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THE HUMAN INTESTINAL MICROFLORA DURING THE FIRST YEAR OF LIFE

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INTRODUCTION

The extensive development of neonatal intensive care has produced a new, surviving population of extremely vulnerable immature infants, adapted to the sterile amniotic fluid, and not to an environment replete with bacteria. Many of them, in addition to receiving broad-spectrum antibiotics, are neither healthy, nor vaginally delivered, nor breast-fed, nor full-term.

In this article, an overview of the initial colonisation and the development of the microflora during the first weeks of life, especially in new-born infants subjected to intensive care management, is given. The consequences of microbial colonisation for these infants are also discussed.

NORMAL DEVELOPMENT OF INTESTINAL MICROFLORA

The normally sterile foetus encounters a "hodgepodge" of microorganisms at the moment of rupture of the foetal membranes. In the study by *Brook* et al. (1979), the microflora of gastric contents of a mere 5-10 minutes old baby was found to reflect the cervical flora of the mother. There was a conspicuous absence of bifidobacteria. In contrast, rectal cultures are normally sterile immediately after birth (*Rotimi* and *Duerden*, 1981; *Ekwempu* et al., 1982). Bacteria start to appear in faeces within 24 hours after birth. *Escherichia coli* and enterococci are frequently isolated even in the very first stool, especially if there has been a premature rupture of the foetal membranes. Colonisation by identical *E. coli* strains in mother and infant occurred in 18 out of 29 cases (*Gothefors* et al., 1976). Anaerobic bacteria belonging to the *Bacteroides* and *Bifidobacterium* genera can be detected in faeces within two days (*Mata* and *Urrutia*, 1971; *Patte* et al., 1979; *Rotimi* and *Duerden*, 1981; *Lejeune* et al., 1984). Bifidobacteria gradually appeared, and by the end of the first week, colonised all infants, and were completely dominating as long as breast-feeding continued in these studies. The initially high counts of *E. coli* declined during the first weeks of life. Starting at weaning, the microflora grows more complex and biochemically active (*Stark* and *Lee*, 1982; *Midtvedt* et al., 1988), but the 1000-fold dominance of anaerobic bacteria seen in adults, as well as adult diversity of bacterial species and biochemical functions, may not be attained for several years (*Ellis-Pregler* et al., 1975, *Norin* et al., 1985).

Conflicting results have been reached in some recent studies, where members of the *Bacteroides fragilis* group were the dominating anaerobic bacteria despite breast-feeding (*Simhon*

et al., 1982; *Lundequist* et al., 1985). In our studies (*Bennet* et al, 1986; *Bennet* and *Nord*, 1987), bifidobacteria still dominated, but not at all to the extent that was reported earlier.

Bacteroides species in neonatal faeces have been found to belong to the *B. fragilis* group, and in these studies *B. fragilis*, *Bacteroides distasonis*, *Bacteroides vulgatus* and *Bacteroides thetaiotaomicron* were the most common species (*Long* and *Swenson*, 1977; *Rotimi* and *Duerden*, 1981; *Bennet* and *Nord*, 1987). Among bifidobacteria, *Bifidobacterium adolescentis*, *bifidum*, *breve* (Sweden), *longum* (Japan), and *infantis* are the most common species (*Benno* et al., 1984; *Bennet* and *Nord*, 1987).

IMPACT OF FORMULA FEEDING ON INTESTINAL MICROFLORA

Type of feeding, i.e. breast milk versus formula, has for many decades been known to influence the faecal flora composition. In formula fed infants, *Bacteroides* dominated among the anaerobes and high counts of enterobacteria were found (*Haenel*, 1961; *Ellis-Pregler* et al., 1975; *Bullen* et al., 1976; *Bullen* et al., 1977, *Stark* and *Lee*, 1982; *Yoshioka* et al., 1983; *Benno*, 1984; *Lejeune* et al., 1984). *Rotimi* and *Duerden* (1981) found moderate numbers of bifidobacteria among infants fed breast milk supplemented with a milk preparation from a cow. *Sakata* et al. (1985) and *Kudinova* et al. (1982) reported that *Bifidobacterium* growth was proportional to the amount of breast milk given. *Lejeune* et al. (1984) found untreated breast milk to be superior to tyndallised and lyophilised breast milk in promoting *Bifidobacterium* growth. There are several factors in breast milk that may influence intestinal microflora. Among them IgA (produced by plasma cells "homing" in the breast glands after activation in the intestinal mucosa of the mother) (*Hanson* et al., 1984), viable white blood cells, lactoferrin, anti-inflammatory factors (*Goldman* et al., 1986), a low buffering capacity (facilitating the production of a low pH), and microorganisms.

Breast milk has been shown to protect against neonatal septicaemia (*Narayanan* et al., 1984). It does not prevent colonisation of the intestine by Gram-negative, potentially pathogenic bacteria, but breast milk IgA prevents contact between these microorganisms and the mucosal membranes (*Mata*, 1971; *Gothefors* et al., 1976; *Hanson* et al., 1984; *Stevenson* et al., 1985).

IMPACT OF CAESAREAN SECTION ON INTESTINAL MICROFLORA

Caesarean section leads to colonisation from the hospital environment rather than from the mother's vaginal and perineal flora. In an attempt to reveal the sources of colonisation of eight Caesarean section delivered newborn infants by *E. coli*, nearly 7000 cultures of samples from eight babies and their environment were analysed (*Lennox-King* et al., 1976). It was found that the most common sources were other infants via nurses' hands, but there was a surprisingly high degree of airborne contamination. Anaerobic colonisation, especially by *Bacteroides*, is delayed and if the infant is transferred to a neonatal unit there is an overgrowth of enterobacte-

ria other than *E. coli* (*Rotimi* et al., 1985; *Bennet* et al., 1986). In our studies (*Bennet*, 1987; *Bennet* and *Nord*, 1987), absence of *Bacteroides* persisted beyond two weeks of life, but both *Bifidobacterium* retrieval and *E. coli/Klebsiella* ratio were similar in vaginally and Caesarean section delivered infants.

In gastric aspirate obtained immediately after birth of babies delivered by Caesarean section after prolonged labour with rupture of membranes, there was no difference compared to vaginally delivered infants except that more streptococci were found (*Brook* et al., 1979).

IMPACT OF HOSPITALISATION AND PRE-TERM BIRTH ON INTESTINAL MICROFLORA

Hospitalisation, also without antibiotic treatment, produces changes of the normal microflora. Thus, colonisation by *Klebsiella*, *Proteus*, *Pseudomonas* and *Candida* was shown to occur in faeces of hospitalised adult patients after a few weeks (*LeFrock* et al., 1979a). Changes in intestinal flora as regards antimicrobial resistance of the bacteria, and also changes of bacterial species, have been shown to be followed by colonisation of both pharynx and skin by the same strains (*LeFrock* et al., 1979b, *Larson* et al., 1986). Also in new-born infants, intestinal colonisation by *Klebsiella*, as well as by other enterobacteria, occurs. It is much more pronounced after Caesarean section (*Long* and *Swenson*, 1977; *Bennet* and Nord, 1987).

In investigations of the anaerobic faecal microflora of hospitalised newborn infants, a delay in *Bifidobacterium* colonisation, a predominance of *Bacteroides*, especially after vaginal delivery, and sometimes an increased incidence of *Clostridium* species recovery is reported (*Graham* et al., 1976; *Goldmann* et al., 1978; *Blakey* et al., 1982; *Rotimi* and *Duerden*, 1982; *Stark* and *Lee*, 1982, *Sakata* et al., 1985). In some of these studies, however, breast milk was not used, or heated to l00°C, or antibiotic treatment given. We found no differences in the anaerobic microflora between term and pre-term infants that could not be explained by neither antibiotic treatment nor a higher rate of Caesarean section in the latter group (*Bennet* and *Nord*, 1987). In the study by *Sakata* et al. (1985), similar results were obtained except for a delay of detection of anaerobes in very low birth weight infants. This was supposed to be a result of the very small amounts of breast milk tolerated by such infants during the first weeks of life. In conclusion, the control of the microflora seems to be intact also in very immature infants, but is easily disturbed by iatrogenic factors.

IMPACT OF ANTIMICROBIAL TREATMENT ON INTESTINAL MICROFLORA

Current knowledge of the effects of various antibiotics on the intestinal flora in adults has been summarised by *Nord* and co-workers (1986). Such effects are the net result of the antimicrobial spectrum of the drug, concentrations in bile, saliva and other secretions, re-absorption, faecal binding,

and antimicrobial inactivation. The effects usually measured are:

1. suppression of anaerobic bacteria,
2. new colonisation, and
3. tendency to induce resistance in bacteria.

Clindamycin, erythromycin and also ampicillin have a strong influence on the intestinal flora, whereas narrow-spectrum penicillins such as phenoxymethylpenicillin and benzylpenicillin have minor effects in clinical doses. Some modern cephalosporins are excreted to a large extent in bile and produce profound changes of the flora (*Bodey* et al., 1983). Aminoglycosides, on the other hand, are excreted in the urine and have no effect on intestinal microflora when given parenterally.

We have studied the influence of various common antibiotic regimens on both aerobic and anaerobic intestinal flora of new-born infants (*Bennet*, 1987). During treatment, there was a suppression of susceptible aerobic bacteria in a predictable way according to the antibacterial spectrum of the drug used. When cephalosporins were used, an overgrowth of enterococci occurred. There was a colonisation by and overgrowth of *Klebsiella* in all treatment groups, including those treated with the narrow-spectrum benzylpenicillin, cloxacillin and flucloxacillin. Other investigators have also found frequent colonisation with various aerobic Gram-negative rods, e.g., *Citrobacter*, *Pseudomonas* and *Proteus* during antibiotic therapy (*Graham* et al., 1976; *Goldmann* et al., 1978; *Lambert-Zechovsky* et al., 1984).

In our study, all regimens led to undetectable levels of anaerobic bacteria in 80-90% of the patients. In half of the remaining children, *Clostridium* species was the only anaerobic microorganism - a condition that was never found in untreated infants.

After antibiotic treatment, there was a slow but steady normalisation of the intestinal flora (*Bennet* et al., 1986). There was a regrowth of bifidobacteria but a continuing absence of *Bacteroides*, also in vaginally delivered infants. The *E. coli/Klebsiella* ratio slowly reverted back to one of *E. coli* dominance (*Bennet* and *Nord*, 1987). There were, however, a few cases where anaerobic bacteria remained absent for several weeks, and in these infants a heavy growth of *Klebsiella* continued. Among the treated infants, there were no differences relating to mode of delivery.

The fact that *Bacteroides* species do not re-establish after antibiotic treatment suggests that they are truly eradicated from the intestinal tract and that little transmission of *Bacteroides* occurs from the external environment. Clinically, *Bacteroides* infections are very rare in new-borns. In only one of 329 cases of neonatal septicaemia in our neonatal intensive care unit during 1979-1983, *Bacteroides* was isolated from blood (*Bennet* et al., 1985). Since *Bacteroides* has usually been shown to be rare in the faeces of new-born infants, its failure to become re-established may not be of any disadvantage.

NEONATAL SEPTICAEMIA

The increasing survival of high-risk infants has created a new population of patients, extremely vulnerable to infection. There are several reasons for this increased infectious risk, both in the environment and within the infants themselves. The abnormal colonisation of various anatomical sites of NICU patients is similar to what is known from adults (*Goldmann*, 1981; *Morgan*

et al., 1984; *Chugh* et al., 1985). The role of hands as carriers and even reservoirs of Gram-negative bacteria has been pointed out by *Knittle* et al. (1975).

Neonatal septicaemia has remained a serious problem. It has become evident that the clinical picture of this disease is changing, both as regards bacterial aetiology and patient characteristics (Davies and *Gothefors*, 1984; *Bennet* et al., 1985; *Bennet* et al., 1987a,b). Many cases of septicaemia are nowadays caused by staphylococci and group B streptococci, probably emanating from the skin and the mother's cervical flora. However, Gram-negative infections remain a serious problem and are coupled to high mortality and rate of sequelae in survivors (*Bennet* et al., 1989). It is likely that Gram-negative infections often start with antibiotic-induced intestinal overgrowth as demonstrated by *Mathieu* et al. (1984). In our intensive care unit, *Klebsiella* infections were always preceded by antibiotic treatment or Caesarean section (*Bennet* et al., 1987a).

IMPLANTATION OF MICROORGANISMS IN INTESTINAL MICROFLORA

Our ignorance of detailed rules of intestinal microbial ecology is reflected when it comes to methods used to "conventionalise" germfree animals. Complete normalisation of the physiological peculiarities of these animals has so far been achieved only by administration of faeces or intestinal contents from conventional animals without intervening cultures.

There are several animal studies showing an ability of the normal microflora to prevent colonisation by other microorganisms. Here, too, undefined caecal contents have been most successful (*Rantala* and *Nurmi*, 1973; *Berg*, 1980a,b; *Dubos* et al., 1984, *Soerjadi-Liem* et al., 1983). Specific mixtures of 48 and 239 strains, respectively, have been shown to replicate this effect (*Schneitz* et al., 1981; *Impey* et al., 1982).

Starting with fermented milk products during the first half of this century, bacterial interference programs or "bacteriotherapy" have also been attempted in humans, in order to treat more or less well-defined gastrointestinal disorders. It seems unlikely that success in humans should be obtained with very simple cultures of only one or a few anaerobic species. Recently, *Reuman* et al. (1986) reported no effect of a *Lactobacillus acidophilus* preparation on colonisation of low-birthweight infants by resistant Gram-negative bacteria. Yet, there are publications that report good results from oral therapy with lactobacilli (*Prado* et al., 1980; *Zoppi* et al., 1982).

Another approach is to give one apathogenic bacterial species, which is ecologically similar to the offending one, as demonstrated with nasopharyngeal alpha-streptococci by *Sprunt* et al. (1980). *Duval-Iflah* et al. (1982) reported the creation of a barrier function against colonisation by resistant *E. coli* by giving another strain of this species to new-born infants.

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