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Metabolism of Free Glutamate in Clinical Products Fed Infants

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One of the tentative conclusions advanced by the Select Committee on GRAS Substances (SCOGS) (11) regarding the health aspects of glutamate salts as food ingredients was as follows:

> The evidence is insufficient to determine that the adverse effects reported are not deleterious to infants should glutamic acid, L-glutamic acid hydrochloride, monoammonium L-glutamate, monopotassium L-glutamate or monosodium L-glutamate be added to infant formulas and/or commercially prepared strained and junior foods.

It is difficult to understand the rationale for this conclusion if it is based on a knowledge of infant feeding practice and the statement by the SCOGS Committee (12) that "there appears to be no hazard of brain damage in the use of casein hydrolysate formulas."

During the neonatorum, infants are fed at intervals of 3 to 4 hr. Because of reduced gastric capacity, low-birth-weight infants are usually fed at 3-hr intervals. Many infants of less than 1,500 g birth weight are provided a source of nitrogen in the form of an amino acid mixture or a protein hydrolysate as part of their parenteral nutrition program. Since infants are fed frequently, they are in a constant postprandial state. If human young were unable to metabolize dicarboxylic amino acids effectively, survival of the species would be jeopardized. In fact, the nature and content of the free amino acids found in human milk and the amino acid content of the proteins of human milk would undoubtedly differ from reported values (1).

Measurement of plasma and erythrocyte concentrations of free amino acids has been used as an index of the ability of infants to metabolize amino acids. Biochemical immaturity and inborn metabolic defects in amino acid metabolism are associated with hyperaminoacidemia. Substrate overload in persons otherwise normal may potentially produce the same results.

In 1970 we initiated an ongoing study of plasma and erythrocyte aminograms of infants fed a variety of nitrogen sources either enterally or parenterally. These observations, collated in this report, show that irrespective of birth weight or age, the infant effectively metabolizes glutamic and aspartic acids provided either as the amino acid, peptides, or intact protein.

ENTERAL FEEDINGS

Term Infants

Healthy, normal term infants, breast or formula fed, are constantly enrolled in studies of normal growth and development within the University of Iowa Pediatric Metabolism Unit (6,7). At monthly intervals a biochemical profile is determined on small blood samples obtained 2 hr postprandially from a majority of all infants. Serum or plasma aminograms and erythrocyte aminograms are among the determined biochemical indices.

In 1971 Stegink and Schmitt (18) reported that healthy term infants, 28 to 33 days of age, showed no statistically significant difference in serum glutamate or aspartate when fed a conventional milk-based formula or Nutramigen (Mead Johnson Laboratories, Evansville, Ind.). The nitrogen source in the latter formula is an enzymatic hydrolysate of casein with approximately equal parts of free amino acids and polypeptides. Despite the relatively large amount of free glutamate (22% of total amino acids) present in the Nutramigen feeding, there was no evidence in the serum aminogram of glutamate overload.

Stegink and co-workers (19) extended their studies to include observations of infants fed a soy protein isolate formula (Isomil, Ross Laboratories, Columbus, Ohio) and older infants, 6 and 11 months of age. While these studies were designed to evaluate the response of infants to formulas fortified with DL-methionine, there was no evidence of any aberrant response in plasma concentrations of glutamate or aspartate.

The responses of term infants to enteral loads of glutamate and aspartate are summarized in Table 1. Two-hour postprandial concentrations of plasma or serum glutamate and aspartate are given for breast-fed infants, infants fed conventional milk-based formula, a soy isolate-based formula, and a formula prepared from an enzymatic hydrolysis of casein. From these data it is quite evident that the term infant responds to a formula containing dicarboxylic amino acids in free or peptide form in a manner comparable to that of the breast-fed infant. Plasma or serum concentrations of glutamate or aspartate are not elevated by the feeding of hydrolyzed versus milk protein or the ingestion of human milk with its relatively high content of free glutamic acid (1).

Concentrations of glutamate and aspartate in erythrocytes of breast-fed and formula-fed infants are summarized in Table 2. These observations, like those reported by Stegink and co-workers (16,17) in adults, do not show preferential accumulation of the dicarboxylic amino acids within the erythrocyte.

Low-Birth-Weight Infants

Since biochemical immaturity is a major problem for low-birth-weight (LBW) infants, we have investigated the plasma aminogram response of healthy, growing LBW infants, 11 to 35 days of age, to four formulas (5). Eight infants weighing 1.3 to 1.8 kg at birth were studied in a Latin square design so that each infant was fed all

TABLE 1. *Plasma glutamate and aspartate concentrations in term infants*

Feeding	Protein source	Intake (mg/kg/feeding)		Plasma concentrations (μmoles/dl)	
		Glutamate	Aspartate	Glutamate	Aspartate
Human milk	Human milk	65	33	12.3 ± 3.1	0.7 ± 0.4
Nutramigen[a]	Casein hydrolysate	80	28	10.1 ± 2.1	3.0 ± 2.5
Isomil	Soy isolate	95	57	13.0 ± 3.1	3.1 ± 2.1
Enfamil[a]	Cow milk	62	15	9.8 ± 2.8	2.4 ± 2.2

[a] Serum samples.

TABLE 2. *Erythrocyte glutamate and aspartate concentrations in term infants*

Feeding	N	Intake (mg/kg/feeding)		Erythrocyte concentration (μmoles/dl)	
		Glutamate	Aspartate	Glutamate	Aspartate
Human milk	13	65	33	42.4	13.3
Soy protein isolate	12	95	57	43.0	8.8

of the formulas. This approach enabled us to determine age effects on amino acid metabolism if they existed. All formulas were isocaloric and isonitrogenous at 67 Kcal and 1.5 g protein/100 ml. The data summarized in Table 3 show no statistically significant differences in 2-hr postprandial plasma concentrations of glutamate or aspartate for the four formulas studied. These observations are very important to the issue of glutamate and aspartate safety for infants because the nitrogen source in the experimental formula, identified as modified Pregestimil (Mead Johnson Laboratories, Evansville, Ind.), was an enzymatic hydrolysate of casein. Infants fed this formula received their dicarboxylic amino acids in both free and peptide form. Even under these circumstances plasma levels of glutamate or aspartate were not excessively elevated.

The data summarized in Table 3 are comparable to the data summarized in Table 1 and are indicative of the fact that LBW infants are as effective as term infants in metabolizing glutamic and aspartic acids.

One-Year-Old Infants

We recently tested the concept that infants may not metabolize a high protein meal as effectively as an adult (3). To test this hypothesis, it was necessary to have a high protein meal that could be eaten by both infants and adults. A custard whose composition is shown in Table 4 was prepared and fed to normal, healthy fasted adults and 1-year-olds. The custard contained 14% protein from milk and eggs and

TABLE 3. *Postprandial plasma glutamate and aspartate concentrations in LBW infants*

Feeding	Protein source	Intake (mg/kg/feeding)		Plasma concentrations (μmoles/dl)	
		Glutamate	Aspartate	Glutamate	Aspartate
SMA	Cow milk	69	10	12.4 ± 3.1	3.1 ± 2.8
Enfamil	Cow milk	82	20	13.5 ± 5.8	3.4 ± 1.3
Modified Pregestimil	Casein hydrolysate	94	32	11.0 ± 2.9	1.7 ± 0.8
5031A	Cow milk	90	24	10.3 ± 3.3	2.3 ± 1.0

TABLE 4. *Custard composition*

Component	Weight (g)	Protein (g)	Fat (g)	CHO (g)
Egg	150	20	17	1
Nonfat dried milk	150	54	1	78
Fructose	30			30
Water	200			
Total	530	74(14%)	18(3%)	109(21%)

TABLE 5. *Custard study on adults and 1-year-olds*

Subjects	*N*	Intake (mg/kg/feeding)		Plasma concentrations[a] (μmoles/dl)			
		Glutamate	Aspartate	Glutamate		Aspartate	
				Fast.	Post.	Fast.	Post.
Adults	6	220	77	3.3	6.4	0.4	0.6
Infants	24	230	80	5.8	11.0	0.6	1.0

[a] Fast., fasting; Post., postprandial.

was fed at a level to provide 1 g protein/kg body weight. A 10-kg infant had to eat 70 g of custard. In contrast, an 80-kg male had to eat 570 g. Serial blood samples were collected from each group of subjects. Adult subjects were bled at frequent intervals over a period of 6 hr using a heparinized indwelling catheter. Blood samples from infants were obtained by heel stick, with each infant providing four specimens at staggered intervals over a period of 4 hr.

Plasma concentrations of free amino acids doubled in both adults and infants as the result of the protein load. Plasma values peaked in approximately 2 hr and remained elevated during the entire study period. Protein intakes of 1 g/kg body weight at one feeding are not unusual for the infant (4) and many adults frequently achieve intakes of this magnitude or more.

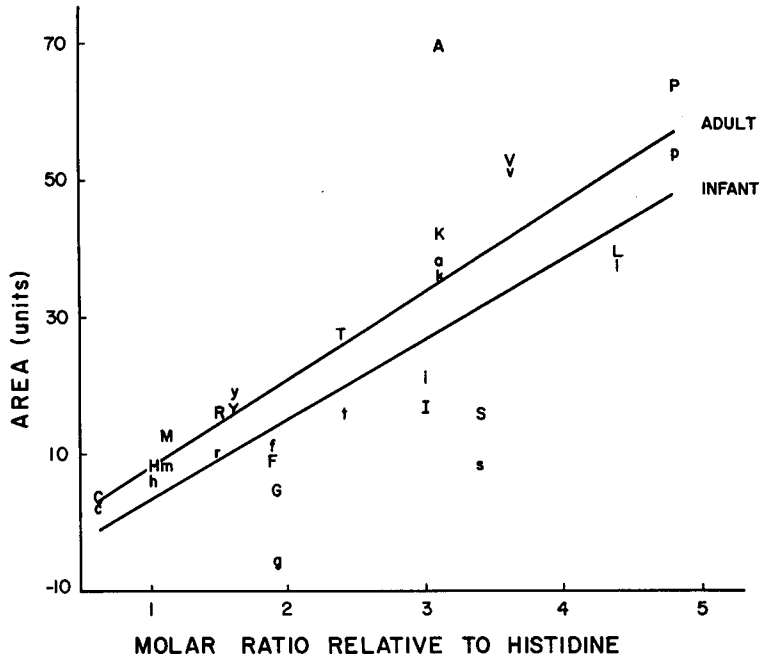

FIG. 1 Area under the curve for specific amino acids as a function of their molar ratio to histidine following ingestion of a high protein meal. Capital letters = adult data, lower-case letters = infant data.

As shown in Table 5, the concentration of plasma glutamate and aspartate in the fasting state is greater for infants than adults. When given identical protein loads, the percentage change in plasma glutamate and aspartate concentration is identical for the two age groups. Plasma concentrations of glutamate and aspartate in the postprandial state of 1-year-olds fed custard are similar to those observed for formula-fed LBW infants or formula-fed and breast-fed term infants.

Marrs and co-workers (8) have suggested a method of comparing the absorption of protein hydrolysates to amino acid mixtures. From timed plasma aminograms an absorption curve is plotted for each amino acid. The area under the curve is determined and this value is expressed as a function of the molar ratio of each amino acid relative to histidine. If protein hydrolysates and amino acid mixtures are similar, the plot will fit a common regression line.

This method of analysis has been applied to the aminogram data obtained on the infant and adult subjects fed custard. The molar ratio for 15 amino acids relative to histidine (indexed at 1) was calculated from the amino acid composition of the custard. Area under the curve in arbitrary units was calculated for these amino acids and plotted as a function of relative molar ratio (Fig. 1). Individual amino acids in this figure are identified according to the one-letter notation employed in the *Atlas of Protein Sequence and Structure* (2). If the infant and adult digest, transport, and

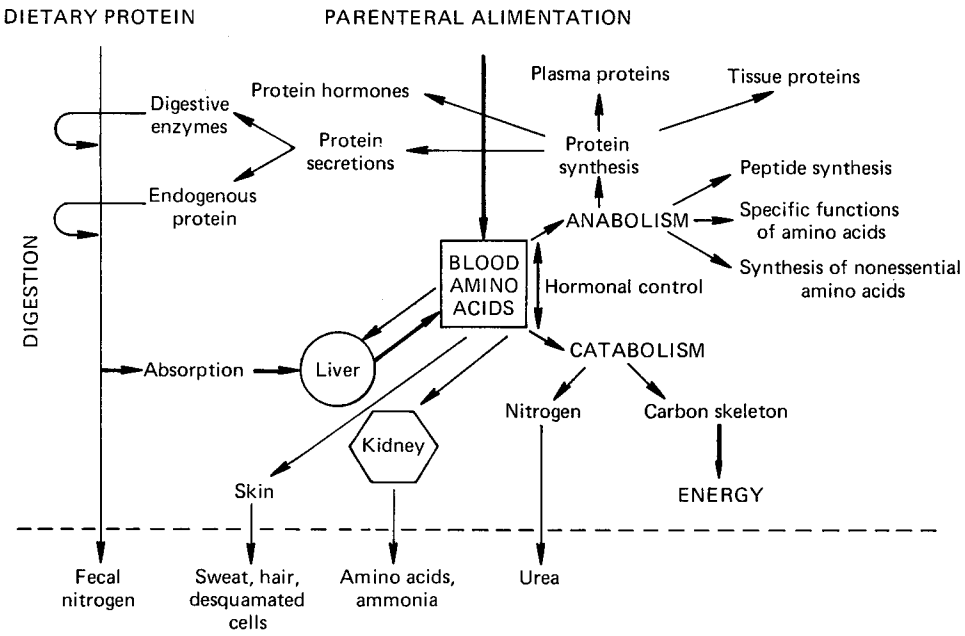

FIG. 2. Interrelationships of amino acid metabolism during enteral and parenteral feeding.

metabolize the egg-milk protein load in a similar manner, the data should fit a common regression line. As shown in Fig. 1, adults and infants have similar regression lines. Calculated coefficients of correlation are $r = 0.73$ for adults and $r = 0.81$ for infants.

PARENTERAL FEEDINGS

During enteral feeding, dietary or exogenous protein entering the gut is diluted by a considerable quantity of endogenous protein secreted into the gut (10) (Fig. 2). Proteins present in the gut are digested to free amino acids and small peptides, both of which enter the intestinal mucosal cell. Mucosal cells metabolize some amino acids, notably glutamic and aspartic acids, thereby offering some protection against excessive intake of these amino acids. Peptides are hydrolyzed to free amino acids during transit through the mucosal cells so that, in general, only free amino acids reach the liver via the portal circulation. Amino acids circulating in peripheral blood and available for tissue uptake are subject to complex hormonal controls that determine their precise anabolic or catabolic fate (13).

During parenteral feeding, both the gut and liver are bypassed since amino acids and peptides are administered directly into the peripheral circulation. From studies of the daily dietary intake and flux of phenylalanine and tyrosine for a 70-kg man, Munro (9) has postulated that man has a considerable capacity to remove via normal metabolic processes amino acids given parenterally.

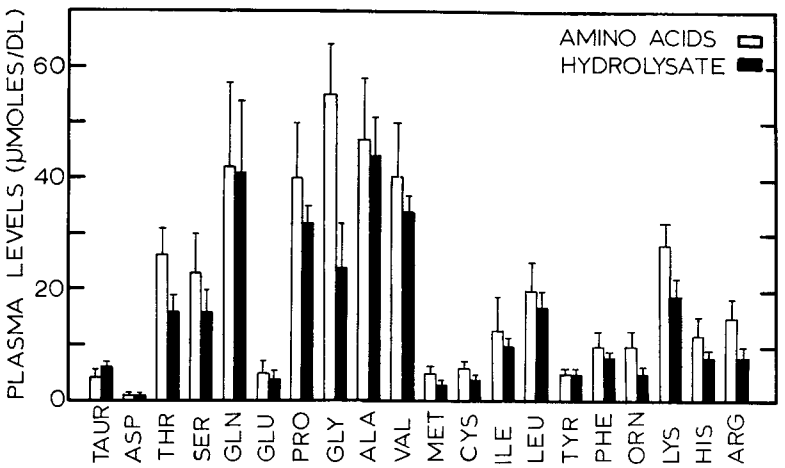

FIG. 3. Plasma amino acid concentrations in normal adult subjects fed parenterally using either a 5% casein hydrolysate-25% glucose solution or a 5% amino acid-25% glucose solution.

Adult Subjects

Stegink and co-workers (14) have studied glutamate and aspartate metabolism in a group of normal, healthy adult volunteers on total parenteral feedings. Subjects were assigned to one of two parenteral solutions. One group of subjects received nitrogen in the form of a casein hydrolysate (Amigen, Baxter Laboratories, Morton Grove, Ill.) containing large amounts of glutamic and aspartic acids. The other group of subjects was given a mixture of crystalline amino acids (Aminosyn, Abbott Laboratories, North Chicago, Ill.) free of glutamic and aspartic acids. Energy requirements were met with a 25% (w/v) solution of glucose. No significant differences in plasma concentrations of glutamate and aspartate were noted, indicative of the rapid metabolism of these amino acids when administered intravenously (Fig. 3). In a parallel study of a group of postsurgical (stressed) patients, no differences were observed in the capacity to metabolize the glutamate or aspartate present in the infused casein hydrolysate.

Term Infants

In 1971 Stegink and Baker (15) reported plasma aminograms on a series of six sick infants receiving total parenteral nutrition. The nitrogen source for these feedings was either an enzymatically digested casein (Amigen) or an enzymatically digested beef fibrin (Aminosol, Abbott Laboratories, North Chicago, Ill.).

Plasma glutamate and aspartate concentrations in such infants infused with protein hydrolysate solutions providing 0.2 g/kg body weight of glutamate and 0.05 g/kg body weight of aspartate were well within the normal postprandial limits observed for enterally fed infants (Table 6). These authors reported that the amino

TABLE 6. *Plasma glutamate and aspartate concentrations in infants infused with hydrolysates*

Infusion	N	Glutamate		Aspartate	
		Intake (mg/kg)	Plasma (μmoles/dl)	Intake (mg/kg)	Plasma (μmoles/dl)
Casein hydrolysate	6	195	6.1	50	1.1
Fibrin hydrolysate	2	35	4.5	65	0.4
Oral feedings of milk-based formula					
Fasting	15	—	2.7	—	0.5
Postprandial	15	62	7.7	15	2.1

TABLE 7. *Plasma glutamate and aspartate concentrations in LBW infants on total parenteral feedings*

Patient	Weight (kg)	Intake (mg/kg)		Plasma concentrations (μmoles/dl)	
		Glutamate	Aspartate	Glutamate	Aspartate
McC	1.38	165	39	3.6	1.6
MA	1.51	201	48	3.9	1.7

Solution: 2.5% casein hydrolysate, 24% glucose.

acid composition of the hydrolysate had a marked effect on plasma amino acid levels, with some degree of amino acid imbalance observed with both products. In general, plasma amino acid concentrations reflected concentrations of specific amino acids in the hydrolysate. For example, the low levels of cystine and tyrosine in the casein hydrolysate caused a reduction in plasma cystine and tyrosine. The beef fibrin hydrolysate, rich in glycine, produced an increase in plasma glycine concentration.

If a population with decreased ability to metabolize dicarboxylic amino acids existed, it might well be that of small premature infants on parenteral feedings. Such infants are biochemically immature and are further compromised by a feeding technique that bypasses the gut. Recently, we have measured amino acid levels in two LBW infants weighing 1.4 and 1.5 kg. The nitrogen source for these parenterally fed infants was a casein hydrolysate. Plasma concentrations of glutamate and aspartate were less than the postprandial concentrations for these amino acids observed in LBW infants following formula feedings (Table 7).

COMMENT

Winters and co-workers (20) have recently concluded that amino acid intakes resulting in plasma concentrations of individual amino acids within the normal

range of postprandial values are both safe and efficacious. Normal postprandial values were defined as those found in breast-fed or formula-fed infants. Intakes of nitrogen as protein, peptides, or free amino acids that yield plasma values significantly above the postprandial range should be considered suspect for safety. Intakes of nitrogen yielding plasma values below this range should be considered suspect for nutritional efficacy.

In this report we have established postprandial values for plasma concentrations of glutamate and aspartate in both breast-fed and formula-fed infants. Like Winters and co-workers, we regard these as normative values.

Relative to these normative data we have demonstrated the following:

1. Term infants fed a casein hydrolysate formula (Nutramigen) have plasma glutamate and aspartate levels that agree with normative data.
2. LBW infants fed conventional milk-based formulas and a special formula prepared from a casein hydrolysate have plasma glutamate and aspartate levels that agree with normative data.
3. Fasting 1-year-old infants fed a high protein meal have plasma glutamate and aspartate levels that agree with normative data. In addition, analysis of plasma aminograms shows that these infants are as efficient as normal adult subjects in metabolizing a protein load.
4. Surgically stressed or ill term infants and LBW infants weighing 1,500 g or less given a casein hydrolysate solution parenterally have plasma glutamate and aspartate levels that agree with normative data.

Although glutamate and aspartate are both metabolically highly reactive amino acids requiring careful study, one cannot escape the fact that abnormally high plasma glutamate and aspartate levels have not been seen following enteral or parenteral administration of formula or intravenous solutions containing high levels of free glutamate and aspartate. On the basis of these clinical observations in man, it is difficult to understand the tentative conclusion advanced by the SCOGS Committee suggesting that insufficient evidence exists to determine whether glutamate affects infants adversely. Available clinical evidence strongly supports the conclusion that glutamate and aspartate ingested in association with an adequate source of carbohydrate are safe for infants.

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