
Protein Source and Microflora

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The human intestinal tract is colonized by a huge number of microbes (around $10^{14}$). These grow predominately in the region of the large bowel (1). These microbes consist of more than 400 different species and subspecies and are either nonpathogenic or pathogenic to the host. In a healthy state, humans live in peaceful coexistence with a well balanced variety of intestinal microflora. This is achieved by a defense mechanism of mucosa-associated bacteria that prevents the translocation of pathogenic germs from the intestinal lumen into the body. The numerical composition of the microflora remains fairly constant (2-4). When a supply of substrate is provided, microbes react by increasing their enzyme activity (5). Homeostasis of the intestinal ecosystem is maintained by the physiological conditions in the colon, by host defense mechanisms, and by the nutrient composition of the chyme that reaches the large bowel. Furthermore, the production of active inhibitory substances, including organic acids such as lactic acid, acetic acid, and propionic acid, suppresses the growth of germs that normally compete with the nutrient sources. This balanced process is maintained largely by changes in the intraluminal pH that reduce microbial enzyme activity and impede substrate utilization. Numerous bacteria of the intestinal microflora produce antibiotic-like substances that can act in a similar way. According to their metabolic properties, intestinal microbes have either anaerobic or aerobic living conditions and predominately utilize either carbohydrates or proteins and other nitrogen sources for growth and energy metabolism.

SOURCES OF NITROGEN FOR MICROBIAL GROWTH

The nitrogen content of the intestinal bacteria is about 10% to 12%. This nitrogen originates from proteins, peptones, peptides, and amino acids that have passed unab sorbed through the small bowel, or from endogenous nitrogen sources.

The digestibility of food proteins is known to be 90% to 98%. This means that a considerable portion of malabsorbed protein is permanently subjected to microbial degradation in the large bowel. Intestinal bacteria appear to have little capacity to split intact proteins, which is remarkable because protein nitrogen administered into the large bowel is to a large extent absorbed (6). It is apparent that ongoing digestion of proteins by chymotrypsin in the chyme of the large bowel makes it unnecessary
TABLE 1. Degrading capacity of different intestinal microbes for protein, polypeptides, amino acids, and urea

<table>
<thead>
<tr>
<th>Microbes</th>
<th>Protein</th>
<th>Polypeptides</th>
<th>Amino acids</th>
<th>Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacilli</td>
<td>(+)</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>—</td>
<td>—</td>
<td>+ (+)</td>
<td>—</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>+</td>
<td>+</td>
<td>+ (+)</td>
<td>—</td>
</tr>
<tr>
<td>Streptococci</td>
<td>—</td>
<td>+</td>
<td>+ (+)</td>
<td>—</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>—</td>
<td>+</td>
<td>+ (+)</td>
<td>—</td>
</tr>
<tr>
<td>Clostridia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Eubacteria</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Peptostreptococci</td>
<td>—</td>
<td>(+)</td>
<td>+ (+)</td>
<td>—</td>
</tr>
<tr>
<td>Peptococci</td>
<td>—</td>
<td>+</td>
<td>+ (+)</td>
<td>—</td>
</tr>
<tr>
<td>E. coli</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Fusobacteria</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

to provide much in the way of additional microbial proteolytic enzyme activity. However, Sütas et al. (7) recently reported on products of casein hydrolysis produced by Lactobacillus GG. These lactobacilli were shown to suppress the proliferation of mononuclear cells and to reduce the production of interleukins in healthy and atopic children. Numerous intestinal bacteria are capable of upgrading inorganic nitrogen, such as ammonia salts, to essential and nonessential amino acids. Some subspecies of Bacteroides are even capable of utilizing elemental nitrogen for the synthesis of organic nitrogen compounds. A survey of microbial metabolic capacity to degrade proteins, peptones, peptides, amino acids, and urea is given in Table 1 (8).

EFFECTS OF MICROBIAL METABOLITES ON THE COMPOSITION OF THE CHYME

The microbial degradation of amino acids and urea results in the formation of ammonia that alkalinizes the chyme in the cecal region of the bowel, if produced in excess. On a mixed diet, fermentation and putrefaction are metabolic processes that run simultaneously in almost all species of intestinal bacteria. Under normal conditions, the ammonia-induced pH shift is therefore compensated by the simultaneous production of organic acids from nonabsorbable carbohydrates. If this condition predominates, as is the case in breast-fed infants, an acidic milieu is established. This acidic milieu converts ammonia from the nonionic to the ionic NH$_4$ condition, which, in contrast to NH$_3$, is poorly absorbed (Fig. 1).

BIFIDOGENIC EFFECT OF HUMAN MILK: A CHALLENGE FOR FURTHER ADAPTATION OF INFANT FORMULAS

The scientific basis for the establishment of the predominant bifidobacterial microflora in breast-fed infants—a unique feature in nature—has still not been explored
in sufficient detail. However, studies on the composition and metabolic effects of the components of human milk may explain the beneficial living conditions for bifidobacteria provided by breastfeeding. The bifidogenic principle of human milk is especially instructive and exciting. To date, it is the only known example of a fundamental, nutrient-dependent change in the composition of the colonic microflora. Mixed diets, such as those consumed after the breastfeeding period, crank diets, and additives such as nonabsorbable sugar substances have all failed to produce comparable effects on the microflora (9,10).

ROLE OF HUMAN MILK PROTEINS IN THE ESTABLISHMENT AND MAINTENANCE OF THE BIFIDOCENRIC MICROFLORA

Though carbohydrates such as lactose and oligosaccharides are the most important factors for the establishment of a predominance of bifidobacteria, there is currently general agreement that the bifidogenic principle of human milk is based not only on single growth factors but on a complex set of interacting factors. Since 1920, the bifidogenic principle of human milk has been known to be connected with the nonprotein fraction of the milk (11). However, there is much evidence for a supportive role of milk proteins. The human milk protein fraction is probably not deleterious for the origination and maintenance of the bifidobacterial microflora, whereas cow’s milk and soy proteins are. The quantity and quality of heterologous food proteins also seem to be important.

Human Milk Protein Concentration

Human milk has the lowest protein concentration of all the mammalian species. Therefore, the chances of complete absorption of the protein component are better
than with milk preparations that are richer in protein. There are significant correlations between the protein intake and the concentration of amino nitrogen in the feces of infants (12) (Fig. 2). Analogous differences between $\alpha$-amino nitrogen and total nitrogen concentrations have also been found in the chyme of the cecal region of infants with colostomies, when human milk feeding was compared with formula feeding (13) (Fig. 3). Consequently, rapidly proliferating bacteria that preferably metabolize proteins and protein degradation products will be afforded better living conditions than slowly proliferating carbohydrate-fermenting bacteria. From this point of view, the protein content of infant formulas—currently almost twice as high as in human milk—may have to be reduced. We have recently shown that $\alpha$-lactalbumin-enriched cow’s milk protein can serve this purpose (14).

### Role of Protein in Enterocyte Proliferation

Protein and protein degradation products detectable in cecal chyme and feces do not exclusively originate from malabsorbed food proteins. The main nitrogen source for the intestinal microflora is from desquamated small and large bowel mucosal cells and digestive enzyme proteins that are secreted into the lumen of the small intestine. The significance of this endogenous nitrogen fraction is not yet known in detail. There is evidence, however, that the enterocyte proliferation rate increases when heterologous food proteins are given. Weaver and Lucas (15) have observed this phenomenon in newborn guinea pigs, and Yeh (16) observed it in newborn rats fed on cow’s milk.

Using newborn pigs fed on a conventional cow’s milk formula compared to hy-
FIG. 3. α-Amino nitrogen and protein concentration in the cecal chyme of two infants with colostomies.

hydrolysate formulas as an experimental model, we found the chymotrypsin activity to be 14 U/g in the chyme obtained from the small bowel of the animals fed on cow's milk formula and 6.5 U/g in the hydrolysate formula group. We conclude that endogenous protein can play an important role in the establishment and composition of the intestinal microflora. The increased proliferation of duodenal and jejunal mucosal cells observed on feeding heterologous food proteins apparently enhances the formation of an antiputrefactive microflora. How these proteins cause the increase in proliferation and desquamation is still unknown; the lower proliferation rate found with hydrolysate formulas suggests a subclinical allergic reaction to food proteins and highlights the potential advantages of hydrolysate formulas in infant nutrition.

Nitrogen Recycling

Nitrogen metabolism in the intestinal microflora is also based on the utilization of protein and other nitrogen-containing products deriving from the decay of intestinal bacteria. The amount of nitrogen produced by this recycling process is not precisely known. The ingestion of large amounts of lactobacilli and bifidobacteria may result in a nominal increase in these organisms within the microflora. This increase may be due either to adherence and multiplication of the bacteria at the surface of the mucosal cells or to stimulation of the resident flora by decay products of the inoculated bacteria. Our studies with 15N-labeled bifidobacteria given to breast-fed infants either orally or by instillation into colostomies showed that more than 80% of the microbial nitrogen is absorbed from the large bowel and that approximately 73% of the nitrogen is retained in the protein pool of the infant (17). This points to
competition between the microflora and the host: the nutrient source released by the decay of intestinal bacteria is made unavailable to the intestinal flora because it is absorbed by the host. In this way, symbiotic support of protein accretion is achieved. This may have special importance in states of marginal protein supply and in protein malnutrition in infancy.

**Proteins with Antimicrobial Activities against Potential Pathogens**

Human milk contains several special proteins, such as secretory immunoglobulin A (slgA), lactoferrin, and lysozyme, that have antimicrobial activity directed against potentially pathogenic bacteria in the intestinal flora. By its effects on cleavage of the murein component, lysozyme causes depolymerization of the mucopolysaccharides in the microbial cell membrane. This leads to the destruction of bacteria and increases their susceptibility to digestive enzymes. Bacteria that have been found to be susceptible to lysozyme degradation include bacteroides, the Enterobacteriaceae, streptococci, staphylococci, and clostridia. We recently showed that incubation of bifidobacteria and lactobacilli with lysozyme enhances the digestion of these bacteria by trypsin (18). Lysozyme from human milk or monocytes probably plays a key role in the destruction of intestinal bacteria, thus making their decay products available for the generation of new bacteria and for symbiotic utilization by the host (19). The iron-binding protein lactoferrin regulates the growth and multiplication of iron-dependent microbes by withdrawing soluble iron from the intestinal fluid. This in turn improves the living conditions of slower-growing and less iron-dependent bifidobacteria and lactobacilli. Secretory IgA in human milk plays an important role in the agglutination of pathogenic bacteria and viruses, as well as in the neutralization of toxins. This prevents intestinal infections in the newborn.

The bioavailability of slgA, lactoferrin, and lysozyme is apparently low in the early newborn period. Later on, less than 1% of the ingested slgA and lysozyme from human milk is excreted with the feces (19). All three protective human milk proteins, as well as α-lactalbumin, which accounts for 29% of total human milk protein, have a high cystine content in common. The release of cystine in the course of microbial proteolytic cleavage of these proteins may have promoting effects on the growth of bifidobacteria. This is known from experience with the cystine-dependent growth of these bacteria in culture media.

These particular features of human milk proteins have no significance under formula-feeding conditions. Cow’s milk contains no or low concentrations of slgA, lactoferrin, lysozyme, or α-lactalbumin, or else the biological activity of these proteins is lost in the heating process. In view of the complexity of the bifidogenic principle of human milk, all attempts to create human milk–like bifidogenic effects by adding these proteins to infant formulas are scarcely promising.

**SIGNIFICANCE OF WHOLE COW’S MILK PROTEIN, CASEIN, AND WHEY PROTEIN**

To try to adapt infant formulas so that they have the composition and properties of human milk, numerous attempts have been made since the beginning of this
century to mimic the bifidogenic effects of human milk (2,10,11). In doing so, the handling of the cow’s milk protein has always proved to be one of the major problems. The high concentration of calcium and casein in cow’s milk was found to be responsible for the formation of so-called Kalkseifen (soapy stools), which have bifidus-inhibiting properties. A reduction in the protein content to 1% to 1.5% in combination with fat, lactose, and starch enrichment was shown to produce bifidogenic effects by Adam in 1922 and by Schönfeld in 1933 (11). In 1925, Bessau recommended a bifidogenic formula based on one part of cow’s milk diluted with two parts of water, to which lysine, lactose, iron and p-aminobenzoic acid were added. It became evident, however, that such far reaching reductions of the protein supply did not meet the amino acid requirements of infants. Cow’s milk dilutions of 2:1 were therefore introduced (10). Further variations including acidified cow’s milk, butter milk, and enrichment of the dilutions with dextrans instead of lactose, as well as the addition of viable bifidobacteria, were claimed to support bifidogenic effects but did not stand a critical evaluation. All these formulas failed to produce the constant predominance of bifidobacteria of greater than 90% to 99% seen on human milk feeding. The large-scale industrial production of cow’s milk protein fractions enabled the producers to adapt the amino acid pattern more closely to that of human milk protein and to reduce the protein content of infant formulas. However, the currently produced formulas still contain protein in great excess of that present in human milk. This may cause protein malabsorption and the resulting origination of putrefactive effects produced by microbial protein digestion.

Though none of the infant formulas currently on the market is capable of originating and maintaining a human milk-like bifidogenic effect, there are detectable differences in the number of bifidobacteria in the feces of infants fed with different proportions of whey protein to casein: Whey protein-dominant formulas tend to increase the number of bifidobacteria compared with formulas rich in casein (20). These data are in agreement with our observation that bifidobacteria grow more rapidly in culture medium when whey protein is provided as the nitrogen source than when casein is provided (9).

Interestingly, acid-precipitated casein was used as a source of protein in the former East Germany to create the bifidogenic infant formula Manasan. However, the bifidogenic effect of this formula disappeared completely when, in accordance with international recommendations, the casein of the formula was replaced by a whey protein-casein mixture (Table 2). Attempts to reduce the antigenicity of cow’s milk proteins by desialinization in order to achieve bifidogenic effects were likewise unsuccessful (21).

From both a theoretical and a practical viewpoint, hydrolysates of food proteins that have been used for the production of infant formulas with reduced antigenicity since 1985 have become interesting microecological research topics.

Predigestion of proteins by treatment with trypsin may enhance small bowel absorption and reduce the release of endogenous nitrogen from the intestinal tract. Consequently, the composition of the nutrient fluid that reaches the bacteria colonizing the large bowel should provide similar growth conditions for the microflora to
TABLE 2. Fecal microflora of infants fed on mother's milk and two casein-based infant formulas (Manasan and modified Manasan)

<table>
<thead>
<tr>
<th></th>
<th>PH</th>
<th>Bacteria (total no.)</th>
<th>Enterobacteriaceae</th>
<th>Streptococci</th>
<th>Staphylococci</th>
<th>Proteoles</th>
<th>Bifidobacteria</th>
<th>Lactobacilli</th>
<th>Bacteroides</th>
<th>Clostridia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mother's milk</strong></td>
<td>5.69</td>
<td>10.51</td>
<td>8.76</td>
<td>6.97</td>
<td>5.87</td>
<td>7.64</td>
<td>10.47</td>
<td>7.13</td>
<td>8.04</td>
<td>6.73</td>
</tr>
<tr>
<td>±0.77</td>
<td></td>
<td>0.34</td>
<td>1.09</td>
<td>1.42</td>
<td>1.02</td>
<td>1.03</td>
<td>0.36</td>
<td>1.69</td>
<td>1.94</td>
<td>1.04</td>
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<td>(n = 11)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Manasan</strong></td>
<td>6.49</td>
<td>10.45</td>
<td>9.15</td>
<td>8.69</td>
<td>6.17</td>
<td>8.27</td>
<td>10.00</td>
<td>7.42</td>
<td>9.12</td>
<td>5.77</td>
</tr>
<tr>
<td>±0.96</td>
<td></td>
<td>0.36</td>
<td>0.64</td>
<td>1.43</td>
<td>0.98</td>
<td>1.10</td>
<td>0.52</td>
<td>1.97</td>
<td>1.64</td>
<td>1.54</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Manasan, modified</strong></td>
<td>6.96</td>
<td>10.25</td>
<td>8.99</td>
<td>8.05</td>
<td>5.37</td>
<td>8.27</td>
<td>9.95</td>
<td>6.69</td>
<td>9.22</td>
<td>7.60</td>
</tr>
<tr>
<td>±1.01</td>
<td></td>
<td>0.45</td>
<td>0.68</td>
<td>0.86</td>
<td>1.18</td>
<td>1.24</td>
<td>0.71</td>
<td>1.09</td>
<td>0.64</td>
<td>2.01</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(>90% = 8/11)

(>90% = 3/10)

(>90% = 0/10)
human milk. However, studies on the microecology of feces from infants fed on hydrolysate formulas showed no increase in the numbers of bifidobacteria (22), though a dominance of bifidobacteria may be found in isolated cases (Table 3). This leads us to conclude that additional factors that optimize the overall composition of currently produced hydrolysate formulas are not sufficiently well balanced, in particular, the protein degradation intensity, the high iron concentration, and the carbohydrate component of such formulas.

PROTEIN QUALITY AND EFFECTIVENESS OF BIFIDOBACTERIAL INOCULATION

The types of bacteria ingested by newborn infants are of great importance in the colonization of the initially sterile intestinal tract. In preterm infants, pathogenic organisms from the maternal genital tract are frequently swallowed. Further inoculation with nosocomial bacteria may cause life-threatening infections in the newborn period. Analogous situations are common in gastrointestinal infections or after treatment with antibiotics that destroy the normal microflora. Under such conditions, inoculation of physiological bacteria such as bifidobacteria or lactobacilli is a rational procedure for reestablishing the normal microflora. Adherence and proliferation of the inoculated bacteria are, however, again dependent on the type of feed given. If the infant is fed on human milk, the classical predominance of bifidobacteria is obtainable more often, and three weeks earlier, than in controls receiving no inoculation. In those preterm infants in our study group who received an extensively hydrolysed infant formula (Alfare, Nestlé) instead of banked human milk, a predominance of bifidobacteria in the feces was observed in four of five infants.

No convincing bifidogenic effects were obtained in term infants fed on a low-phosphate, whey-adapted formula that was subjected to acidification with *Streptococcus thermophilus* and *Lactobacillus helveticus* and subsequently enriched with $10^6$ viable bifidobacteria (23). Studies on yogurt administration in infant diarrhea have shown that the duration of the diarrhea is reduced and the reestablishment of the microflora is encouraged (24,25). It is, however, uncertain whether and to what extent the biochemical and nutritional changes after fermentation, including the curd particle size, contribute to these dietetic effects.

SUMMARY

Bifidobacteria and other carbohydrate-fermenting microbes in the intestinal tract find optimal living conditions for growth and overgrowth in the colonized part of the bowel when the chyme is rich in malabsorbed carbohydrates and poor in protein. Such conditions are fulfilled with breastfeeding. Though the bifidogenic principle of human milk is attributed to the protein-free fraction, the quality and quantity of its proteins play an important role in the origination and maintenance of the microflora. The low concentration of protein in human milk, the properties of this protein, and the presence of specific proteins that inhibit competing microbes in the
TABLE 3. Fecal microflora of 10 infants fed on Beba HA and a Beba HA-variant (1.3% protein, 1.5% starch, 0.35% iron)

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Bacteria (total no.)</th>
<th>Entero-</th>
<th>Streptococci</th>
<th>Staphylococci</th>
<th>Proteolites</th>
<th>Bifidobacteria</th>
<th>Lactobacilli</th>
<th>Bacteroides</th>
<th>Clostridia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beba HA</td>
<td>n = 9</td>
<td>6.65</td>
<td>10.85</td>
<td>9.79</td>
<td>9.15</td>
<td>6.08</td>
<td>9.00</td>
<td>10.29</td>
<td>6.97</td>
<td>9.97</td>
</tr>
<tr>
<td></td>
<td>± 0.89</td>
<td>0.36</td>
<td>0.49</td>
<td>0.69</td>
<td>1.45</td>
<td>0.82</td>
<td>0.68</td>
<td>1.79</td>
<td>0.76</td>
<td>1.91</td>
</tr>
<tr>
<td>Modified Beba HA</td>
<td>n = 14</td>
<td>5.80</td>
<td>10.79</td>
<td>9.01</td>
<td>8.71</td>
<td>5.14</td>
<td>8.79</td>
<td>10.50</td>
<td>6.76</td>
<td>10.09</td>
</tr>
<tr>
<td></td>
<td>± 0.67</td>
<td>0.59</td>
<td>0.58</td>
<td>0.71</td>
<td>1.20</td>
<td>0.89</td>
<td>0.61</td>
<td>1.76</td>
<td>0.72</td>
<td>1.07</td>
</tr>
</tbody>
</table>

(>90% = 2/9)

(>90% = 5/14)
intestinal microflora are essential components of the bifidogenic principle of human milk. Cow's milk proteins in infant formulas have contrary actions because of partial malabsorption and an increase in the endogenous protein fraction of the chyme. Further adaptation of infant formulas to allow the establishment and maintenance of a predominance of bifidobacteria should therefore be directed to a reduction in the concentration and antigenicity of cow's milk proteins. Without this, supportive measures such as bifidus factors, inoculation of bifidobacteria, lowering the buffer capacity, and a reduction in the iron content of formulas are of limited value.

REFERENCES


DISCUSSION

Dr. Haschke: If I understood you correctly, when you compare a casein-dominant formula with a 60/40 whey/casein formula you find that more infants on the casein-predominant formula have a bifidogenic flora. However, you also said that certain whey fractions can induce a bifidogenic formula. Could you clarify that?

Dr. Heine: If you coagulate the milk proteins with rennilase, you get a release of glycomacropeptide from the κ-casein fraction of the casein, and it goes to the whey protein fraction. So you have high concentrations of glycomacropeptide as a source of carbohydrate and glycoprotein within your whey protein fractions. If you then use acid-flocculated casein, you will obtain an additional amount of glycomacropeptide that remains in the κ-casein. So the amount doubles if you use this mixture.

Dr. Haschke: The other question I had is on the hydrolysates. You speculate from your data that hydrolyzed formulas more often induce a bifidogenic flora than regular formulas. Do you think a high degree hydrolysate is more likely to induce a bifidogenic flora than a partial hydrolysate? Do you have any data on that?

Dr. Heine: These hydrolyzed formulas are produced by subjecting the protein component to trypsin digestion, and there are always residues that are not split by this procedure. We expect that the baby will be able to do that, but that may not always be the case. So I believe that when you use partially hydrolyzed proteins, any malabsorbed polypeptide residues that may be present may interfere with the establishment and maintenance of the bifidogenic flora. That is my hypothesis.

Dr. Lentze: In many countries, milk fortifiers are used for feeding premature babies, where the protein sources are high-degree hydrolysates. Do babies fed on these have the same bifidogenic flora as babies fed on unfortified breast milk?

Dr. Heine: There are small differences. The number of preterm infants who develop a bifidogenic flora when fed on these human milk fortifiers is somewhat lower, but nevertheless a bifidogenic flora is obtainable. However, we have also shown that when preterm infants are fed on human milk and are given additional suspensions of bifidobacteria in the milk, the establishment and maintenance of a bifidogenic flora occurs much earlier than in infants not treated in this way. In some preterm infants, this effect lasted 3 weeks until they were able to maintain a normal bifidogenic flora on human milk. So, in my view, you can protect the bifidogenic microflora by using this inoculation treatment. This is relevant to probiotics of course, since the baby is constantly swallowing these bacteria from the mother’s skin surface when breastfeeding.

Dr. Lentze: Do the infants given bifidobacteria show any benefit? Has anybody looked at whether they have less infection or less necrotizing enterocolitis?

Dr. Heine: Yes. During the study, there were 20 cases of septicemia, but not one in infants who had established a dominant bifidobacterial flora. The vulnerable period is between birth and day 7, when the flora is not established. Enabling bifidobacteria to colonize the large
bowel during this period seems to prevent apparently pathogenic germs from growing and translocating.

Dr. Haschke: Is this a speculation, or is your sample size big enough to rule out a Type 2 error? We don’t want to get into the same dilemma as with Long Chain Polyunsaturated Fatty Acids (LCPUFAs), where 15 studies showed an effect but the final outcome was that the studies were too small to avoid a Type 2 error; with large studies, there was no effect. Can you please clarify the focus of this study?

Dr. Heine: The problem was that when we compared the two groups—those who were inoculated and those who were not—the gestational age was different. We used an alternating regimen, so unfortunately the groups were not exactly comparable. However, interestingly enough, the group with the beneficial effect had a mean birth weight of 1600 g, and the control group, 2100 g. This difference is in the right direction to support a beneficial effect, since the smaller babies would be expected to have more infection. I think it was the very low-birth-weight infants who profited most from this inoculation therapy. We will continue this study, although we had 50 infants in each group, and for me the results are convincing. It is worth remembering that we now have to pasteurize the milk we obtain for the human milk bank. Formerly, this milk was given raw and would have contained bifidobacteria. Now that it has to be pasteurized, the bacteria die. Therefore, it is essential in my opinion to inoculate preterm infants with bifidobacteria because of their high risk of infection in neonatal intensive care units.

Dr. Isolauri: To be able to claim that your treatment is effective, you must first define the rate of septicemia in your unit.

Dr. Heine: I cannot tell you the exact incidence of septicemia, but the incidence was not influenced during the period between birth and the day when the bifidus dominance was obtained. So there was a relatively high incidence of septicemia in both groups, but after the establishment of the bifidus microflora dominance, there were no more cases.

Dr. Zoppi: In your conclusions, you said that adapted infant formulas should have a lower protein content. I do not agree with this statement. The aim of an infant formula is not to feed the bifidobacteria, but to feed the infant!

Dr. Heine: The protein content of current infant formulas is almost 200% of that of human milk. We have to give these high concentrations to make sure that the baby receives sufficient essential amino acids. And the limiting amino acids are most importantly tryptophan, but also cystine and others. What we suggest is to supplement formulas with \(\alpha\)-lactalbumin, at least in infants at risk, which will allow us to lower the protein concentration to the lower limit of the recommendations, that is, 1.28 g protein per 100 ml. So it’s a question of quality. We are capable of producing formulas that are identical in amino acid composition and pattern to human milk, but so far this has not been done.

Dr. Freter: Indigenous anaerobes are said to be maintained by endogenous nutrient forces. However, it appears that their numbers decrease substantially in starving animals of different species. If I heard you correctly, you said that the proliferation of epithelial cells is increased by foreign proteins. Is it known whether other possible nutrients for anaerobes are increased too, such as mucus production?

Dr. Heine: These data have not been well elucidated. Only two investigators have claimed that there is a correlation between feeding foreign proteins to newborn rats and guinea pigs and increased proliferation rates of the enterocytes (1,2). It is not known whether this is the case in human infants. However, in infants suffering from malnutrition because of reduced food intake, the microflora is certainly involved, and this is explainable on the basis of lack of substrate for the anaerobic microflora.
Dr. Freter: In malnutrition, the anaerobic population decreases substantially, which is why the enterobacteria increase; usually they’re inhibited by the anaerobes.

Dr. Heine: In earlier studies, we have also shown that these bacteria make use of the urea in human milk (3,4). If you label urea in human milk with $^{15}$N, the label is found within the bacteria; so they utilize this nitrogen source for producing amino acids, nucleotides and so on. That is very interesting.

Dr. Cezard: I’d like to make a comment about hydrolysates from a nutritional point of view. We need to be careful about hydrolysates because there are many factors that interfere with their nutrition value. This depends on the origin of the protein, as you said. It also depends on the hydrolysis technique—whether you use papain or trypsin or pepsin plus trypsin, and so on—and on the degree of hydrolysis. There have been many studies on these factors that have shown that the absorption rate and the nutrition balance are very different—even when you use the same proteins—depending on the technique and degree of hydrolyzation.

Dr. Heine: I fully agree. Of course, we have to analyze the amino acid pattern of the hydrolysates we use for nutritional purposes. If the pattern is correct and compatible with that of human milk protein, I do not see any difficulties in using hydrolysates.

REFERENCES