

Intestinal flora during the first months of life: new perspectives

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Increasing awareness that the human intestinal flora is a major factor in health and disease has led to different strategies to manipulate the flora to promote health. The complex microflora of the adult is difficult to change in the long term. There is greater impact of diet on the infant microflora. Manipulation of the flora particularly with probiotics has shown promising results in the prevention and treatment of diarrhoea and allergy. Before attempting to change the flora of the infant population in general, a greater understanding of the gut bacterial colonisation process is required. The critical stages of gut colonisation are after birth and during weaning. Lactic acid bacteria dominate the flora of the breast-fed infant. The formula-fed infant has a more diverse flora. The faeces of the breast-fed infant contain mainly acetic and lactic acid whereas the formula fed-infant has mainly acetic and propionic acid. Butyric acid is not a significant component in either group. The formula-fed infant also has higher faecal ammonia and other potentially harmful bacterial products. The composition of the microflora diversifies shortly before and particularly after weaning. The flora of the formula-fed infant develops more quickly than that of the breast-fed infant. Before embarking on any strategy to change the flora, the following questions should be considered: Should we retain a breast-fed style flora with limited ability to ferment complex carbohydrates? Can pro- and prebiotics achieve a flora with adult characteristics but with more lactic acid bacteria in weaned infants? Are there any health risks associated with such manipulations of the flora?

Infants: Gut microflora: Breast-feeding: Weaning

Introduction

The bacterial microflora of the human intestine is complex and numerous. The bacteria ferment unabsorbed carbohydrate to short-chain fatty acids (SCFA) which have many health benefits related to heart disease and cancer prevention. However, they have also been implicated in the aetiology of colonic disorders such as colitis and cancer (Onoue *et al.* 1997; Campieri & Gionchetti, 2001).

There has been increasing scientific, medical and commercial interest in the intestinal microflora. This began with the awareness of the actions of the SCFA, was increased by the resurgence of the health benefits of lactic acid bacteria (probiotics) and is maintained by the emergence of food products containing probiotics and prebiotics (materials which increase the growth of probiotic bacteria).

This increase in awareness has also been fuelled by the development of new techniques for identifying and investigating the flora. New molecular techniques based on bacterial RNA and DNA have rekindled the mystery of the gut flora (Vaughan *et al.* 2000). These methods have shown that a significant proportion of bacterial

DNA in human faeces is not accounted for by known and culturable species (Sghir *et al.* 2000).

The current understanding of the importance of the gut microflora has led to different strategies to manipulate the bacterial populations to promote health. These include changes to the diet, inclusion of unabsorbable carbohydrates and ingestion of live bacteria with potential health benefits (probiotics). However, the complex microflora of the adult human is difficult to manipulate in the long term. The flora is made up of at least 400 different species in the dominant flora (Moore *et al.* 1978; Tannock, 2000) which create a stable ecosystem resistant to new bacterial species.

There is a greater impact of diet on the intestinal microflora of the human infant. Several groups have explored the interactions between diet, the intestinal bacteria, diarrhoea (Howie *et al.* 1990; Pathmakanthan *et al.* 2000) and allergy (Wold, 1998; Bjorksten *et al.* 1999; Ouwehand *et al.* 2001). Manipulation of the gut flora of the infant has shown promising results (Saavadra *et al.* 1994; Arvola *et al.* 1999; Isolauri, 2001; Kalliomaki *et al.* 2001). Optimising the microflora of the infant may also have long-term benefits if the flora of the adult

Abbreviation: SCFA, short-chain fatty acids.

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is determined by events occurring in this critical period of gut colonisation. However, before the flora of infants in the general population is altered, for example by the addition of probiotics or carbohydrates that promote their growth (prebiotics) to normal infant formula or weaning foods, it must be clear what is intended and what the long-term consequences might be. To predict the long-term effects, a much greater understanding is required of the bacterial colonisation process and the metabolic activity of the flora.

Bacterial colonisation in the newborn

The critical stages of gut colonisation are in the days after birth and during weaning (Fig. 1). At birth the gut is sterile and as the infant is exposed to bacteria in its environment, the birth canal, maternal faecal bacteria and other sources (Tannock *et al.* 1990; Zetterstrom *et al.* 1994), the colonisation process begins. In the first months of life, diet has a crucial influence on the bacteria and their metabolism. Several studies, using conventional culture techniques, have shown that the flora of the breast-fed infant is dominated by bifidobacteria and lactobacilli, whereas the flora of the formula-fed infant contains more bacteroides, clostridia and Enterobacteriaceae (Balmer & Wharton, 1989; Fuller, 1991). Some studies have not seen this difference (Simhon *et al.* 1982; Lundquist *et al.* 1985) which may be due to changes in obstetric practice. However, the differences have been confirmed by new molecular techniques (Harmsen *et al.* 2000; Martin *et al.* 2000). Most infants in the UK are fed both breast and formula milk during their first months of life due either to an inability of the mother to produce sufficient milk, or cessation of breast-feeding by the mother due to a return to work or for other reasons. Very little is known of the bacterial flora of infants fed a mixture of breast and formula milk.

The mechanisms for the difference in the flora of infants fed human milk and modern formula are numerous and

difficult to reproduce despite great efforts taken by infant formula manufacturers to mimic human milk as closely as possible. Immunological factors such as secretory immunoglobulin A and lysozyme in human milk prevents the growth of some bacteria. The lower faecal pH of breast-fed infants (Bullen & Willis, 1971) may promote bacteria such as the lactobacilli and bifidobacteria, which are more tolerant of acid. Fe-related factors may also be important. The iron content of human milk is low (about 0.5 mg/l; Bullen *et al.* 1972), but the Fe present has a high bioavailability (Siimes *et al.* 1979; Calvo *et al.* 1992). This is increased by the presence of lactoferrin, which aids Fe absorption and binds any unabsorbed Fe making it unavailable to bacteria in the colon. Bifidobacteria and lactobacilli do not need Fe in contrast to bacteroides and enterobacteria (Archibald, 1983). Fe added to infant formula has been found to increase the growth of clostridia and enterococci (Balmer & Wharton, 1991). However, Fe-free formula, although promoting a flora more like that of the breast-fed infant, still did not produce a flora dominated by lactic acid bacteria. The effects of added lactoferrin have been inconsistent perhaps related to the use of bovine rather than human lactoferrin (Balmer *et al.* 1989*a*). Increasing the whey to casein ratio in the protein fraction of the milk, however, does promote a more breast-fed style flora although there are still major differences (Balmer *et al.* 1989*b*; Roberts *et al.* 1992).

There are several bifidogenic factors in human milk and these might be considered natural prebiotics. They include non-absorbable oligosaccharides (3–6 g/l in mature milk; Kunz & Rudloff, 1993). The amount of oligosaccharides in colostrum is even higher. These oligosaccharides mainly consist of a lactose core substituted with *N*-acetyl glucosamine, galactose, fucose and sialic acid resulting in over 100 different compounds (Kunz & Rudloff, 1993). Recently infant food products, which

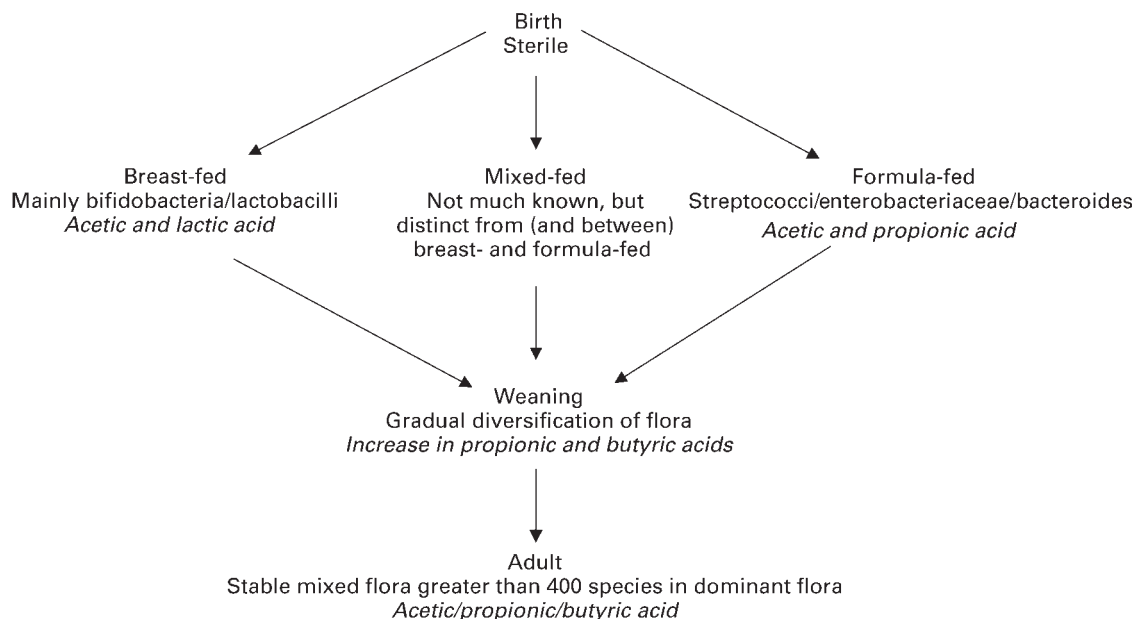


Fig. 1. A scheme of the development of the intestinal microflora in humans.

include oligosaccharides and are aimed at specific groups of infants with intestinal symptoms, have been launched in Europe. However, at present there is little published information on their impact on the faecal flora. Human milk also contains nucleotides (Gil *et al.* 1986; Balmer *et al.* 1994) and gangliosides (Rueda *et al.* 1998) which when added to formula milk have in some studies been shown to increase colonisation by bifidobacteria.

Bacterial metabolism

The metabolic activity of the adult microflora is equivalent in size and diversity to that of the liver but is often involved in reversing actions of the liver. For example, the liver conjugates toxic compounds with sugars or amino acids to make them safer; in the colon these conjugations are broken down and the toxins and other compounds reactivated. Many of the complex bacterial activities are a function of bacteroides, clostridia and other bacteria present in only low concentrations in the infant gut.

Short-chain fatty acids

The fermentation of carbohydrate is a major function of the intestinal flora. Carbohydrate provides most of the energy for the bacteria but the SCFA produced provide the host with an alternative energy source (Livesey, 1990), a mechanism to absorb water in the large intestine (Ruppin *et al.* 1980) and an inhibitory agent against pathogens (Fay & Faries, 1975).

The profile of SCFA in the faeces of infants differs substantially from that in adult faeces. In meconium, SCFA are very low, about 10% of adult values, increasing in the first four days of life. SCFA concentrations in the breast-fed infant are still low and increase significantly in the first year of life (Parrett *et al.* 2000). The molar proportions of the individual SCFA are also very different. In adults the major faecal SCFA are acetic, propionic and n-butyric acid in the proportions 57:22:21 (Szyliit & Andrieux, 1993). Acetic and lactic acids dominate the SCFA profile of the breast-fed infant. There is little or no propionic or butyric acid (Edwards *et al.* 1994), whereas the faeces of the formula-fed infant is dominated by acetic acid and propionic acid with a higher proportion of butyric acid than breast-fed infants but still much less than adult faeces. There is generally no lactic acid. Butyric acid is believed to be the major fuel for the colonocytes (Roediger, 1982) and thought to be essential for colonic health. Butyrate has also been shown to have anti-neoplastic properties by stimulating apoptosis (programmed cell death) of colonic cells (Hague & Paraskeva, 1995), differentiation of cancer cell lines *in vitro* (Augeron & Laboisse, 1984), and inhibiting other important stages in cancer development (Candido *et al.* 1978; Borenfreud *et al.* 1980). The lack of butyric acid in infant faeces may mean that butyrate is not critical for the infant colon or that most of the butyrate necessary is absorbed or utilised by the colonic mucosa before it reaches the faeces. However, studies of fermentation *in vitro*, using infant faeces, also show very low butyric acid production (Parrett *et al.*

1997). A high production of propionic acid and its ratio to acetic acid may help promote healthy plasma lipid levels (Venter *et al.* 1990; Wolever *et al.* 1991, 1996; Berggren *et al.* 1996). How this relates to the low propionic acid levels in the breast-fed infant is unclear.

As stated earlier, infants in many Western societies are fed a mixture of breast milk and formula milk. Some are fed breast and formula milk at different feeds and infants who begin with breast milk often are given formula milk within a few days or weeks after birth. In the UK, the number of mothers who are breast-feeding at discharge from hospital is substantially more than the number 1–6 weeks later (White *et al.* 1992). The impact of the introduction of formula milk to an initially breast-fed infant is not well studied. There is a belief that one bottle of formula milk will convert the flora of the breast-fed infant to that of an infant fed exclusively on formula milk. Bullen *et al.* (1977) reported that faecal pH was less acidic in mixed-fed infants than those exclusively breast-fed, but was still lower than in formula-fed infants. In a recent longitudinal study of nineteen mixed-fed infants, the SCFA in faeces indicated a distinct pattern of fermentation from either breast-fed or formula-fed infants before and during the weaning process (Parrett *et al.* 2001).

The simplicity of the infant flora limits its fermentation capacity for complex carbohydrates. When the fermentation capacity of the faecal flora from breast-fed and formula-fed infants for simple and complex carbohydrates was compared with that of adult faecal flora using a simple *in vitro* model, the infant flora from both groups of infants was much less able to ferment complex carbohydrates (Parrett & Edwards, 1997a) than the adult flora. The total SCFA produced by the infants' microflora was not affected by the type of milk fed but the proportions of individual SCFA (acetate, propionate, lactic) in the culture supernatant reflected the different patterns of SCFA seen in infant faeces (Parrett & Edwards, 1997a).

Other bacterial activities

There are many colonic bacterial activities, not associated with carbohydrate fermentation, some of which may have undesirable effects in the human colon. Most of these activities are associated with the adult colonic flora and do not develop until later in the first or second year of life.

Midtvedt *et al.* (1988) have studied a range of bacterial activities in early life. Mucin degradation ability was not established before 3 months of age. This ability was first seen in many children later in the first year of life and in all children by the second year (Midtvedt *et al.* 1994). The ability to deconjugate bile acids was already present at 1 month (Jonsson *et al.* 1995) whereas the ability to convert cholesterol to coprostanol developed in the second half of the first year and was delayed by breast-feeding (Midtvedt & Midtvedt, 1993). At 6 months urobilinogen production was very low with most infants gaining this activity between 11 and 21 months (Norin *et al.* 1985).

The metabolites of protein degradation such as ammonia, cresol and paracresol have been associated with harmful effects in the colonic mucosa and systemically

after absorption. Phenol and cresol had growth-depressing effects in young pigs (Yokoyama *et al.* 1982). Before weaning formula-fed infants had higher faecal urease activity (Gronlund *et al.* 1999) and faecal ammonia (Heavey *et al.* 2000) than breast-fed infants. Phenol and cresol levels were also higher (Heavey *et al.* 2000).

The enzymes β -glucuronidase and β -glucosidase may be involved in the activation of carcinogens and other toxins in the large intestine. The activity levels of these enzymes are low in the breast-fed infant before weaning but are increased in formula-fed infants possibly reflecting the more diverse flora. In a recent study β -glucuronidase activity was significantly higher in formula-fed than in breast-fed infants (Gronlund *et al.* 1999; Heavey *et al.* 2000), β -glucosidase was also higher in the formula-fed infants but this did not achieve statistical significance. The level of all these bacterial activities increased substantially after weaning as the flora diversified (Heavey *et al.* 2000).

Effect of weaning

The next critical stage after birth in the development of the flora occurs during the slow process of weaning (Fig. 1). There are several factors which change during weaning, including the addition of non-milk foods to the diet and the continued development of intestinal function. The amount of dietary carbohydrate which enters the colon in the infant depends on the digestive and absorptive ability of the small intestine (Christian *et al.* 1999). The intestine of the neonate is immature with low salivary amylase and low pancreatic function. Salivary amylase reaches adult levels between six months and one year (Rossiter *et al.* 1974). Pancreatic amylase is very low (1.6% of the adult level at birth) until four months and does not increase until the end of the first year, with mature levels only reached by the fifth year (Gillard *et al.* 1989). Mucosal enzymes such as glucoamylase may compensate for this and amylase and lipase in breast milk may aid digestion and absorption. Pancreatic sensitivity to cholecystokinin and secretin (Zoppi *et al.* 1972; Lebenthal & Lee, 1980) is much reduced in infancy and transport of sugars across the mucosa may also be much lower than in the adult gut (Younoszai, 1974; McNeish *et al.* 1983). In neonates, lactose may be a major substrate for the colonic microflora. Kien *et al.* (1992) estimated that up to 74% of lactose might be metabolised in the large intestine of premature babies but other studies suggest that lactase activity is already present at adult levels in the small intestine of most term infants. However, the very acid nature of the breast-fed infant stool and the high SCFA and lactate content would suggest that a substantial amount of rapidly fermentable carbohydrate is escaping digestion in the small intestine.

As weaning begins, infants are exposed for the first time to many different complex carbohydrates. A significant proportion of the starch will escape digestion because of the lack of chewing ability and immature pancreatic exocrine function in these young infants. This will enter the colon along with any dietary fibre. We have shown that significant amounts of starch are detectable in the faeces of children up to the age of 3 years (Verity & Edwards,

1994; Parrett *et al.* 2000). This starch will have escaped both digestion and fermentation. If colonic fermentation capacity develops sufficiently rapidly, the colonic salvage of energy from unabsorbed complex carbohydrates may contribute significantly to daily energy needs in these infants. In adults the energy gained from fermentation of complex carbohydrate is estimated as 8.36 kJ/g (Livesey, 1990). As weaning progresses, both intestinal function and fermentation capacity mature and the amount of faecal starch peaks in early weaning and then decreases (Parrett *et al.* 2000). However in some breast-fed infants the level is still significant at 1 year. In another study using *in vitro* incubation methods, infants during early weaning appeared to have a greater and faster capacity for fermenting starch than adults, perhaps reflecting the greater likelihood of starch entering the colon of the infants (Christian *et al.* 2000). In the developing world, where starchy foods such as cassava with low digestibility are eaten by pre-school children, faecal starch is high and may be a cause of high faecal energy losses (Hamaker *et al.* 1991). However, in the developed world the amount of starch excreted is too small to have any impact on energy losses (Parrett, 2001).

The concentrations and profiles of SCFA in the faeces of human infants change as weaning progresses (Midtvedt & Midtvedt, 1992). The rate of change is related to initial feeding practice. In the breast-fed infant there is a gradual increase in total SCFA concentration with a decrease in lactic acid production, an increase in acetic and propionic acid and by late weaning a gradual increase in butyric acid production. In the formula-fed infant the change is less profound, mainly because of the more mature SCFA production before weaning. The proportion of propionic acid decreases as butyric acid increases (Parrett, 2001).

The ability of the colonic flora of the breast-fed infant to ferment complex carbohydrates appears to develop more slowly than that of the formula-fed infant. This is understandable in the light of the greater diversity of species in the formula-fed infant colon and the greater populations of Gram-negative anaerobes. In a cross-sectional *in vitro* study of fermentation capacity of infants at different stages of weaning (Parrett *et al.* 1997; Fig. 2), it was found that before weaning breast-fed infants could ferment the simple sugars lactose and glucose well. However, raffinose, a fructo-oligosaccharide from inulin, was less well fermented and the complex carbohydrate soya polysaccharide was hardly fermented at all. In early weaning (one month after the introduction of solid foods) the ability to ferment raffinose increased but the ability to ferment complex carbohydrates did not increase until late weaning (seven months to one year). The differences in fermentation capacity of the formula-fed infants at the different stages of weaning were not significant indicating a faster maturation of their colonic flora (Parrett & Edwards, 1997b). In a more recent longitudinal study, breast-fed infants had a similar slow development of the capacity to ferment complex carbohydrates although there was much variation in individual infants (Parrett, 2001). Pectin was more readily fermented than other complex carbohydrates such as resistant starch and guar gum at each stage of weaning.

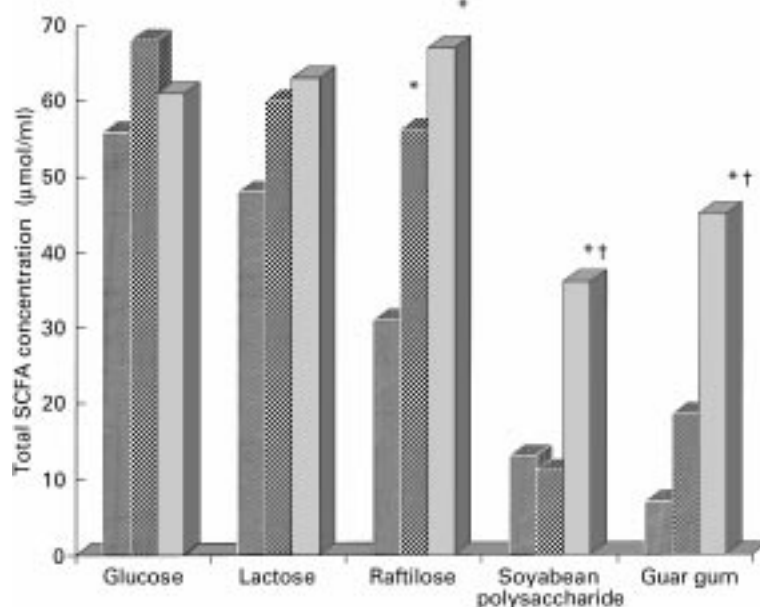


Fig. 2. Short-chain fatty acid (SCFA) production from different carbohydrates by faecal bacteria of breast-fed infants at different stages of weaning *in vitro*. (■) before weaning; (▨), early weaning, (□) late weaning; *Significantly different compared with before weaning; †significantly different compared with early weaning. (From Parrett & Edwards, 1997a,b).

The impact of a weaning diet is likely to persist into adulthood. The amount and type of dietary fibre in the weaning diet of rats influenced their fermentation capacity for these fibres and consequent effects on stool output when the same fibres were fed to adult rats (Armstrong *et al.* 1992). More SCFA were present in faeces when pectin was fed to adult rats for a month if the rats had been fed this dietary fibre during weaning than if they had been weaned onto a diet containing other dietary fibre. Moreover, rats produced more SCFA from a low fibre diet as adults if they were fed dietary fibre at weaning. In a study in South Africa, faecal bacteria from children, under the age of three, from Soweto township produced more butyric acid from different carbohydrates *in vitro* than the bacteria from white children in Potchefstroom. This may have been a result of a higher intake of resistant starch in their diet at weaning (Edwards *et al.* 1998).

As the gut is developing throughout the first year of life, it is important to consider if the changes in the intestinal flora occur as a result of weaning or just at the time that weaning occurs. Recent longitudinal studies of human infants using rRNA probes show that the bacterial flora diversifies with greater dominance of bacteroides and clostridia groups even before weaning in both breast-fed and formula-fed infants (Martin *et al.* 2000). The effects of weaning therefore need to be studied carefully, taking this into account.

Conclusion

The flora of the infant develops slowly during the first year

of life. The flora of the breast-fed infant has been shown to be beneficial for the infant reducing the incidence of diarrhoea. It is possible that manipulation of the flora, by use of food products containing prebiotics, probiotics and synbiotics (a mixture of pro- and prebiotics), may allow promotion of a more beneficial flora which could reduce gastrointestinal problems, allergy and other debilitating conditions, as well as potentially helping to prevent chronic adult diseases such as heart disease and colon cancer. However, there have been very few good studies of the likely impact of these food products on the infant flora and its metabolism. In particular, long-term effects are unknown. At this important time in the infant's development, we must be clear of our intentions. Should we change the microflora of the infants during weaning? This may have greater implications than just increasing the numbers of some bacterial species. If we try and maintain a breast-fed style flora for as long as possible we may limit the ability of the weaning infant to cope with the complex carbohydrates and dietary fibres in their diet. The most sensible manipulation may be to encourage the development of fermentation capacity for complex carbohydrates but at the same time increase the dominance of the lactic acid bacteria. This would promote the production of butyric acid, which may become more important in the older child despite being low in infancy. The levels of lactic acid bacteria could then be promoted through childhood and into adulthood by dietary means. However, much detailed research is needed. At present most research is aimed at infants with specific gastrointestinal and allergy problems. We need to know much more about the normal population and 'normal' colonic function.

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