Establishment of a Normal Intestinal Microflora in the Newborn Infant

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The intestinal microflora of an adult person is a complex ecosystem, estimated to harbor about 400 different bacterial species. Anaerobic bacteria outnumber aerobic and facultative bacteria by a factor of 100 to 1000. The population level of each species is thought to be strictly regulated by the competition for nutrients and space. Thus, potential pathogens such as Escherichia coli and other enterobacteria are kept at moderate numbers. Since the normal microflora makes it difficult for newly arrived bacteria to become established, it also provides a first line of defense against pathogenic bacteria, a function described as colonization resistance (1).

Although colonization of the skin and mucosal surfaces of the infant begins directly after birth, the establishment of the microflora ecosystem is a slow and gradual process that takes several years. The development of the ecosystem can be followed by assessing biochemical reactions performed by certain species of indigenous intestinal bacteria, for example, the production of short-chain fatty acids, the conversion of cholesterol to coprostanol, and the degradation of mucin. Yet at 2 years of age, some of these functions are not fully established in all children, which shows that several bacterial species do not populate the gut until later (2). The intestinal microflora in early life has certain features that may make the infant vulnerable to infections. At the same time, the early intestinal microflora might be of importance for the maturation of the gut immune system, including the development of oral tolerance to harmless antigens such as food proteins. In this chapter, I review neonatal intestinal colonization and its relation to infant morbidity.

SOURCES OF BACTERIA COLONIZING THE NEONATE

The bacteria that become established in the neonatal intestine include aerobic bacteria, facultative anaerobic bacteria (i.e., bacteria capable of growing in the presence or absence of oxygen) such as E. coli and other enterobacteria, and a wide range of different anaerobic bacterial species. These bacteria may be acquired from various sources. Most studies identifying sources of bacteria colonizing newborn infants have focused on E. coli and other enterobacteria.
Maternal Microflora

After the rupture of the fetal membranes and during the passage through the birth canal, the infant is exposed to the mother’s vaginal flora. However, although vaginal bacteria are ingested during delivery, they do not normally seem to settle in the infant’s intestinal tract. Thus, in a small study involving five mother/infant pairs, lactobacilli present in maternal vaginal flora did not colonize the intestines of any of the babies (3).

Conversely, the maternal intestinal flora is a recognized source of bacteria colonizing the newborn’s intestine. Bettelheim and Lenox-King (4) found that two-thirds of the neonates in a maternity ward acquired at least one E. coli strain originating in the mother’s fecal flora, but most other studies report lower vertical transmission rates, with about one-third of infants acquiring maternal E. coli strains (5). The low transmission rate has been attributed to extensive hygienic measures applied during delivery. However, we have recently observed a vertical transmission rate of E. coli of less than 50% among Pakistani infants delivered without the use of aseptic techniques (6). Possibly even the limited hygienic measures practiced during home deliveries in Pakistan (washing of the mother’s perivaginal and perianal area with warm water and avoiding direct contact between the baby and maternal fecal material) are sufficient to substantially reduce contact with the maternal fecal flora during delivery. It is less likely that E. coli strains acquired from the environment counteracted the establishment of maternal strains, since strains acquired from the mother had a much greater tendency to become firmly established in the infant’s microflora than other E. coli strains (6).

Transfer from mother to infant of enterobacteria other than E. coli (e.g. Klebsiella, Enterobacter, and Citrobacter species) is infrequent (7), probably because of their low population levels in the intestinal microflora of adults.

It seems reasonable to assume that anaerobic bacteria are also transferred from mother to infant during a normal vaginal delivery. This is supported by the fact that colonization with anaerobes, especially Bacteroides, is delayed in infants delivered by cesarean section who do not come into contact with the maternal fecal flora at delivery (8–11). However, the transfer of specified strains of anaerobic bacteria from mother to infant has received little study. Using plasmid profiles to identify bifidobacteria, Tannock et al. (3) isolated strains of supposed maternal-fecal origin in the intestinal flora of two of the five infants studied. The species or biotypes of bifidobacteria most common in infants, that is, Bifidobacterium bifidum type B, B. infantis subsp. infantis, and B. longum subsp. longum type B, rarely occur in adult individuals (12), indicating that sources other than the maternal microflora are important. The proportional distribution of different Bacteroides species in neonates, however, is similar to that found in adults, with Bacteroides vulgatus, B. thetaiotaomicron, B. fragilis, and B. distasonis being the species most commonly isolated (13).

Environment

In maternity wards and nurseries, E. coli strains are spread between neonates by nurses’ hands (4). Such horizontal transfer is greatly reduced when the mothers themselves are the predominant caretakers.
Klebsiella, Enterobacter, and other non-\textit{E. coli} enterobacteria are also frequently shared among neonates, and these bacteria survive better than \textit{E. coli} outside the human host in the hospital milieu (7). Feeds given to Pakistani newborn infants, for example, were commonly contaminated with Klebsiella and Enterobacter species, but never with \textit{E. coli} (6).

Anaerobic bacteria may also be acquired from the environment, especially aerotolerant ones such as bifidobacteria and lactobacilli, which may survive for a considerable time outside the human host, and bacteria such as clostridia, which have spores that resist most sterilizing procedures. Thus, colonization with bifidobacteria has been shown to differ among different maternity wards both in the rate of colonization and the species distribution, suggesting spread in the ward (12,14). In contrast, strict anaerobes such as \textit{B. fragilis} require very close contact for transfer (8) and are spread between neonates only with difficulty.

ESTABLISHMENT OF DIFFERENT BACTERIAL GROUPS IN THE INTESTINE

Facultative and Aerobic Bacteria

Although the newborn infant is commonly exposed to a wide range of different bacteria, not all are able to establish themselves in the neonatal intestine. The implantation of bacterial strains into the intestinal microflora is regulated through the limitations of the intestinal milieu, which change with the successive establishment of different bacteria. Thus, bacteria capable of oxidative metabolism, such as enterobacteria, streptococci, and staphylococci are the first to proliferate in the intestinal tract (10,15,16). These bacteria thrive well in the intestinal milieu at this time, which shows a positive oxidation reduction potential. Strictly anaerobic bacteria, in contrast, require a negative oxidative reduction potential (16) and cannot compete favorably during the initial phase of intestinal colonization. As a consequence, facultative and aerobic bacteria reach higher population numbers in the intestine of the newborn infant (10^10 to 10^{11} bacteria per gram of feces) than in the adult individual (10^6 to 10^9 bacteria per gram) (16,17).

Enterobacteria are commonly isolated from the intestinal tract of neonates from the first days of life. \textit{E. coli} is the dominant enterobacterial species in healthy neonates (15,18), but other enterobacteria, for example, Klebsiella, Enterobacter, and \textit{Citrobacter} species, which are less common in the microflora of adults, are present in high population numbers (10^7 to 10^{10} bacteria per gram of feces) in 20% to 60% of newborn infants during the first weeks of life (9,10,14). Pathogenic enterobacteria, including salmonellae, transiently colonize up to 20% of neonates in developing countries (19). In the long run, however, \textit{E. coli} is probably better adapted to the human intestine than other enterobacteria. Klebsiellae and other non-\textit{E. coli} enterobacteria are successively replaced in the intestinal microflora by \textit{E. coli} after a few weeks (6) or months (7,18).

Enterococci (\textit{Streptococcus \{Enterococcus\} faecalis} or \textit{Streptococcus \{Enterococcus\} faecium}) are isolated from most neonates, reaching population levels of 10^{10}
bacteria per gram of feces (16,20). Nonhemolytic streptococci are found equally early but less frequently (20).

Staphylococci, that is, Staphylococcus epidermidis and in some infants Staphylococcus aureus, are also isolated from the first days of life (10,20) at levels of $10^5$ to $10^{10}$ bacteria per gram of feces (10).

**Anaerobic Bacteria**

When the facultative bacteria expand, they consume oxygen and lower the redox potential to negative values. This enables the anaerobic bacteria to proliferate and reach much higher levels than possible during the first week (16,17). Anaerobic bacteria commonly found in the newborn infant include bifidobacteria, clostridia, and Bacteroides (10,16,21–24). Clostridia have been shown to reach higher population levels in neonates ($10^9$ per gram of feces) than in adults ($10^{7.5}$ per gram) (25), and Clostridium difficile, for example, is commonly isolated from healthy neonates and infants, while being present in the intestinal microflora of less than 5% of adult individuals.

Many other anaerobic bacteria that usually form stable populations in the intestine of adults, such as peptostreptococci, peptococci, eubacteria, and lactobacilli, are less commonly isolated during the first months of life, with great differences in isolation rate and numbers seen among different studies (11,14–16,21,25). When present, veillonellae may reach higher population levels in the intestine of the newborn than later in life (25).

**Establishment of a Dominance of Anaerobic Bacteria in the Neonatal Intestine**

When the anaerobic bacteria expand, the facultative bacteria usually decline in numbers (15,16). However, relatively high numbers of both facultative and anaerobic bacteria may be present concomitantly during the first months (16) or years of life (25) before a clear anaerobic predominance is established. As mentioned, the anaerobic bacteria outnumber the facultative bacteria by 1000:1 or 100:1 in the adult. Mice that are mono-associated with *E. coli* can be used as a model of the early neonatal intestinal flora. When *E. coli* is present as the only colonizing species, levels of $10^{10}$ to $10^{11}$ bacteria per gram of feces are obtained. To bring down the *E. coli* population to the levels found in conventional animals, 95 different anaerobic strains isolated from conventional mice were required (26). The mechanisms behind the suppression of facultative bacteria are not fully understood. Competition for nutrients appears to be the most important factor regulating the population sizes of different intestinal bacteria, and a decreased oxygen availability restricts the range of substrates that can support growth of *E. coli*, for example (26). Anaerobic bacterial groups may also have inhibitory effects on facultative bacteria by the elaboration of metabolites, such as hydrogen sulfide and fatty acids. Klebsiellae may be more sensitive to the
suppressive effect of certain anaerobic bacteria than \textit{E. coli} (9), as suggested by the fact that klebsiellae are replaced by \textit{E. coli} with increasing age of the infant.

**INTESTINAL MICROFLORA AND NEONATAL INFECTIONS**

The risk of developing certain infections is much higher during the neonatal period than later in life. This is true, for example, of urinary tract infection, septicemia, and diarrhea—three major health hazards for infants and children globally. This increased risk is in part related to the special features of the intestinal microflora in early life.

**Urinary Tract Infection**

Urinary tract infection is caused by bacteria originating in the intestinal flora that colonize the periurethral area and ascend into the urinary tract. Urinary tract infections may be confined to the bladder (cystitis) or ascend to the ureters and kidneys (pyelonephritis). In the latter case, bacteria may spread into the blood stream and cause urosepsis.

\textit{E. coli} accounts for a majority of urinary tract infections in neonates and infants. Certain \textit{E. coli} strains are more likely than others to cause disease due to their expression of various "virulence factors"—bacterial factors that contribute to the infectious process (27). \textit{E. coli} strains causing pyelonephritis usually express P fimbriae, which mediate adherence to the urinary tract epithelium (28) but also promote large intestinal colonization, especially in the neonate (6,29). Such uropathogenic strains are easily spread in neonatal wards, which may result in epidemic outbreaks of acute pyelonephritis (30).

**Septicemia**

Septicemia without prior focal infection is a much more common feature in neonates than in older children or adults. The incidence of neonatal septicemia in Western countries is 1 to 4 per 1000 live births (9), whereas in Pakistan the incidence is probably around 2% and 1% of the newborn infants die from septicemia (31). \textit{E. coli}, klebsiellae, and other enterobacteria, as well as pseudomonads and enterococci, are causative agents in up to 80% of these infections in developing countries. In Pakistan, \textit{Klebsiella} alone accounts for 30% of all cases (32). In Western countries, Gram-negative bacteria, including enterobacteria, account for only 30% to 40% of the cases, while the majority are caused by \textit{S. aureus}, \textit{S. epidermidis}, and group B streptococci (9). Staphylococci are mostly regarded as members of the skin flora, and group B streptococci as colonizers of the respiratory tract of the neonate, but \textit{S. epidermidis} (33), as well as \textit{S. aureus}, is also a common inhabitant of the intestinal microflora of newborn infants.

Neonatal septicemia caused by intestinal bacteria is likely to result from direct translocation of the bacteria over the intestinal barrier. Bacteria that escape phagocy-
Establishment of a normal intestinal microflora

Translocation and destruction by complement can reach the bloodstream (34). Enterobacteria, staphylococci, and enterococci are able to translocate, that is, pass viably over the intestinal epithelium to the mesenteric lymph nodes, blood, and other organs, while most anaerobic bacteria do not have this capacity (35). Translocation is promoted by high population levels in the gut; it is likely to occur when a bacterial species with the inherent capacity to translocate reaches concentrations of $10^8$ bacteria per gram of feces (35). Although translocation has not been formally documented in infants, it is likely to occur more often during the neonatal period because many aerobic and facultative bacteria reach high levels in the intestine during this period. Treatment with certain antibiotics may reduce the number of anaerobes and allow enterobacteria to attain unusually high numbers (9,35).

Diarrhea

Diarrhea is the major cause of infant mortality worldwide. The intestinal microflora plays a key role in protection against diarrheal disease by providing colonization resistance against the incursion of pathogens or by regulating the population levels of potentially pathogenic bacteria in the intestine (1). Lack of a protective microflora is regarded as an important factor in increased susceptibility to diarrhea among neonates and infants. The frequent and uncontrolled use of antibiotics in the treatment of infantile diarrhea in developing countries worsens the situation by altering the normal intestinal microflora, thus rendering the infant more susceptible to new episodes of diarrheal disease. Furthermore, diarrheal disease results in disturbances of the intestinal microflora, including, for example, a decrease in the numbers of anaerobic bacteria (36).

Necrotizing Enterocolitis

Necrotizing enterocolitis is a life-threatening intestinal disease primarily affecting premature neonates. The clinical manifestations include abdominal distension, rectal bleeding, and ultimately intestinal perforation. The pathogenesis is unknown, but the condition is thought to emerge as a result of gut immaturity, ischemic injury, and the action of intestinal bacteria. The common clustering of cases has suggested an infective etiology, but no direct evidence has been obtained. The bacteria isolated from the intestines of affected infants are normal members of the neonatal gut flora, most commonly klebsiellae, E. coli, and clostridia (37).

Differences in Colonization Pattern Among Groups of Infants

Delivery Mode

As mentioned, a normal vaginal delivery commonly permits transfer of bacteria from the mother to the infant. During cesarean deliveries, this transfer is completely
absent. Thus, infants delivered by cesarean section are colonized with anaerobic bacteria, especially *Bacteroides*, later than vaginally delivered infants (9). *Clostridium perfringens* is the anaerobic bacterium most frequently isolated after cesarean deliveries (8). Colonization with enterobacteria may be somewhat delayed, but these bacteria are easily acquired from environmental sources. When colonized, cesarean-delivered infants less frequently harbor *E. coli*, and more often klebsiellae and enterobacteria, than vaginally delivered neonates (9), probably because of the sparse colonization with anaerobic bacteria.

**Industrialized versus Developing Countries**

The degree of bacterial exposure during the neonatal period is likely to influence the intestinal colonization pattern. Infants born in developing countries are often heavily exposed to bacteria from birth onward, and the few studies performed show that these infants acquire bacteria very early. In Indian infants from Guatemala, a majority of the meconium samples passed at 4 to 7 hours after birth contained bacteria (15). Enterobacteria and streptococci were the first bacterial groups encountered. These bacteria reached high numbers during the first days of life, but already by the end of the first week a pronounced bifidobacterial dominance was established, and the facultatives were suppressed. A similar bifidobacterial predominance has been observed, for example, in Nigerian breast-fed infants (21).

Whether delivered in the hospital or at home, and regardless of delivery mode, Pakistani infants from poor areas were colonized significantly earlier than Swedish hospital-delivered infants (6,38). Within a few days after birth, many Pakistani infants harbored several different enterobacterial species, an uncommon finding in Swedish infants (38). Very early colonization with enterobacteria regardless of delivery mode has also been described in Nigerian infants (39), indicating pronounced exposure to environmental bacteria from the day of birth.

Among the Pakistani infants, the isolation rate of enterobacteria other than *E. coli* was high during the first week, but from the second week of life, *E. coli* dominated (6). The replacement, for example, of klebsiellae and enterobacteria by *E. coli* thus occurs earlier than among Swedish infants (18), possibly reflecting an earlier establishment of anaerobic bacteria. However, a stable *E. coli* flora had not been acquired at 6 months of age in most Pakistani infants, and a large number of different *E. coli* strains succeeded each other in their intestinal flora (6). This contrasts sharply with the stable pattern observed among infants in industrialized societies. When colonized, these infants acquire an *E. coli* strain that persists for prolonged periods; few additional strains are isolated from such infants (5,40).

In industrialized societies, the obstetrical practices and the hygienic routines aimed at reducing the spread of pathogenic bacteria in maternity and neonatal wards have instead probably synergized to result in a delayed or absent colonization by certain groups of intestinal bacteria. For example, we observed that around one-fourth of a group of Swedish neonates had not been colonized by enterobacteria after 1 week in the hospital (38). Low colonization rates with enterobacteria have also been ob-
served in other Swedish studies (14,18), and el Mohandes et al. (33) reported that only 65% of preterm infants in the United States harbored any aerobic bacteria in their stools while still in the hospital at 2 weeks of age. Similarly, low colonization rates with, for example, bifidobacteria have been reported in other studies (12,14).

The health consequences of the Pakistani and Swedish respective colonization patterns are unknown. One could speculate that the exposure to a large number of enterobacterial strains in the Pakistani infants would increase the risk of encountering pathogenic ones. On the other hand, the limited exposure to bacteria indigenous to the intestinal tract seen in Sweden and other highly industrialized societies may result in reduced stimulation of the gut immune system (41).

Antibiotics and Neonatal Intensive Care

The ill or preterm newborn cared for in neonatal intensive care units (NICUs) acquire an intestinal flora that differs from that of healthy newborn infants. *Klebsiella* or *Enterobacter* species dominate in these infants more often than *E. coli* (7,42). This may be partly related to the fact that many of these infants are delivered by cesarean section (9).

The intestinal microflora obtained in NICUs is also much influenced by the frequent use of antibiotics in neonatal intensive care. Antibiotics profoundly influence the colonization pattern of neonates (9). The effects observed include a pronounced suppression of anaerobic bacteria, commonly leaving clostridia as the only anaerobes present at detectable levels (9). Further observations include decreased levels of *E. coli*, increased levels of other enterobacteria such as *Klebsiella*, *Enterobacter*, and *Citrobacter*, as well as pseudomonads (9,42), and an emergence of antibiotic-resistant strains (43). The local antibiotic policy of the neonatal ward influences the enterobacterial colonization pattern not only of treated but also of untreated neonates (43).

A high percentage of neonates in NICUs are premature and of very low birth weight. It is uncertain if prematurity per se influences the intestinal colonization pattern. Probably, this is of minor importance when one takes into account other factors such as antibiotic treatment, mode of delivery, and feeding pattern (9).

INTESTINAL MICROFLORA IN BREAST-FED VERSUS BOTTLE-FED INFANTS

Early Studies

Since the early work of Tissier (44), who identified bifidobacteria as the predominant microorganisms in the feces of breast-fed infants, these bacteria have been regarded as especially prominent members of the intestinal flora acquired during breastfeeding; the low buffering capacity of human milk would selectively favor the proliferation of the acid-tolerant bifidobacteria. They have also been thought to play an important role in resistance to infection in breast-fed infants by inhibiting
the growth of enterobacteria and Gram-negative anaerobes. However, many more recent studies report similar counts of bifidobacteria in breast-fed and bottle-fed infants (12,16), sometimes with very low counts of bifidobacteria in both groups (14,21). Moreover, few studies have convincingly demonstrated that high bifidobacterial counts result in low counts of enterobacteria in the intestines of breast-fed infants (22); the fecal pH seems to be lower among breast-fed infants, irrespective of the bifidobacterial counts (10,21,45).

Differences Between Breast-fed and Bottle-fed Infants in the Major Bacterial Groups Colonizing the Intestine

Despite the questionable relevance of bifidobacteria as a special marker of the breast-fed infant's microflora, there are several other differences in the intestinal microflora between breast-fed and bottle-fed infants that are better documented. A summary of the results from 23 different studies published between 1973 and 1995 is given in Table 1.

The most consistently observed difference is that breast-fed infants have lower counts of clostridia and enterococci (10,16) and higher counts of staphylococci (10,14) than bottle-fed infants. There is also a tendency toward lower counts of enterobacteria in breast-fed infants (10,22).

Although most relatively recent studies have detected no significant differences in bifidobacterial counts or colonization rates between breast-fed and formula-fed infants, more breast-fed than bottle-fed infants may harbor a flora dominated by bifidobacteria, an effect of marginally increased bifidobacterial counts in combination with lower proportional levels of other bacterial groups (10,24). Bacteroides do

<table>
<thead>
<tr>
<th>TABLE 1.</th>
<th>Reported differences between breast-fed and bottle-fed infants concerning bacterial counts or colonization rates of different bacterial groups in the intestinal microflora</th>
<th>Number of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td>More in breast-fed</td>
<td>No significant differences</td>
</tr>
<tr>
<td><strong>Anaerobic bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Clostridia</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Veillonella</td>
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<td>2</td>
</tr>
<tr>
<td>Eubacteria</td>
<td>—</td>
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<tr>
<td><strong>Facultative/aerobic bacteria</strong></td>
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<td>Enterococci</td>
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<td>3</td>
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<tr>
<td>Staphylococci</td>
<td>5</td>
<td>5</td>
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*The table summarizes the results from 23 studies published between 1973 and 1995, including references numbers 10, 12, 14, 16, 21, 22, 24, and 45. A complete list of references may be obtained from the author.*
not seem to be much influenced by breastfeeding, and even less so do the lactobacilli (11,22).

**Influence of Breastfeeding on Intestinal Bacteria at the Species or Strain Level**

An influence of breastfeeding on the intestinal microflora can also be observed at the species or strain level within certain bacterial groups. Breast-fed infants are less often colonized with enterobacteria other than *E. coli*, for example with *Klebsiella* or *Enterobacter* organisms (10,38,46), and harbor fewer enterobacterial species per individual than bottle-fed infants (18) or fewer *E. coli* serotypes concomitantly (46,47). The *E. coli* flora also seems to be more stable over time in breast-fed infants than in bottle-fed infants (47).

Some studies indicate selective favoring of enterobacterial strains of low virulence in the intestines of breast-fed infants. *E. coli* isolated from breast-fed infants are thus more sensitive to the bactericidal effect of human serum (48) and less frequently carry K1, a virulence-associated capsular antigen, than *E. coli* from bottle-fed infants (46). Furthermore, recent studies have shown that breastfeeding promotes the expression of the mannose-binding type 1 fimbrial adhesin by intestinal *E. coli* strains (49), probably because of an interaction between type 1 fimbriae and mannose-containing carbohydrate chains on secretory immunoglobulin A (IgA) in human milk (50). In contrast, *E. coli* that express mannose-resistant adhesins are isolated less often from breast-fed than from bottle-fed infants (18,49), possibly as a result of the selection for type 1 fimbriated strains. Type 1 fimbriae of *E. coli* have not been linked to pathogenicity, whereas mannose-resistant adhesins include, for example, P fimbriae and other adhesins regarded as virulence factors (27).

**Protection by Breastfeeding against Infectious Diseases**

There is convincing evidence that breastfeeding protects against diarrheal disease, urinary tract infection, septicemia, and necrotizing enterocolitis in both developed and developing countries. Furthermore, breastfeeding reduces infant mortality in developing countries (reviewed by Wold and Hanson [50]). The protection against infection afforded by breastfeeding is probably in part due to a modulation of the intestinal microflora. Thus, a reduction in enterobacterial numbers and a selection for less virulent enterobacterial strains are likely to protect against urinary tract infection and septicemia (50). In an animal model, breast milk reduced enterobacterial counts in the small intestine, resulting in a decreased translocation rate (51). In addition, breastfeeding seems to be able to modulate the behavior of intestinal bacteria. Fully breast-fed infants may thus be colonized with diarrheal pathogens and still remain perfectly healthy (15).

Factors in the milk of suggested importance for protective effects include a low casein and phosphate content, lactoferrin (1.5 g/l), lysozyme (0.3 g/l), secretory IgA (0.3 to 1 g/l), and complex oligosaccharides (4 to 6 g/l) (50). Immune factors in
human milk may have limited effects on bacterial growth in the intestine but still play a key role in protection against infection. For example, in a neonatal rabbit model, human secretory IgA was found to decrease the translocation of *E. coli* over the intestinal epithelium, although not altering its population levels in the gut (52).

**PROBIOTICS AND BACTERIOTHERAPY IN NEONATES AND INFANTS**

The administration of probiotics, that is, "live microbial feed supplements that beneficially affect the host by improving its microbial balance" (53), may prove useful in neonates and infants, for example, after antibiotic treatment, infections, or other conditions resulting in an abnormal intestinal microflora. It is an attractive alternative to the uncontrolled and inappropriate use of antibiotics in the treatment of infantile diarrhea.

At present, most probiotics contain lactic acid-producing bacteria: lactobacilli, bifidobacteria, and streptococci. Despite the variable rates of colonization with lactobacilli in neonates, it seems possible to achieve persistent colonization with lactobacilli in the newborn after oral administration. *Lactobacillus rhamnosus* GG persistently colonized four of seven premature neonates, but no reduction in growth of *Enterobacteriaceae*, enterococci, yeasts, or staphylococci was reported (54). However, this and some other strains of lactic acid-producing bacteria have been successful in the treatment or prevention of acute diarrhea in infants (55,56).

Nonpathogenic *E. coli* strains may also have probiotic potential. There are well characterized strains that colonize and persist after oral administration to newborn infants (57–59) and also seem to protect from subsequent colonization with other strains of *E. coli* or related enterobacteria with pathogenic potential (58,59). Such strains may also reduce infection; peroral colonization with *E. coli* O83:K24:H31 reduced the infection rate from 37% to 20% in premature infants (59). Naturally, such bacteria must be carefully investigated to prove their nonpathogenic nature and guarantee their safe administration.

**SUMMARY**

Acquisition of an intestinal microflora begins after the rupture of the fetal membranes and proceeds over several years. Before the firm establishment of anaerobic bacteria, potentially pathogenic facultative bacteria may reach high numbers in the neonatal intestine, which may promote spread to extra-intestinal sites and the development of infections such as septicemia/meningitis. In addition, the incompletely developed microflora of neonates and young infants is likely to be a poor barrier against enteropathogenic bacteria.

Various environmental factors may influence the colonization process. In Western societies, modern obstetrical practices and neonatal care may profoundly reduce bacterial exposure and possibly delay the establishment of a functionally active and protective microflora. In developing countries, a pronounced and uncontrolled
bacterial exposure from environmental sources may result in unstable colonization with a wide range of potentially pathogenic bacteria and a high rate of neonatal infections. The administration of antibiotics to neonates profoundly disturbs the intestinal microbial balance and may predispose to new episodes of infectious diseases.

It would be desirable that a flora with a low pathogenic potential develop in neonates—a flora that could nevertheless confer resistance to intestinal pathogens. The best documented way of achieving this is through exclusive breastfeeding, which is especially important in developing countries. In addition, the controlled administration of carefully investigated bacterial strains lacking pathogenic potential may be useful, especially as an alternative to the often hazardous overuse of antibiotics.

ACKNOWLEDGMENTS

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REFERENCES


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DISCUSSION

Dr. Roulet: Can you comment about bacterial translocation in neonates? For me, bacterial translocation has been well proven in animal models, particularly in rats, but it's very difficult to prove in humans except in very special situations such as in patients with cirrhosis and ascites, and so on. Do you think that bacterial translocation may play a role in neonates. Also, do you think that intestinal permeability changes according to the number of bacteria in the digestive tract?

Dr. Adlerberth: Bacterial translocation has not been proven in human neonates, but it has been shown in surgical patients. I think bacteria have been found in mesenteric lymph nodes of patients operated on for colonic carcinoma. Also, there was a study by Albers (1) in healthy neonates that showed that blood cultures were positive in 20% of these infants, though they were asymptomatic and had no signs of septicemia. This bacteremia was occurring at the same time as the colonization of the gut and was presumably related to the permeability of the intestine. I don't know if there are any studies on how this early bacterial flora in neonates...
could change gut permeability, but there are indications that, for instance, lactobacilli may influence permeability and the uptake of macromolecules in the gut. And in animals, it is known that during the neonatal period there is an increased uptake of macromolecules. I think this has also been shown in humans.

Dr. Baerlocher: You explained the urinary infection in newborns by the ascending route from the anogenital region. How do you explain the fact that it is mainly male newborns who are infected during the neonatal period?

Dr. Adlerberth: It has been shown that this is due to differences in the immune system between females and males. Studies have also shown that P-fimbriated E. coli, which are the most common cause of urinary tract infection in neonates, adhere to the male prepuce from where they colonize the area and can probably ascend into the bladder. It is also said that male neonates have decreased urinary flow rate during this period, which could also predispose to urinary tract infection.

Dr. Guesry: The main argument from the people who would like to add lactoferrin to infant formula is that lactoferrin in breast milk keeps iron away from intestinal bacteria such as staphylococci that need iron to grow. But you showed that breastfeeding increases the numbers of staphylococci. What is your interpretation of this, which I find a bit bothersome?

Dr. Adlerberth: I don't know why breastfeeding increases staphylococci. It has been suggested that staphylococci may be transferred from the nipples during breastfeeding. It has also been shown that during storage and freezing of human milk, staphylococci are the main contaminants recovered. But I don't know of any factors in breast milk that would increase the growth of staphylococci in the intestine.

Dr. Isolauri: I would like to know how it has been shown—and compared to what kind of control group—that breastfeeding increases staphylococcal colonization in the way you said. An abstract to be presented at the next European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) meeting describes a group of infants with severe atopic disease during exclusive breastfeeding (2). These babies were divided into two groups: the first continued breastfeeding, while the mother changed her diet; the second went on to a substitute formula.

In the substitute-formula group, signs of atopic disease disappeared. Of the 20 patients in this group, only three had normal flora. Candida, staphylococci, or clostridia were detected in the feces of all of them.

In the breast-fed group, only one infant had staphylococci in the stool. So according to this study, breastfeeding prevents colonization.

Dr. Adlerberth: There are several different studies that have shown this increase in staphylococci in breast-fed infants (3–6). A very pronounced difference has been observed in these studies—a difference of two logs in concentration between breast-fed and bottle-fed infants. These are quite recent studies performed in the 80s and 90s.

Dr. Isolauri: What age were these infants?

Dr. Adlerberth: Mostly during the first weeks of life.

Dr. Isolauri: I'm talking about 4 to 6 months.

Dr. Adlerberth: That is a very important difference, because staphylococci usually decline in the intestinal flora during the early months.

Dr. Klish: There is a minor controversy going on in my country regarding the use of supplementary iron in breast-fed children, in the sense that if you add iron to their diet and saturate the lactoferrin, it suppresses the acidophilic bacteria and gives rise to other bacteria—I think E. coli and some of the other bacteria that were mentioned. As a result, there is a feeling that there should be a special formula for complementing the feeding of a breast-fed infant.
Yet your data do not seem to support that view, because if you look at the breastfeeding data you presented in your paper, the acidophilic bacteria are, if anything, present in the same or perhaps even somewhat lower concentrations in the breast-fed group compared with the bottle-fed group. Could you comment on that?

Dr. Adlerberth: The majority of studies show that colonization with lactobacilli is not influenced by the feeding pattern, though there are some studies that show that it is somewhat decreased during breastfeeding.

Dr. Lönnadal: This is with regard to both Dr. Guesry’s question and Dr. Klish’s. I think we need to dissociate hypotheses derived from in vitro observations from real-life observations. There is no reason, from a bacteriostatic point of view, why bovine lactoferrin should not have an effect in human infants.

But to date, virtually all studies have been inconclusive or even negative. When it comes to iron, I think a lot of the discussion is confused. In particular, it is claimed that a low-iron formula should help, but the low-iron formula in the United States is a misnomer—even that one contains 1000% of the amount of iron in breast milk. So the whole field is very murky, and the in vivo results very rarely support the very nice in vitro observations that have been made.

Dr. Mao: Do you think there is any difference in the establishment of intestinal microflora between a normal newborn baby and a premature baby?

Dr. Adlerberth: Some studies have shown that there is a delay in anaerobic colonization in premature babies. However, there are also studies that show that there is no difference when you allow for things like increased rates of cesarean section, treatment with antibiotics, and so on (7). So I doubt that prematurity per se would change the pattern, but premature infants are transferred to the neonatal units, and they will acquire the flora that prevails in these neonatal units, which is often dominated by enterobacteria other than E. coli.

Dr. Hanson: Just briefly, there is some suggestion from very new data that there are differences in placental cytokines in small-for-gestational-age and postmature infants, and it is very likely that this has consequences for the immune responses. This might influence colonization, but that has not yet been analyzed.

REFERENCES