept of orally administering viable preparations of desirable microbes, referred to as probiotics or direct-fed microbes (121). In theory, this concept should be effective when the subject is at risk of colonization by undesirable microbes or when transfer from maternal sources cannot occur (generational discontinuity). For humans, these situations include cesarean-delivered infants, prematurely-born infants, and full-term infants requiring intensive care all of whom are at risk of acquiring hospital-derived microbes and whom may carry antibiotic resistance. Oral ingestion of probiotics may be beneficial to the host and influence succession at different stages because microbial succession is an ongoing process. Opportunities for administration of probiotics are created during times of stress or antibiotic treatment when administration of a desirable organism may prevent colonization by a transient or pathogenic organism. In addition, probiotics may assist or promote reestablishment of the indigenous microbiota with health benefits for the host. However, a serious consideration related to the use of probiotics is whether they are opportunistic pathogens, especially in immunodeficient hosts. This question has particular relevance to new-

### TABLE 2

Selected fecal microbiota-associated characteristics used as indicators of colonic microbial activity

<table>
<thead>
<tr>
<th>Indicator and reference</th>
<th>Bacteria</th>
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<tbody>
<tr>
<td>Conversion of cholesterol to coprostanol (103, 104)</td>
<td>Eubacterium</td>
</tr>
<tr>
<td>Dehydroxylation of bile acids (105, 106)</td>
<td>Eubacterium and Clostridium</td>
</tr>
<tr>
<td>Transformation of conjugated bilirubin (107)</td>
<td>Clostridium</td>
</tr>
<tr>
<td>Inactivation of tryptic activity (108)</td>
<td>Bacteroides</td>
</tr>
<tr>
<td>Mucin breakdown (109, 110)</td>
<td>Ruminococcus, Peptostreptococcus, and Bifidobacterium</td>
</tr>
<tr>
<td>Dipeptidase activity (111)</td>
<td>Bacteroides</td>
</tr>
<tr>
<td>Presence of short-chain fatty acids (112)</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td>Presence of intestinally produced methane in expired air (113, 114)</td>
<td>Methanobrevibacter</td>
</tr>
</tbody>
</table>

1 Adapted from Midtvedt (102) and Conway (42).
born infants. A recent study that assessed the capacity of probiotic bacterial species to colonize, infect, stimulate immune responses in, and affect the growth and survival of congenitally immunodeficient gnotobiotic mouse strains emphasizes the need for additional research (122). The implication from this work is that although probiotic species may be innocuous for adults, caution and further studies to assess the safety of probiotic bacteria for immunodeficient hosts, especially neonates are required.

Lactic acid bacteria are the most frequently used probiotic organisms, although their beneficial effects have not been conclusively proven (123, 124). However, several studies have shown beneficial effects from probiotics containing lactic acid bacteria in humans (126–128), pigs (129), chicks (67) and preruminant calves and lambs (130). In addition to lactic acid bacteria, several other microbes have been studied as probiotic organisms to reduce acquisition of undesirable pathogens from the hospital environment by high-risk newborn infants (63). This approach utilizes the competitive exclusion principle where the harmless probiotic organism purportedly occupies the same site as the potential pathogen. An example is the implantation of a streptococcus strain in the pharynx to reduce the incidence of colonization of this site by Staphylococcus aureus (131). Animal experiments have shown successfully that oral administration of E. coli strains that have no K capsular antigens and are nonpathogenic lowered incidence of colonization of newborns by antibiotic-resistant E. coli strains. The strains most likely to colonize are the ones introduced soon after birth, i.e., within the first 2 h (132). Further research showed that babies dosed orally with nonpathogenic E. coli had fewer antibiotic-resistant E. coli strains than did a control group in an open study (133). These results indicate that a control mechanism such as competitive exclusion or colonization resistance operates in the neonatal gut with established strains outcompeting introduced strains.

Whereas pure bacterial cultures or simple defined mixtures have generally been used, success has also been achieved with complex mixtures of organisms. Complex mixtures of anaerobic bacteria have been introduced into humans in an effort to reestablish desirable gut microbiota in patients colonized by the nosocomial pathogen C. difficile as a result of antibiotic therapy (134). Development of accurate identification procedures to precisely monitor the population of specific probiotic strains during passage through the gastrointestinal tract is essential for a realistic evaluation of attempts to manipulate the development and composition of the intestinal microbiota. Recent research on lactobacilli and bifidobacteria in healthy human adults using molecular fingerprinting techniques highlights individual differences in native populations (135). Variation among individuals in the composition of the intestinal microbiota is certain to effect the ability of an introduced strain to colonize or otherwise affect host benefit.

SUMMARY

The gastrointestinal tract is essentially sterile at birth and is subsequently colonized by microbes acquired from the mother and the surrounding environment. The successional pattern is similar for both humans and animals in that the gut is initially colonized by lactic acid bacteria, enterobacteria, and streptococci. After the introduction of solid foods, obligate anaerobes increase in numbers and diversity until a pattern similar to that in adult humans is achieved by 2 y of age. Importantly, succession of species and strains, which is apparently ongoing throughout life, has not been well described. This is confirmed by analysis of MACs that has been valuable in identifying differences in the development of breast-fed and formula-fed infants, especially after supplementation; routine microbiological techniques lack the sensitivity required to monitor these population shifts. This emphasizes the importance of modern molecular ecology techniques for studying acquisition and succession of the microbiota in the neonatal intestine. In providing specific, sensitive, and culture-independent evaluation of all members of the gastrointestinal ecosystem, new molecular technologies will change fundamentally our understanding of microbial ecology in the gut and allow a complete description of this key physiologic system for the first time.

We acknowledge the detailed suggestions and comments of an anonymous reviewer that resulted in substantial improvement to the manuscript.

REFERENCES


82. Wold AE, Caugent DA, Lidin-Janson G, de Man P, Svanborg C.


85. Gustafsson BE, Midtvedt T, Norman A. Isolated fecal organisms capa-