Nutritional Importance of Non-protein Nitrogen

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The class of compounds called non-protein nitrogen (NPN) is not a homogeneous group of similar chemicals but rather an operational term for the remainder of nitrogen in milk and formula once the protein fraction has been removed. NPN therefore consists of widely diverse types of compounds, ranging from urea to complex nitrogen-containing oligosaccharides. It is obvious that the capacity of an infant to utilize these compounds and the potential physiological effects that they can exert will also vary considerably.

CHARACTERIZATION OF NPN IN BREAST MILK

Human milk was early found to have a large proportion (about 20–25%) of total nitrogen in the form of NPN (1,2). It should be noted, however, that the absolute concentration of NPN in breast milk is similar to that of cow’s milk; the reason for the high relative percentage is rather the low protein concentration of human milk, making total nitrogen considerably lower than in cow’s milk (3).

The overall concentration of NPN in human milk does not vary significantly during the lactation period (4); the concentration is usually about 0.4–0.5 mg N/ml (Table 1). However, as a relative percentage, NPN comprises about 5–10% of the nitrogen in colostrum and in milk produced during early lactation, but 20–25% in mature milk.

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<th>Table 1. Total nitrogen and NPN in human milk</th>
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<tr>
<td>Total N (mg N/liter)</td>
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<td>----------------------</td>
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<tr>
<td>Preterm milk</td>
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<tr>
<td>Colostrum</td>
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<td>Mature milk</td>
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* Mean values from several studies (reviewed in ref. 9).
This is due to the high protein concentration of colostrum and early milk, not to a low concentration of NPN. Similarly, human milk from women delivering premature infants has a low percentage of NPN due to the higher protein concentration of the milk (so-called preterm milk) (5). Some effect of maternal protein intake on NPN has been observed: women having a high proportion of protein in their diet had a higher concentration of NPN in their milk, part of which was due to a higher level of urea (6). The time after ingestion of a meal by the mother can also affect milk NPN levels (7). Postprandial increases in milk NPN can be more than 30% above baseline levels (Fig. 1).

**FIG. 1.** Postprandial changes in milk NPN. Concentrations were normalized by the premeal values for each subject. Bars represent the mean (±SEM) for all subjects. [From Donovan SM, et al. (7)].

![Gel filtration chromatography of human whey on Sephadex G25. (From Donovan SM, & Lönnerdal B Am J Clin Nutr 1989; 50: 53–57.)](image)
Since NPN is operationally defined as non-protein, this milk fraction can be isolated and studied by removing all proteins from human milk. This can be achieved by gel filtration chromatography, ultrafiltration, or dialysis, but gel filtration has been shown to be the most efficient (8). It is important to note, however, that proteins are defined as polypeptides with molecular weights higher than about 6000 Da. This means that smaller polypeptides, dipeptides, and amino acids are part of the NPN fraction, even if the net result of digestion is that proteins and polypeptides in the NPN fraction all will yield dipeptides and amino acids available for absorption.

Practically, NPN is usually analyzed by precipitation of milk proteins by trichloroacetic acid (TCA) at 12% (wt/vol) and analysis of nitrogen in the supernatant (9). Results from this method are very similar to those obtained by gel filtration, while ultrafiltration and dialysis underestimate the NPN fraction (8). When separating human whey on Sephadex G-25, three peaks can be detected (Fig. 2). The first contains all the proteins and no NPN, while peaks 2 and 3 contain only NPN compounds. Of the NPN peaks, the first (peak 2 in the chromatogram) contains substances of higher molecular weight than the second and constitutes about 65% of all the NPN (Fig. 3). This fraction is likely to contain larger peptides (1000–6000 Da) and nitrogen-containing oligosaccharides. The last peak contains smaller compounds (<4000 Da), including free amino acids (among them taurine), small peptides, urea, and so on (Fig. 4). The identity of many of these NPN compounds remains to be established.

**NPN IN INFANT FORMULA**

Since cow's milk is generally considered to be low in NPN (2%), formulas based on skim milk powder and whey protein have often been assumed to contain only very...
small amounts of NPN. While this is correct to some degree for casein-predominant formulas, which usually contain about 5–7% of total nitrogen as NPN, whey-adjusted milk formulas can have much more NPN, depending on the whey protein source used (10). Thus the NPN fraction of whey-predominant formula can vary from 5% up to 16%. Ultrafiltered whey (UF whey) contains the lowest proportion of NPN, 6–8%, followed by electrodialyzed whey (ED whey), 14–18% NPN, and finally, ion-exchanged whey with the highest NPN content, about 26% (Table 2). For the latter two whey protein sources, urea constituted 65–82% of the NPN content. Since the protein content of formula often is determined by the nitrogen content multiplied by a factor (6.25 or 6.38), the true protein content of a formula is overestimated considerably (Table 3) if a significant part of total nitrogen consists of NPN (11). As term infants cannot utilize urea nitrogen to any significant extent (see below), this overestimation of formula protein can have important implications with regard to fulfilling amino acid requirements of infants.

The proportion of peptide and free amino acid nitrogen in formulas also varies with the raw materials used. Some formulas using whey protein have relatively high

<table>
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<th>TABLE 2. NPN in formula protein sources (%)</th>
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<tr>
<td>Electrodialyzed (ED) whey</td>
</tr>
<tr>
<td>Ultrafiltered (UF) whey</td>
</tr>
<tr>
<td>Ion-exchanged whey</td>
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<tr>
<td>Skim milk powder</td>
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TABLE 3. "True protein" level of infant formulas (g/liter)

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<tr>
<th>Ingredient</th>
<th>Analyzed</th>
<th>Label claim</th>
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<tr>
<td>Enfamil</td>
<td>12.8</td>
<td>15</td>
</tr>
<tr>
<td>SMA</td>
<td>13.8</td>
<td>15</td>
</tr>
<tr>
<td>Similac</td>
<td>13.7</td>
<td>15</td>
</tr>
<tr>
<td>Milkotal 2</td>
<td>11.1</td>
<td>13</td>
</tr>
<tr>
<td>Babysemp 2</td>
<td>12.6</td>
<td>13</td>
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proportions of peptides, at around 100–160 μmol/liter, while casein-predominant formulas contain about 40–50 μmol/liter (10). As a comparison, human milk (and cow’s milk) contains about 80–90 μmol of amino acid nitrogen as peptides per liter. The proportion of free amino acids also varies but is much less than of peptides, at about 7–12 μmol/liter. These values should be compared to the total concentration of amino acid nitrogen in formulas (1700–2500 μmol/liter) and in human milk (1300 μmol/liter).

UREA

The main constituent of NPN in human milk is urea; about 0.18 mg/ml urea nitrogen is present, which is about 50% of total NPN (10) (see above). During early lactation, urea levels can be somewhat higher (12). Cow’s milk contains about 0.08 mg/ml urea nitrogen, while formulas have concentrations of 0.04–0.19 mg/ml, depending on which whey protein source is used.

Since the protein content of human milk is believed to be close to the estimated protein requirement of infants and urea nitrogen is a significant part (~12%) of total nitrogen in human milk, it has been speculated that urea could be a utilizable nitrogen source for the infant (13,14). Using stable isotope labeling ($^{15}$N,[$^{15}$N]urea) to trace the metabolic fate of dietary urea, it was found that healthy term infants retained about 13% of the labeled urea nitrogen (15,16). This retention was independent of whether the infant was given the labeled urea in breast milk (16) or in infant formula (15). It is possible that the proportion of urea nitrogen utilized by the infant is dependent on its nitrogen requirement. It has been shown that term infants recovering from infection (and therefore having higher nitrogen requirements than normal) can utilize a considerably higher fraction of urea nitrogen, around 39–43% (13,14). Low birthweight infants, who also have a high nitrogen requirement, were shown to retain about 28% of labeled urea nitrogen (17). Thus part of the urea nitrogen can be utilized by infants, although this fraction is relatively small in term infants (Table 4). The retained urea nitrogen can also be utilized by intestinal microorganisms (19), serving as a substrate for protein and nucleic acid synthesis by the gut microflora—up to 7% of labeled urea nitrogen was found incorporated into fecal bacteria in one study (13). However, part of urea is probably converted to ammonia by bacterial and mucosal enzymes and then metabolized by the infant. In summary, it is likely that the...
degree of NPN utilization will be dependent on the growth rate of the infant, the amount and quality of dietary protein given, and the composition of the microflora.

NITROGEN-CONTAINING CARBOHYDRATES

The nitrogen-containing oligosaccharides and the smaller amino sugars in breast milk have received considerable attention, due primarily to their effect on intestinal bacterial growth, particularly on lactobacilli. Since the finding of a high degree of colonization of breast-fed infants with *Lactobacillus bifidus*, there has been an intense search for the so-called "bifidus factor" (20). Although the difference in intestinal bacterial flora between breast-fed and formula-fed infants may be less pronounced than was earlier believed, several amino sugars have been shown to stimulate the growth of *L. bifidus* (20). It should also be noted that there are many different oligosaccharides in human milk, some of which have very complex structures (21). Many of these have not yet been characterized and the potential biological activity has been evaluated for only a few.

All the milk oligosaccharides terminate with lactose or *N*-acetyl-lactosamine at the reducing end (20) and are classified as acidic or neutral, based on the presence or absence of *N*-acetylneuraminic acid (NANA, sialic acid). Many milk oligosaccharides contain the basic lacto-*N*-tetrose sequence (Gal-GluNAc-Gal-Glc, or a derivative), which has been shown to be a potent "bifidus factor" (20). As these oligosaccharides are not precipitated by acid, they are found in the acid-soluble NPN fraction of human milk.

The concentration of NANA in human milk changes considerably during lactation (22); NANA content in the NPN fraction is as high as 1100 mg/liter in early lactation.
and then falls to about 100–150 mg/liter in mature milk (Fig. 5). N-Acetyleneuraminic acid in human milk is found both in oligosaccharides and glycoproteins; oligosaccharide NANA appears to be the dominant form, particularly during early lactation (Table 5). Thus NANA constitutes about 10% of the NPN in colostrum but only 1.5% in mature milk. In addition to the possible role of NANA as a nitrogen source, it has been shown that brain ganglioside synthesis in young animals is modified by dietary NANA, suggesting a possible role for milk-derived NANA in early infancy (23). Liver enzymes needed for NANA biosynthesis have low activity in the newborn period (24), emphasizing a possible need for an exogenous supply of NANA. Since cow’s milk and infant formulas contain much less NANA than human milk, breast-fed infants may benefit from this supply during early life.

Glucosamine nitrogen (GluN) contributes a significant part of NPN in human milk. Similar to NANA, the concentration of GluN is higher in early lactation than in mature milk and has been found to contribute as much as 30% of NPN (9); however, this value should be viewed with caution as no free glucosamine (or galactosamine)
is found when performing analysis of free amino acids in human milk. It is possible that the method of preparing the NPN fraction, in this case acid precipitation, liberates GluN from oligosaccharides and glycoproteins. Concentrations of GluN have been reported to be about 250 mg N/liter and that of galactosamine (GalN) to be about 15 mg N/liter (9,23). The considerably lower proportion of GalN is in agreement with its lower abundance in milk oligosaccharides and glycoproteins. Infant formulas contain very small quantities of GluN and GalN (8). This difference in GluN intake of breast-fed and formula-fed infants may also affect the growth of *L. bifidus*. The fate of GluN in the infant's gut remains uncertain. It is possible that it is absorbed and serves as a nitrogen source for synthesis of non-essential amino acids. Another possibility is that GluN is utilized within the enterocyte for intestinal glycoprotein synthesis. Furthermore, intestinal bacterial may utilize GluN for their growth and make this nitrogen source unavailable to the infant. Studies with labeled GluN are needed for a better evaluation of these different possibilities.

NUCLEOTIDES, NUCLEIC ACIDS, AND POLYAMINES

Nucleic acids have been reported to be present in human milk at concentrations of 100–5600 mg RNA/liter and 10–120 mg DNA/liter (25-28). Most of these nucleic acids are likely to originate from intact or lysed cells in the breast milk; in agreement with this, colostrum, which contains many more cells than mature milk, has a much higher nucleic acid concentration. Whether ribonuclease activity in human milk contributes to the degradation of RNA to nucleotides is not yet known. However, it is well known that human milk contains significant concentrations of preformed nucleotides (25-28). Most of these nucleotides appear to be monophosphates (AMP, CMP, IMP, GMP, UMP) and diphosphates (UDP, ADP, GDP), but cyclic nucleotides (cAMP, cGMP) are also present (25,26,29). Although the amount of nitrogen provided by nucleotides in human milk is low (~3 mg N/liter), it has been proposed that these nucleotides may play a physiological role under situations of nutritional stress. This aspect of nucleotides in human milk and their potential effect when added to infant formula is described in detail in the chapter by Uauy and Quan.

Polyamines such as putrescine, cadaverine, spermidine, and spermine have been described and quantitated in human milk (29). Although a physiological role of these compounds cannot be excluded, their concentrations are very low and the amount of nitrogen provided by them (0.05–0.2 mg N/liter) is minute. It is possible that their main role(s) is in milk synthesis within the mammary gland.

BIOLOGICALLY ACTIVE PEPTIDES

The peptide fraction of NPN constitutes about 60 mg of N/liter, which is about 4–5% of total amino acids in human milk (9,30). In this fraction, many peptides with known or suggested biological activity are found; epidermal growth factor (EGF), insulin, insulin-like growth factor (IGF), nerve growth factor, prolactin, calcitonin,
and so on, have been reported in human milk at low but physiologically significant concentrations (31–34). Several of these have been shown to be absorbed by suckling rat pups (33); however, little is known about their fate in the gastrointestinal tract of human infants and the potential physiological effects in the infant. It should also be recognized that several of these peptides may exert their physiological effect within the mammary gland (34).

CHOLINE AND OTHER AMINO ALCOHOLS

Unesterified choline, phosphatidylcholine, and sphingomyelin have been found in human milk (35). About 3–9 mg N/liter of the NPN is derived from unesterified choline; the other forms are more likely to be associated with the lipid fraction. The concentration of choline (unesterified) is higher in early lactation (600–700 μmol/liter) than in mature milk (100–200 μmol/liter), but does not vary during a feed (35), showing that it is found in the water-soluble fraction. In contrast, choline-containing compounds are much higher in hind-milk, demonstrating their association with lipids (which also are higher in hind-milk). Concentrations of these compounds do not show the same pronounced changes during lactation as that of unesterified choline (Fig. 6). Choline from human milk provides an infant with about 6 mg/kg/d, which is similar to the adult diet. Choline has not yet been defined as an essential nutrient; however, it has been suggested that the premature infant may benefit from endogenous choline.

Other amino alcohols, such as phosphoethanolamine, phosphoglyceroethanolamine, and phosphoserine, have been detected in human milk (5,12). Accurate analysis

of these compounds is difficult and little is known about the physiological significance of the contribution of these compounds from the diet.

CREATININE, CREATINE, URIC ACID, AND AMMONIA

The presence of creatinine, creatine, uric acid, and ammonia in human milk has been well documented (9,30). The amount of nitrogen provided from all these compounds together is very low and their contribution to the total NPN fraction in human milk is minor. There have been no indications of any physiological significance of these compounds provided from the diet.

CARNITINE

Carnitine is found in the milk of all species and its concentration does not appear to be affected by maternal diet (36). It is a quarternary amine which is essential for the transport of long-chain fatty acids into mitochondria for ß-oxidation (37). In human milk, most of the carnitine (80%) is present in free form. The amount of nitrogen provided by carnitine in human milk is about 1.5 mg N/liter. It has been suggested that breast milk or milk-based formula could supply adequate quantities of carnitine to infants with limited capacity to synthesize endogenous carnitine (37). Soy formulas are naturally low in carnitine, and infants fed such formulas were earlier found to have low plasma carnitine levels (38). This led to supplementation of soy formulas with carnitine.

CONCLUDING REMARKS

The NPN fraction of human milk contains many compounds of diverse chemical composition. Several of these, like some peptide hormones and nucleotides, may act as growth factors, while others, like oligosaccharides and amino sugars, may influence the intestinal microflora. Other compounds, such as urea, may be of little significance for the healthy term infant but could be of importance as a nitrogen source for the compromised infant. The biological activities of several NPN compounds remain to be explored.

REFERENCES


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DISCUSSION FOLLOWING THE PRESENTATION OF DR. LÖNNERDAL

Dr. Garlick: I would like to comment on the $^{15}$N experiments that have been reported where, from the fact that some of the labeled nitrogen appeared in amino acids and protein, it was concluded that urea nitrogen was utilized. This is equivalent to giving labeled bicarbonate or labeled CO$_2$—you will find the label in many body constituents but this doesn’t mean that CO$_2$ is in any way being utilized as a nutrient. The fact that $^{15}$N gets into the components of protein metabolism can be explained by exchanges during transamination; it does not mean that it has been used as a nutrient. Other experiments will be needed to prove that.

Dr. Lönnerdal: That is a valid point. While we are certainly no experts in the labeled isotope field, we used well-established methodology. We found only 20% utilization, and this is the maximum value, not the real value.

Dr. Raihä: I would like to point out that the very high non-protein nitrogen (NPN) you showed in the Swedish formula—I think it was 14% or 15%—was changed about a year ago when the manufacturers changed their source of whey protein. It is now under 10%. Your analysis was done with the old formula. This may be important. We have done studies with old formulas that had NPN values well over 10%, which means that the protein intake in the infant was probably much lower than we thought and they were still growing perfectly normally, with normal plasma amino acids and urea. When formulas are designed we need to look at the NPN fraction.

Dr. Lönnerdal: Many of these clinical studies just cite the protein level claimed on the label. It is impossible to judge the NPN from that.

Dr. Marini: You showed that urea was higher in whey protein formulas than in casein-based products. Could Dr. Guesry tell us how Nestlé prepare the whey?

Dr. Guesry: NPN (urea, creatinine, and so on) comes mainly with the whey and less with the casein. Since for term baby formulas, though not for premature formulas, we use 100% whey, this means that the quantity of NPN will be higher than in standard formulas containing a mixture of whey proteins and casein.

Dr. Marini: Another company has estimated that the urea content in a hydrolysate formula is about 5% of the NPN.

Dr. Uauy: When we consider NPN we should spend some time looking at the biological activity of some of these compounds as well as at their role as potential sources of nitrogen. What is your view as to the potential significance of some of these biologically active compounds? The fact that they are present doesn’t mean that they play a nutritional or metabolic role. Which compounds do you think we should be focusing on? Should we consider the possibility of mass producing some of these compounds? Theoretically, they could be added to formulas.
**Dr. Lönnertal:** I think there is strongest evidence for biological activity among the peptides (epidermal growth factor, EGF, for example) and the nitrogen-containing oligosaccharides. The latter are now being produced by genetic recombinant techniques and also by carbohydrate synthesis. We must await more clinical studies, but my bet would be that these compounds have biological importance. There is speculation that some non-protein compounds may be important for the premature newborn infant but I haven't seen any data that would convince me that they are important for the term infant.

**Dr. Rassin:** You raised the issue of glycosylation precursors. We need to think a little bit more about these in view of the suggestion that we may eventually achieve transgenic protein production. Even if we make a cow produce a human protein, a cow is still going to glycosylate like a cow. I think this illustrates some of the complications in trying to make human milk from another species. The glycosylation precursors and enzymes are going to be different in the cow than in the human. If you really want to make transgenic human milk, not only will you have to produce human caseins and human whey proteins but you will have to produce human glycosylation proteins and human glycosylation precursors. This is a good illustration of how complicated that kind of a product is going to be.

**Dr. Lönnertal:** I agree. It is all becoming very complex, but it is quite certain that bovine recombinant glycosylated protein will never be like the human counterpart. We do, however, have proteins like a-lactalbumin which are neither glycosylated nor phosphorylated. Some of the caseins are phosphorylated and the bovine mammary gland can probably phosphorylate in a fashion similar to the human mammary gland. With regard to the glycosylated proteins, we have shown that the carbohydrate side chain is irrelevant for lactoferrin binding to the receptors, and in that sense it is not really important. On the other hand, the glycan of human lactoferrin may be part of the reason why human lactoferrin is less digested than bovine lactoferrin, by protecting it against proteases. It may not therefore be totally irrelevant.

**Dr. Pettifor:** What role do you believe the active peptides, particularly EGF, may play in maintaining or improving gut function, particularly in the premature infant, and do you have any comments on the role of some of the newer peptides, such as PTH-related peptide, which is present in high concentration in breast milk?

**Dr. Lönnertal:** Starting with EGF, Leanna Read (unpublished) has done a nice set of experiments in premature newborn rhesus monkeys where she gave human recombinant EGF *in utero* and also postnatally and then looked at maturation. The gut mucosal protein growth (DNA and RNA) was significantly enhanced and lung maturation was improved. I think that the evidence is fairly strong that the EGF may enhance the maturation of certain organs.

The PTH-related peptide is very fascinating. Why is there so much in breast milk? I have not seen many studies on its potential function in the infant.

**Dr. Uauy:** One general point regarding the biologically active peptides is that, in order for them to be active, they need to be preserved from digestion and absorbed intact, otherwise you would have just a local effect. For EGF this makes sense because one can envisage it acting locally. What is your view about the potential for intact absorption of some of these peptides, for example insulin, insulin-like growth factor, thyroid hormones, and so on?

**Dr. Lönnertal:** Many of these compounds do not occur in milk in the free form but are associated with binding proteins. I am just speculating here, but I look on these binding proteins as a kind of bait for proteases; they are attacked first, so to speak, and therefore may provide some protection for the active peptides down the gut. Most of the studies that have been done have been in suckling rat pups, and several peptide molecules have been able to survive gut transit in this species. I have seen very little yet to convince me that the
same occurs in the human infant, but with duodenal intubation one could potentially look at the
degree to which intact survival may occur in the upper gut, with the possibility of subsequent
absorption.

Dr. Rassin: When we looked at some of our own amino acid constituents we found that
a large proportion of the cysteine and glutathione is actually bound to the protein during TCA
precipitation. Tryptophan could behave like this as well. Sometimes I wonder whether we
underestimate the NPN fraction through losing some of it during TCA precipitation or in
filtration types of separation. About 85–90% of glutathione in human milk would be precipi-
tated by TCA and would not appear in the NPN fraction.

Dr. Bremer: The proteins of the fat globules contain some enriched nutrients such as
selenium. No one has mentioned the proteins of the fat globules. Do they play a role in
addition to selenium or are these constituents waiting to be investigated?

Dr. Lönnerdal: Fat globule proteins are specialized carbohydrate-rich high molecular
weight structures with similarity to some of the glycocalix proteins. There is speculation that
they could have an effect on both bacterial proliferation and bacterial attachment, but there is
little evidence for this at present.

Dr. Pettifor: Would you like to speculate on what infant food manufacturers should be
looking at as far as supplementation of cow's milk formula is concerned? Which of the non-
protein nitrogen sources do you believe are the most important to be considered for inclusion
in a cow's milk formula?

Dr. Lönnerdal: If I were a company I would step very carefully. They should wait until
there is conclusive evidence for biological activity and only then consider supplementation.
There is always likely to be the eternal dilemma that we had in the lactoferrin field; it is very
difficult to show benefit in industrialized societies because the general level of health is high.
You have to have huge cohorts in order to show biological activity. In developing countries,
on the other hand, there is almost no limit to the number of confounders. Urea is not high
on my list of possible additives, but there may be peptides and carbohydrates that will be
proven to be of value.

Dr. Rassin: One NPN constituent that is widely supplemented is taurine. We could proba-
bly spend a couple of hours debating whether this is only window dressing. It is very difficult
to prove that it has any useful function, yet it is a widely used supplement.

Dr. Marini: The same thing is happening with carnitine. We found that normal full-term
neonates fed with soya milk, which is devoid of carnitine, can synthesize carnitine very well.
The problem of carnitine deficiency is probably limited to preterm babies.

I should like to ask your opinion about the peptides and other substances. Some people
say that polyamines may be important for closure of the intestinal barrier. It has been sug-
gested that they could be of value in the prevention of allergy by promoting gut closure. I
am also concerned about growth-promoting factors for infants born with intrauterine growth
retardation. There is evidence that maternal blood levels of insulin-like growth factor (IGF)
are low in pregnancies complicated by fetal growth retardation. It has been claimed that the
possibility of catch-up growth in these babies can be predicted by IGF levels in the blood
after 15 days or 1 month of age (1).

Dr. Lönnerdal: I believe, although I am not an expert on this, that gut closure is something
that happens very quickly. Whether or not it can be accelerated by feeding formula with
polyamines I do not know.

With regard to the other part of your question, there are more and more studies on IGF-
I and IGF-II and their importance for growth. For example, synthesis of IGF-I is dependent
on zinc supply and there may quite often be an inadequate zinc supply during pregnancy and
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lactation. Therefore, there may be confounding variables, but nevertheless, IGF appears to be a very important component.

Dr. Guesry: Cow's milk whey is full of carnitine and all infant formulas based on cow's milk contain a level of carnitine equal to or higher than breast milk. It is only in soy formulas that you need to add carnitine. To add it to cow's-milk-based formula is nonsense.

Dr. Heine: What are the practical consequences of your findings that the protein concentration in some formulas is below 12 g/liter, when most international committees on nutrition limit the lowest value of the protein concentration to around 12 g/L?

Dr. Lönnerdal: Most companies do not need to make any changes. They are at present fairly close to the level where they should be. Their protein values are lower than they believe them to be since they are used to thinking in terms of crude protein rather than true protein. Niels Raihã has evidence that even the lowest levels found in commercial formulas will be adequate. I think that based partly on Fomon's early studies, a protein concentration of 11 or 12 g/liter of true protein may well be the lower limit.

Dr. Rassin: With regard to soy formulas, how much does the non-protein nitrogen fraction reflect the methionine that has to be added to improve the protein quality?

Dr. Lönnerdal: It is part of it. I can't tell you how much.

Dr. Rassin: I think it is likely to result in a very different NPN fraction from that seen with other milks.

Dr. Heine: We have done some studies on the sialic acid concentration in different formulas. Soy formulas were found to have only 3% of the human milk sialic acid concentration. This may be crucial in the nutrition of low birthweight infants. You pointed out a connection between the development of the brain and the presence of sialic acid as a central constituent in the synthesis of gangliosides and glycoproteins.

Dr. Pandit: Since we have digressed from animal proteins to soya, I wonder what would be the status of other proteins, for example chicken- or meat-based protein. Would these be a good alternative? We have tried chicken-based formula in low birthweight babies and the clinical tolerance and weight gain have been excellent. The osmolarity comes to about 217 mOsm/liter and the protein content is 2.5 g per 100 ml. Protein utilization is almost 90%, and amino acid analysis is satisfactory. In India there is an enormous amount of poultry meat available and it is fairly cheap.

Dr. Marini: In Italy we have used lamb-based formulas over the past three decades, even in small infants as little as 2 weeks old, when there is severe food intolerance. We find it very effective.

Dr. Guesry: We are always on the lookout for good-quality chicken meat powder, but it is very difficult to find because it has either been heat treated (and the heat treatment has usually impaired the protein quality) or it has not been sufficiently heat treated and is full of salmonella.

Dr. Pandit: I have been working on this for the last 4 years. I can assure you it is possible to produce good-quality dried chicken meat protein with current technology without salmonella. However, I still don't know what its role is in human nutrition.

Dr. Househam: Developing countries cannot afford to buy commercial hydrolysates; they are simply too expensive to use, particularly for children who have chronic diarrhea who need rehabilitation. We have also made extensive use of chicken-based formula and the children do extremely well on it. I think this is an issue that needs to be addressed because chicken meat powder is cheap and can be used as the basis for a cheap formula which has a real application in the developing world.
Dr. Rassin: There are one or two things that you might want to look at in relation to chicken meat. One is that chicken muscle has more post-translational changes in its amino acids, for example histidine, than other kinds of proteins, and I have no idea what this does. Another thing is that chicken muscle has an enormous carnitine pool and the non-protein nitrogen pool originating from chicken sources would be very different from cow’s milk.

REFERENCE