Plasma amino acids in small preterm infants fed on human milk or formula

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SUMMARY Plasma amino acids were measured in 35 preterm infants, of whom 11 weighed less than 1000 g and 24 weighed between 1000 g and 1500 g at the time of sampling. Repeat samples were obtained in 18 at least seven days later. Seventeen infants were fed with preterm formula milk and 18 with expressed maternal breast milk at one to two hourly intervals during the study period. Formula fed infants gained weight faster than those fed on breast milk but there was little difference in amino acid patterns. Infants fed on breast milk were more likely to have concentrations of essential amino acids below the mid trimester fetal range than formula fed infants, but few infants in either feeding group had values above the fetal range. Those that did were equally distributed between both groups. Only two samples approached toxic concentrations, both in the group fed breast milk. The ratio of branched chain to aromatic amino acids was higher in the group fed on formula after correction for post conceptional age, implying that liver maturation may be accelerated by formula feeding. No correlations were found between plasma amino acid concentrations and nitrogen retention or metabolisable energy intake.

The survivals of very small preterm infants are improving all the time. It is common now for babies weighing less than 1000 g at birth to survive, and there is a steadily increasing chance that an infant born weighing less than 750 g will leave hospital well. There are many problems in looking after such babies, not the least being those associated with metabolic immaturity, which makes the type, amount, and route of administration of nutrients critical. Because it is important that infants whose central nervous system is in a state of rapid development should receive adequate nutrition, the clinical management of such babies must strike the right balance between the provision of nutrients in adequate amounts and quality for optimal growth and the possibility of harming the baby by giving more than can be satisfactorily metabolised.¹

It is still uncertain whether very immature infants are best nourished with their own mothers' milk or with a specially designed formula.²⁻⁴ Preterm mothers' milk may often have quite high protein concentrations,⁵ and preterm formulas are designed to have higher protein concentrations than mature breast milk or formulas for full term infants. In either case protein intake by a very immature infant could be too high when fed at the volume of intake generally recommended (180 ml/kg/day), leading to

undesirable or dangerous concentrations of potentially toxic amino acids,⁶ hyperammonaemia,⁷ and metabolic acidosis.⁸ Harmful concentrations of one or more amino acids in the blood of a very immature infant can occur without any clinical evidence that the infant is in difficulty, and the smaller and more immature the infant, the more likely are such disturbances to be silent. High plasma concentrations of amino acids have been reported previously in relatively mature preterm infants fed on high protein formulas.⁹⁻¹¹

Although preterm formulas are now widely used in neonatal nurseries, there are few data on their effects on plasma amino acid concentrations, neither are such data available for very small infants fed on breast milk. The purposes of this study were to examine the range of plasma amino acids in infants of very low weight (<1500 g) and extremely low weight (<1000 g) to find out whether potentially toxic concentrations occurred with breast milk or formula feeding; to try to determine from the plasma aminograms whether there was any evidence of protein malnutrition; and to see if it was possible to find any associations among plasma amino acid patterns and nitrogen balance, energy balance, and growth rate or maturation, or both.

Patients and methods

We studied 35 preterm infants of whom 11 weighed less than 1000 g and 24 weighed less than 1500 g at the time the first blood sample was taken. All were managed in the neonatal unit at this hospital throughout the study during which 17 were fed on preterm formula milk (15 on SMA LBW, one on Prematalac, and one on Preaptamil), and 18 on expressed breast milk mainly obtained from their mothers. With the exception of three very small infants in the group fed expressed breast milk, no infant was studied until he was on full enteral feeding at an intake of 180–200 ml/kg/day. The three exceptions had been fed expressed breast milk from birth but had not yet reached full enteral intake at the time the first blood sample was taken. Table 1 shows composition of the formulas. The composition of the breast milk was not determined except during balance periods when nitrogen and energy were measured. Feeds of 180–200 ml/kg/day were given by nasogastric tube at one to two hourly intervals.

Gestational age was determined from the menstrual history and from the results of ultrasound measurements performed before 20 weeks' gestation. In cases of doubt Dubowitz's postnatal assessment was used but reliance was not placed on this in infants of less than 29 weeks' gestation. None of the infants had any congenital abnormality and none was in a critical condition at the time of blood sampling.

The weights at the times of sampling were obtained from the weight record, all infants being weighed daily on a Marsden spring balance (Marsden Weighing Machines Ltd, London) accurate to within 5 g. The rate of weight gain at each blood sampling date was calculated from the weights recorded for seven to 14 successive days (including the date of sampling) during which the type of

feeding was not changed. The line of best fit and the slope representing the rate of weight gain were computed by the method of least squares, and weight gain was expressed as g/kg/day using the median weight as the reference.

The blood samples were taken when blood was needed for clinically important investigations. All samples were taken shortly before a feed was due to be given, and never more than two hours after the previous feed. The study had the approval of the hospital ethical committee and the permission of the mothers was obtained before blood sampling.

Blood samples for amino acid analysis were obtained from all infants. In nine infants from each feeding group samples were obtained on a second occasion at least seven days after the first, a total of 53 amino acid profiles being obtained. Most of the blood samples (volume 100–200 µl) were obtained from peripheral veins but in a few cases heel prick samples were used. All samples were taken into heparinised tubes and immediately separated. Plasma was stored at –20°C until deproteinised and analysed.

Protein was removed from the plasma samples overnight at 4°C with 25 mg 5-sulphosalicylic acid/ml of sample. They were then centrifuged at 8800 g for three to four minutes and the supernatants passed through a 0.22 µm millipore membrane before being returned to the –20°C freezer. Centrifugation and millipore filtration were repeated immediately before amino acid analysis. The preferred volume for amino acid analysis was 40 µl, but smaller volumes had to be used in some cases.

Free amino acids were measured in an LKB 4400 automatic analyser (LKB Biochrom Ltd, Cambridge, United Kingdom) using a lithium buffer system. Hamilton amino acid calibration standards (Hamilton Co, Reno, Nevada, United States of America) were used at a concentration of 10 nmol for most residues. Standards were run before and after each batch of four or five samples. Retention times and peak areas were calculated automatically on a Spectra Physics integrator (Spectra Physics, St Albans, Hertfordshire, United Kingdom).

Amino acid residues in both standard and sample amino acid profiles were identified by their order of appearance and amino acid concentrations were calculated from the printout of the peak area provided by the integrator. Proline and hydroxyproline peaks were measured by hand. Glutamine standard was freshly prepared and loaded separately from the mixed amino acid standard because of its poor stability. The glutamine peak area of some standards from which glutamine had been omitted was calculated from the mean ratio of glutamine to six other amino acids (aspartic acid, asparagine,

Table 1 Composition of preterm formulas (per litre except osmolality)

	SMA LBW	Preaptamil	Prematalac
Protein (g)	20	21	24
Fat (g):	44	36	50
Saturated (%)	49	47	48
Unsaturated (%)	51	53	52
Carbohydrate (g)			
(as disaccharide):	82	83	66
Lactose (g)	41	83	66
Maltodextrin (g)	41	Not given	Not given
Energy (kJ)	335	310	331
Osmolality (mOsm/kg)	268	350	342

glutamic acid, cysteine, ornithine, and arginine) in the standards where glutamine had been included. The mean interassay coefficient of variation for amino acids at 10 nmol in standards run within the same week and with the same batch of ninhydrin reagent was 6.1% (range 3.1–18.7).

Total amino acids comprise the sum of taurine, threonine, serine, proline, glycine, alanine, citrulline, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ornithine, lysine, histidine, and arginine. Tryptophan could not be measured in our assay. Glutamic acid, glutamine, aspartic acid, and asparagine were not included in the total amino acids because storage and analysis affect their concentrations. It was not always possible to measure hydroxyproline, and most investigators do not report ethanolamine, so these were also omitted from the analysis of total amino acids. Cystathionine was not detectable in most of the samples, so we have not reported the results. Total essential amino acids comprise threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, and histidine. Cysteine, arginine, and glycine are considered to be 'semi-essential' amino acids for preterm infants by some authors.^{12–14}

In 24 infants (15 fed on formula and nine fed breast milk) energy and nitrogen balances were also performed. Faeces and urine were collected for three days between carmine markers as previously described,¹⁵ stored at –24°C before analysis, freeze dried and homogenised. Energy was calculated by ballistic bomb calorimetry¹⁶ and nitrogen by Kjeldahl analysis (Kjeltec, Tecator AB, Högenäs, Sweden). Samples of feed were also analysed. Nitrogen retention was defined as the difference between nitrogen intake and measured losses in

urine and stools, not including integumentous losses which are unknown and likely to be small. Metabolisable energy was defined as the difference between energy intake and measured losses in stools and urine.

Comparison of independent samples was carried out by the two tailed Student's *t* test or the Mann-Whitney U test as appropriate. Paired samples were compared by the two tailed Student's paired *t* test or Wilcoxon's matched pairs signed ranks test as appropriate. If significant linear correlations were suspected the regression lines were calculated using the method of least squares. Analysis of covariance comparing formula fed and those fed expressed breast milk for each of the amino acids using birthweight, gestation, and postnatal age as covariates; cluster analysis; and stepwise multiple regression analysis were also performed.

Results

Table 2 shows the ages and weights of the infants by type of feed. There were significant differences ($p < 0.02$) between the postnatal age and weight at sampling of those fed on formula and those fed expressed breast milk, though the mean (SEM) post conceptional age at the time of sampling (gestational age plus postnatal age) was not significantly different being 31.6 (1.9) and 29.9 (2.2), respectively. Smaller and more immature infants were disproportionately over-represented in the group fed expressed breast milk. This was unavoidable because of the need to respect the mother's choice of feeding for her infant, and because of the commonly held view among the nursing staff that expressed breast milk was safer for infants of extremely low birth

Table 2 Age and weight data of infants studied classified by weight at first blood sampling and by type of feed

Measurement	Formula fed (n=17)		Fed on breast milk (n=18)	
	<1000 g* (n=2)	1000–1500 g* (n=15)	<1000 g* (n=9)	1000–1500 g* (n=9)
Mean birth weight (g):	795	1119	870	1164
SD	—	222	167	149
Range	690, 900	850–1520	550–1100	940–1460
Mean gestational age (weeks):	27	28.4	28.2	28.1
SD	—	2.3	2.7	1.2
Range	26, 28	25–35	25–33	27–30
Mean postnatal age at first blood sample (days):	16	24.8	9.6	16
Range	13, 19	6–48	3–29	4–36
Mean weight at first blood sample (g):	810	1297	859	1261
SD	—	118	156	146
Range	740, 880	1020–1480	500–990	1080–1460
Mean weight gain (g/kg/day)	13.2	21.3	10.5	14.6
Range	8.8, 17.5	10.4–31.6	–2.24–22.3	–5.93–25.4

*Weight at time of first blood sample.

weight. No consistent relation could be found, however, between postnatal age, birthweight, or gestation and amino acid concentrations by multiple regression analysis or by analysis of covariance, except in the case of threonine and methionine. Formula fed infants gained weight significantly faster than those fed on expressed breast milk ($p=0.01$).

All infants weighed less than 1500 g at the time of first sampling. Fifteen infants in the formula group and nine in the group fed expressed breast milk weighed between 1000 and 1500 g at this time; two infants fed on formula and nine fed expressed breast milk weighed less than 1000 g.

Table 3 shows amino acid concentrations (mean and range) in the first blood samples obtained from all infants, compared with the amino acid composition of the main formula. There were few significant differences between the feed groups. Threonine ($p<0.01$) and citrulline ($p<0.05$) were present in greater concentrations in the plasma of formula fed infants than in the plasma of those fed on breast milk. Methionine concentrations were significantly higher in the group fed on formula ($p<0.05$) only

when analysis of covariance was performed and not when the uncompensated data were compared. Glutamic acid was higher in the group fed breast milk ($p<0.02$) but because glutamine is easily degraded to glutamic acid when stored the values for these two amino acids tend to be unreliable. Analysis of the sum of glutamine plus glutamic acid showed no significant difference between the groups.

Postnatal age at the time of sampling did not differ between those infants fed on expressed breast milk weighing below 1000 g and those weighing between 1000 and 1500 g (table 2). We found no significant differences in amino acid concentrations between them.

Table 4 shows the highest measured concentrations of those amino acids known to be toxic to immature infants and the ranges found in clinically important hyperaminoacidaemias. Two samples from the group fed breast milk reached the lower end of the clinically important ranges (one for tyrosine, and one for tyrosine and valine). In comparison with Pohlandt's ranges for full term breast fed babies, many infants in our study had raised concentrations of one or more potentially

Table 3 Amino acid concentrations in $\mu\text{mol/l}$, mean (range)

Amino acid	Group fed on formula		Group fed breast milk		SMA LBW formula*
	<1000 g (n=2)	1000-1500 g (n=15)	<1000 g (n=9)	1000-1500 g (n=9)	
Taurine	175 (60, 290)	146 (38-373)	171 (28-360)	187 (58-503)	Not given
Aspartic acid	30 (10, 50)	30 (trace-88)	31 (trace-85)	30 (trace-93)	17 800
Hydroxyproline	42 (30, 53)	52 (trace-87)	41 (trace-133)	54 (trace-110)	Not given
Threonine	197 (178, 215)	434 (49-923)	196 (15-476)	217 (73-473)	11 800
Serine	183 (145, 220)	238 (58-493)	200 (81-436)	244 (75-700)	13 000
Asparagine	63 (58, 70)	78 (46-158)	63 (trace-105)	79 (30-168)	Not given
Glutamine	110 (40, 180)	92 (trace-251)	233 (39-485)	159 (48-308)	31 400
Glutamic acid	333 (195, 470)	403 (158-1110)	259 (112-424)	466 (249-900)	Not given
Proline	164 (118, 210)	248 (144-668)	281 (89-556)	231 (107-633)	17 400
Glycine	224 (218, 230)	321 (160-688)	266 (34-504)	305 (146-625)	6300
Alanine	270 (205, 335)	357 (205-650)	271 (68-464)	333 (105-555)	11 500
Citrulline	13 (trace, 12.5)	36 (10-83)	14 (trace-28)	26 (trace-83)	Not given
Valine	137 (103, 170)	170 (96-323)	168 (62-364)	144 (68-290)	12 600
Cysteine	30 (30, —)	53 (20-110)	54 (23-85)	64 (33-123)	1500+
Methionine	35 (10, 60)	36 (10-88)	25 (4-80)	24 (trace-45)	3200
Isoleucine	26 (13, 40)	72 (44-119)	66 (17-148)	51 (12-123)	10 500
Leucine	73 (60, 85)	126 (70-229)	116 (47-184)	99 (35-230)	18 900
Tyrosine	57 (28, 85)	128 (35-258)	140 (56-320)	141 (49-393)	6100
Phenylalanine	94 (140, 48)	60 (35-115)	78 (25-192)	62 (36-105)	6500
Ethanolamine	65 (trace, 65)	98 (23-165)	101 (trace-223)	117 (48-193)	Not given
Ornithine	99 (175, 23)	138 (53-275)	81 (37-125)	164 (45-658)	Not given
Lysine	223 (380, 65)	180 (120-340)	126 (33-216)	156 (75-345)	14 200
Histidine	82 (95, 68)	84 (48-130)	83 (49-127)	83 (37-160)	3300
Arginine	37 (60, 13)	50 (16-1081)	53 (3-116)	61 (3-128)	4000
Total:					
Essential amino acids	864 (543, 1185)	1184 (733-2095)	855 (373-1708)	831 (384-1770)	Not given
Total amino acids	2093 (1363, 2823)	2899 (2123-4487)	2474 (959-4394)	2569 (1067-5750)	Not given

*Manufacturer's information; +Value for cystine.

Table 4 Highest measured plasma concentrations of potentially toxic amino acids

Amino acid	Highest measured concentration ($\mu\text{mol/l}$)		Disease range ²⁵
	Formula	Expressed breast milk	
Valine	323	364	354-646 (classic maple syrup urine disease)
Methionine	88	80	1930 (cystathione β synthetase deficiency)
Isoleucine	119	148	168-1366 (classic maple syrup urine disease)
Leucine	229	230	1107-4000 (classic maple syrup urine disease)
Tyrosine	258	393	280-2760 (transient neonatal tyrosinaemia)
Phenylalanine	140	192	>1200 (classic phenylketonuria)
Histidine	130	160	200-1160 (histidinaemia)

toxic amino acids.¹⁷ There was no difference between the feeding groups in the incidence of high values. When we compared our results with McIntosh's mid trimester fetoscopy values, we found higher values in our study only for methionine (two samples in the group fed on formula) and tyrosine (four samples in the group fed on formula and six in the group fed expressed breast milk).¹⁸

Low essential amino acids concentrations were found in several infants. Table 5 shows the number of measured values of essential amino acids that were below Pohlandt's¹⁷ and McIntosh's¹⁸ ranges, and the number of infants in whom these low essential amino acids concentrations were measured. Values for glycine were below McIntosh's range in one formula fed infant and three infants fed expressed breast milk.

Whitehead's ratio of essential to non-essential amino acids was calculated from the molar concentrations of glycine plus serine plus glutamic acid plus taurine, divided by those of leucine plus isoleucine

plus valine plus methionine, values above 3.0 being commonly found in kwashiorkor.¹⁹ Of the infants in whom the ratio could be calculated, 10 of 17 in the group fed on formula and six of 14 in the group fed expressed breast milk had values above 3.0. The ratio of glycine:valine was also calculated. Values of greater than 2.0 were found in seven of 17 infants in the group fed on formula and in eight of 17 in the group fed expressed breast milk.

The data were examined for correlations which might have been of biological importance. No significant association was found among the following: weight gain and total amino acids, total essential amino acids, valine:glycine ratio, or tyrosine:phenylalanine ratio; postnatal age and valine:glycine ratio, or tyrosine:phenylalanine ratio; nitrogen retention and valine or valine:glycine ratio; or metabolisable energy intake and valine or valine:glycine ratio. Significant correlations were found in both feeding groups between the concentrations of valine, threonine, and total essential

Table 5 No of low values of essential amino acids in two feeding groups

Amino acid	Values below Pohlandt's range for full term breast fed infants ¹⁷		Values below McIntosh's range for mid trimester fetoscopy samples ¹⁸	
	Formula (n=17)	Breast milk (n=18)	Formula (n=17)	Breast milk (n=18)
Threonine	1	3	3	9
Valine	2	4	9	13
Methionine	2	4	0	2
Isoleucine	1	4	1	4
Leucine	2	4	1	4
Phenylalanine	0	0	0	1
Lysine	2	4	8	11
Histidine	0	1	4	9
No of infants with one or more essential amino acids below the stated range	7	9	12	16

amino acids, and the concentrations of total amino acids ($p < 0.001$ in each case).

The subgroups of nine infants from each feed group in whom second plasma amino acid analyses were carried out had birth weights and gestational ages that were not significantly different from the remaining infants in their feed group. In the group fed on formula their mean (SD) weights at first and second samplings were 1271 g (182) and 1863 (428), and their median (range) postnatal ages were 18 days (6–48) and 37 days (17–88), respectively. In the group fed breast milk the mean weights were 1126 g (217) and 1633 g (403) and the median (range) postnatal ages were 13 days (7–36), and 42 days (21–67) at first and second samplings, respectively. There were no significant differences in birth weight and gestational age, nor in postnatal age, weight, and rate of weight gain, between these two subgroups at either date of sampling.

In the infants fed on formula hydroxyproline ($p < 0.05$) and cysteine ($p = 0.05$) concentrations were higher in the first sample than in the second, and taurine was lower ($p < 0.05$). In the group fed breast milk citrulline concentration was lower in the first sample than in the second ($p = 0.05$), and the valine:glycine ratio was increased in the second sample ($p < 0.05$). When all infants in whom more than one sample was analysed were combined ($n = 18$), the comparison of early and late samples showed only two significant differences in amino acid concentrations: taurine was lower in the second sample ($p < 0.02$) and the valine:glycine ratio was higher ($p < 0.05$). Plasma urea concentration was higher in the first sample than in the second ($p < 0.02$).

The association between plasma concentration and postnatal age was examined for four potentially toxic amino acids using data from early and late samples. For methionine, phenylalanine, and histidine there was no clear association between plasma concentrations and postnatal age. There was, however, a pronounced decrease in the median (range) plasma concentration of tyrosine and in variability as postnatal age increased, declining from 160 $\mu\text{mol/litre}$ (27.5–392.5) in infants less than 13 days old ($n = 8$) to 94 $\mu\text{mol/litre}$ (55–105) in infants over 45 days old ($n = 6$).

Glasgow and Moore described the usefulness of the ratio of plasma branched chain amino acids (valine, leucine, and isoleucine) to the aromatic amino acids (tyrosine and phenylalanine) in determining hepatic maturity, because branched chain amino acids are metabolised mainly in muscle and aromatic amino acids are metabolised in the liver, and their plasma concentrations tend to decline as the liver matures.²⁰ We found no signifi-

cant correlation between this ratio and post conceptional age in either feeding group or in both groups combined. The mean (SD) ratio was, however, significantly higher in infants fed on formula than in those fed breast milk (1.96 (0.44) and 1.54 (0.40), respectively, $t = 2.9$, $p < 0.01$), implying greater hepatic maturity among infants fed on formula. When the branched chain amino acids:aromatic amino acids ratio was corrected for post conceptional age to compensate for the shorter gestational period of those fed breast milk, the mean value was still higher in the group fed on formula ($t = 2.2$, $p < 0.05$).

Discussion

The overall distribution of the amino acids in our two groups was similar to that reported in other studies of preterm infants (mostly less immature), our values (except for threonine) usually coming within the range of the other published values.

There were surprisingly few differences in the aminograms between the two feeding groups, the main ones being the significantly higher concentrations of methionine (when corrected for age and weight), threonine, and citrulline in the group fed on formula, and of glutamic acid in the group fed expressed breast milk. It is doubtful whether the differences in non-essential amino acids have any biological importance and they are likely to be related mainly to differences in intake. Of greater potential importance was the fact that threonine, methionine (corrected for age and weight), and the sum of the total essential amino acids were significantly higher in the formula fed infants. This may be an advantage or a disadvantage for this group, but these infants were less likely to have values below the range found in the mid trimester fetus (table 5) than were the infants fed expressed breast milk.¹⁸ Rigo *et al*, showed that threonine metabolism is impaired in preterm infants and advised a restriction of threonine intake to prevent hyperthreoninaemia.²¹ In none of our samples, however, did threonine concentrations exceed those found in the mid trimester fetus. We did find two samples in which methionine concentrations exceeded fetal values, but neither came near the range found in diseases associated with hypermethioninaemia. Nevertheless, this finding emphasises the need for caution concerning the methionine content of formulas for very immature infants.

Potentially hazardous rises in amino acid concentrations occur only rarely under normal circumstances, even in the smallest infants (below 1000 g) when the protein intake is below 4 g/kg/day. We found only two samples in which potentially toxic

amino acids approached the range of concentrations normally associated with diseases. This finding must be viewed with caution because immature infants, particularly if they have some other metabolic disturbance (such as hypoxaemia or acidosis), might be vulnerable to damage at lower plasma concentrations than are found in mature infants with inborn errors of metabolism. Furthermore, our measurements may have missed the peak postprandial concentrations. It is likely, however, that continued rather than brief and intermittent rises are the most important factors in toxicity, and our results certainly show no evidence of cumulative effects associated with short feeding intervals. It is also reassuring to note that hardly any values exceeded the range of mid trimester fetal values at fetoscopy and analysed in the same laboratory, and those that did were equally distributed between both groups.

Amino acid ratios do not seem to be particularly helpful in determining the state of protein nutrition in very immature infants, in contrast to older infants and children, presumably because plasma concentrations of amino acids in preterm babies are influenced by immature metabolic pathways. Hence Whitehead's essential:non-essential amino acid ratio¹⁹ produced as many 'abnormal' values in the group fed on formula as in the group fed breast milk (and more than were expected from the growth and clinical progress of the babies). The valine:glycine ratio also produced too many 'abnormal' values, and was unhelpful in discriminating small for gestation infants from appropriately grown infants.²²

No significant differences in plasma amino acid concentrations were shown between very low birthweight and extremely low birthweight infants, though the great variability of amino acid concentrations makes it hard to obtain significant differences without much larger numbers. The longitudinal data showed a decline in taurine concentration in formula fed infants (probably representing its utilisation in the synthesis of bile salts²³), and the valine:glycine ratio increased, a change that is more likely to reflect enzyme maturation than improvement in protein nutrition.

We found a significant difference in the ratio of branched chain:aromatic amino acids between the two groups, the ratio being higher in the group fed on formula. Glasgow *et al*²⁰ calculated that this ratio increased with maturation because increasing hepatic metabolism of the aromatic amino acids resulted in lower plasma concentrations in the more mature infants, while the branched chain amino acids changed comparatively little. The higher values of the ratio in our formula fed infants persisted even after correcting for maturity, so there must be some other variable. It is tempting to suggest that formula

feeding itself stimulates hepatic maturation. The lower incidence of physiological jaundice in full term formula fed infants in comparison with breast fed infants is well known, but has generally been ascribed to inhibition of conjugation by breast milk rather than to stimulation by formula feeding.

Although certain correlations between weight gain and amino acid concentrations have previously been reported in low birthweight infants—for example, an inverse association between plasma branched chain amino acids and growth velocity²⁴—we were unable to show any in our study. We also could not correlate nitrogen retention or metabolisable energy intake with amino acid concentrations. Where the amino acid supply is not limiting growth the plasma concentration will be more likely to reflect individual variability in enzyme maturation and the pathways of metabolism than the relation between supply and demand (for instance the deposition in new tissues). Our results indicate that in very preterm infants plasma amino acids measurements are not useful indicators of protein nutritional state.

Preterm formulas with adapted whey:casein ratios and protein concentrations not exceeding 20 g/l are unlikely to cause dangerous rises in plasma amino acid concentrations when fed at 200 ml/kg/day or less, even in extremely small and immature infants. Moderate rises of some amino acids are as likely to occur when maternal breast milk is used. We found no convincing evidence that maternal breast milk was associated with protein deficiency although weight gain was not so fast as on formula feeding, but plasma concentrations of essential amino acids below the fetal range were more likely to occur with breast milk feeding. Until the importance of the immunological properties of breast milk is clarified, particularly with regard to babies nursed in a clean environment, the decision as to whether to use mother's milk (when available) or a preterm formula in these very small infants should be based primarily on the importance of ensuring adequate gut tolerance and on the desirability of maintaining a good rate of growth.

We thank Mr PA Johnson, Medical Statistics Department, Wyeth Research (UK) Ltd, who performed those statistical tests that required main frame computer analysis.

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Received 14 July 1987