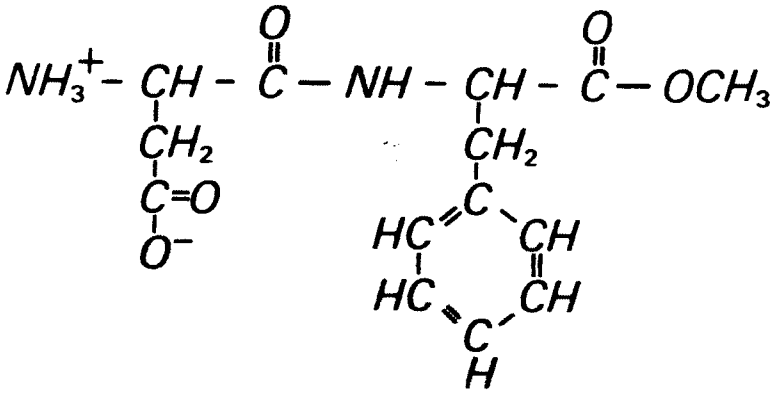
ASPARTAME

Physiology and Biochemistry

edited by

Lewis D. Stegink • L. J. Filer, Jr.



26

Aspartame Metabolism in Humans: Acute Dosing Studies

Lewis D. Stegink
University of Iowa College of Medicine, Iowa City, Iowa

Toxicology is based on the premise that all compounds are toxic at some dose. Salt, water, sugar, and even a mother's love produce deleterious effects when given in inappropriate amounts. Thus it is not surprising that very large doses of aspartame (Fig. 1) or aspartame's component parts (aspartate, phenylalanine, and methanol) produce deleterious effects in sensitive animal species. The critical question is whether the compound is potentially harmful at normal use and potential abuse levels.

Aspartame may be absorbed and metabolized in one of two ways (Fig. 2). It may be hydrolyzed in the intestinal lumen to aspartate, phenylalanine, and methanol by proteolytic and hydrolytic enzymes (1-5). These compounds are absorbed from the lumen and reach the blood in a manner similar to that of amino acids and methanol arising from dietary protein or polysaccharides. Alternatively, aspartame may be absorbed directly into mucosal cells by peptide transport mechanisms (4,5) with subsequent hydrolysis within the cell to aspartate, phenylalanine, and methanol. In either case, large doses of aspartame release aspartate, phenylalanine, and methanol to the portal blood, and these components must be metabolized and/or excreted.

Olney (6-9) and Reif-Lehrer (10) expressed concern about the safety of aspartame because of its aspartate content. Administration of high doses of aspartate to neonatal mice or rats results in elevated plasma aspartate concentrations (11-16) and hypothalamic neuronal necrosis (14-18). Aspartame administered in large amounts (1-2.5 g/kg body weight) to infant mice produces neuronal necrosis

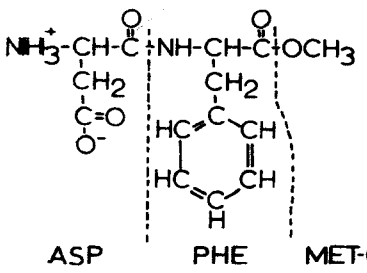


Figure 1 Chemical structure of aspartame. The dotted lines divide the structure into its component parts: aspartate (ASP), phenylalanine (PHE), and methanol (MET-OH).

(19,20), presumably as a result of elevated blood aspartate concentrations. However, aspartame administration (2 g/kg body weight) to infant nonhuman primates, with or without added monosodium L-glutamate (1 g/kg body weight), did not produce neuronal necrosis, even though plasma aspartate and glutamate levels were elevated (20,21).

Turner objected to aspartame (22) because of its phenylalanine content. He speculated that aspartame ingestion would markedly elevate plasma phenylalanine concentration. Grossly elevated plasma phenylalanine concentrations, such as those found in children with classic phenylketonuria, are associated with mental retardation (23-26).

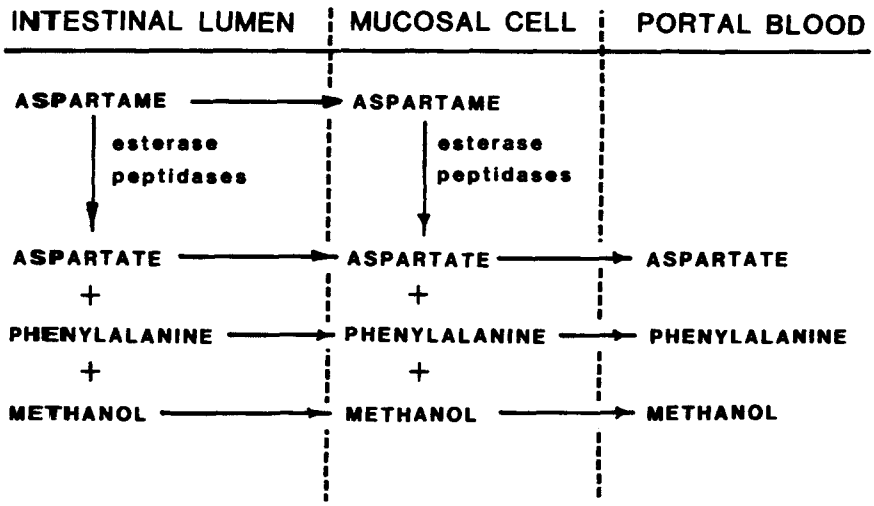


Figure 2 Aspartame hydrolysis occurs in both the intestinal lumen and in mucosal cells, releasing aspartate, phenylalanine, and methanol to the portal blood.

Since aspartame is a methyl ester, its metabolism releases methanol to the circulation. Ingestion of large doses of methanol is associated with adverse effects in sensitive species (27-30). Thus the potential toxicity of aspartame due to its methanol content must be considered.

In considering the toxicity of each of aspartame's three components, it is important to recognize that the blood concentration of each component (aspartate, phenylalanine, or methanol) must be markedly elevated to produce toxic effects. Certain facets of methanol toxicity, in fact are associated with formate rather than methanol accumulation (30,31).

Evaluation of potential toxicity of new food additives must deal with the question of sensitive species. Some species are more resistant than others to adverse effects resulting from the administration of a specific compound. Although rodents are commonly used in toxicology studies, nonhuman primates are often a better model of the human response. Investigators must deal with conflicting toxicity data obtained in different species and determine how these data relate to toxicity in humans. Tests evaluating the potential toxicity of aspartate, phenylalanine, and methanol in humans must be indirect; however, it is possible to examine the effect of large doses of aspartame on human blood amino acid and methanol concentrations to determine whether such doses produce the excessively high blood concentrations of these compounds associated with toxicity in animals.

This chapter reviews plasma and erythrocyte levels of amino acids as well as blood methanol and formate concentrations that result from the administration of large doses of aspartame to normal adults and individuals heterozygous for phenylketonuria.

The first section will discuss the effect of aspartame loading on plasma phenylalanine concentration, the second its effect on plasma aspartate concentration, and the third section its effect on blood methanol concentration.

PROJECTED ASPARTAME INTAKE

Projected levels of aspartame ingestion have been calculated by the Food and Drug Administration (FDA), the Market Research Corporation of America (MRCA), and our research group (32-35). Table 1 summarizes these data. If

Table 1 Summary of Projections for Aspartame Intake

Source	Aspartame totally replaces mean sucrose sweetness (mg/kg body weight)	Maximum (mg/kg body weight)
FDA (32,33)	Not calculated	22-28
MRCA (34)	3-11	25-34
Stegink et al. (35)	7-9	23-25

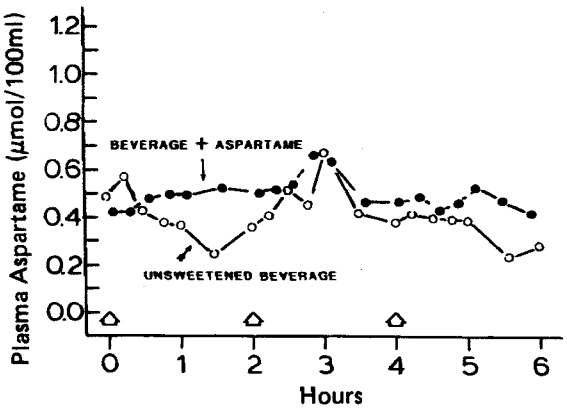


Figure 26 Mean plasma aspartate concentrations in normal adults ingesting repeated servings of either unsweetened beverage (o- - -o) or beverage providing aspartame at 10 mg/kg body weight (●—●).

load produced by aspartame loading is not completely cleared and/or metabolized prior to ingestion of a second dose of aspartame, a cumulative increase in plasma aspartate concentration might occur with the ingestion of successive aspartame doses. To test this, we studied the effects of successive ingestion of three servings of aspartame-sweetened beverage upon plasma aspartate concentration.

Eight normal healthy adults were studied, four male and four female (72). The study was conducted in two stages in a standard crossover design. Subjects ingested three successive 12-oz servings of beverage, with a 2-hr interval between servings. In one stage of the study, subjects drank three servings of unsweetened beverage, while in the other stage the three servings of beverage each provided aspartame at 10 mg/kg body weight.

As shown in Figure 26, the addition of aspartame to the beverage had no significant effect on plasma aspartate concentration. Similarly, plasma glutamate concentrations were not affected by the addition of aspartame to the beverage.

METHANOL

Aspartame is a dipeptide methyl ester, and during absorption and metabolism of the peptide, methanol is released. The ingestion of large quantities of methanol is known to result in elevated blood methanol and formate concentrations and leads to a variety of adverse effects, including metabolic acidosis and blindness (27-30). Blood methanol concentrations were measured in normal subjects administered aspartame at 34, 50, 100, 150, and 200 mg/kg body weight (99).

In the first study, blood methanol levels were measured in 12 subjects ingesting aspartame at 34 mg/kg body weight in a randomized crossover design with

aspartic acid at a dose of 13 mg/kg body weight. Blood methanol concentrations were below the limits for detection (0.35-0.4 mg/dl).

Blood methanol concentrations were also measured in the six female subjects administered aspartame at 50 mg/kg body weight in a randomized crossover design with lactose at a dose of 50 mg/kg body weight. Blood methanol concentrations were below the detection limit when lactose was ingested; however, blood methanol was detected in normal adults administered 50 mg/kg body weight aspartame. The mean (±SEM) peak blood methanol level was 0.34 ± 0.12 mg/dl.

Blood methanol concentrations in subjects administered aspartame at 100, 150, and 200 mg/kg body weight are shown in Figure 27. Blood methanol concentrations were increased significantly over preloading values ($p < 0.05$) from 15 min to 7 hr after aspartame ingestion, with the increase in blood methanol proportional to the aspartame dose. Mean (±SD) peak methanol concentrations were 1.27 ± 0.48 mg/dl at the 100 mg/kg body weight dose, 2.14 ± 0.35 mg/dl at the 150 mg/kg body weight dose, and 2.58 ± 0.78 mg/dl at the 200 mg/kg body weight dose of aspartame. Similarly, the area under the blood methanol concentration-time curve increased in proportion to the increase in aspartame load. Blood methanol concentrations returned to preloading values 8 hr after administration of the 100 mg/kg body weight dose. Blood methanol was still detected 8 hr after subjects received aspartame at 150 or 200 mg/kg body weight; however, no methanol was detected 24 hr after administration of aspartame.

Recent studies (30,31) indicate that many of the toxic effects of methanol in the nonhuman primate are due to formate accumulation rather than to formaldehyde or methanol. Accordingly, blood and urine samples from subjects administered the highest aspartame dose were assayed for formate content by the method

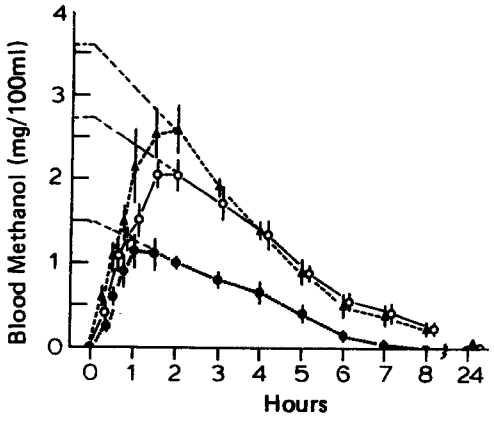


Figure 27 Mean (±SD) blood methanol concentrations in normal adults administered aspartame at 100 (●· · ·●), 150 (○- - -○), or 200 mg/kg body weight (▲- - -▲). (From Ref. 99.)

of Makar et al. (100). No significant changes in blood formate concentration were noted after aspartame administration at 200 mg/kg body weight (Fig. 28); however, urinary formate excretion (Table 9) was significantly increased over preloading values in urine samples collected 0-4 and 4-8 hr after aspartame loading. Urinary formate excretion returned to preloading values in those samples obtained 8-24 hr after loading. The urinary excretion data indicate conversion of methanol to formate. Since the rate of formate synthesis apparently did not exceed the rate of formate excretion, blood formate levels were not detectably elevated. Thus there appears to be little risk from aspartame's methanol content at the doses studied.

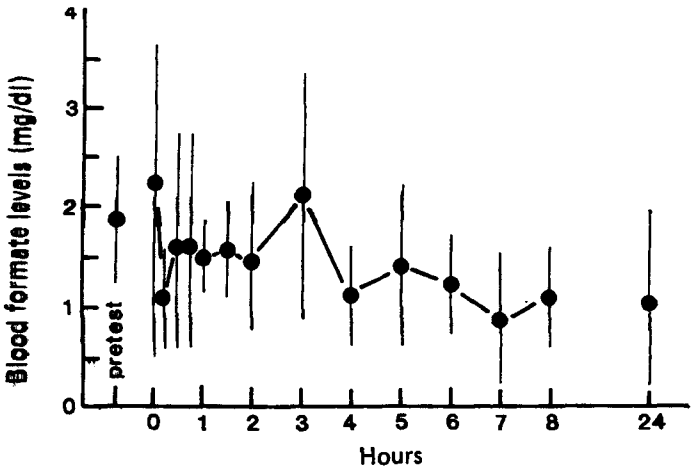


Figure 28 Mean (±SD) blood formate concentrations in normal adults administered aspartame at 200 mg/kg body weight.

Table 9 Urinary Formate Excretion in Normal Adults Ingesting 200 mg/kg Body Weight Aspartame

Urine sample (hr)[a]	Formate excreted (μg/mg creatinine)
-8 to 0	34 ± 22[b]
0 to 4	101 ± 30[c]
4 to 8	81 ± 22[c]
8 to 24	38 ± 12

[a]Negative values are preloading times.
[b]Data are expressed as the mean ± SD.
[c]Differs statistically from base-line levels; $p < 0.01$.

Methanol is a normal constituent of saliva and expired air and can be detected in blood (101,102). Because there is considerable variability in breath methanol concentration, Eriksen and Kulkarni (101) concluded that dietary sources are only partial contributors to the total body methanol pool. Indeed, Axelrod and Daly (103) reported the presence of a methyltransferase enzyme in pituitary extracts catalyzing the conversion of S-adenosylmethionine to methanol and S-adenosylhomocysteine. Later work (104,105) demonstrated that this methanol-forming enzyme was protein carboxylmethylase, an enzyme that methylates free carboxyl groups of proteins. Methanol is formed as the end product of this protein methylation through the action of protein methylesterases (106,107), as illustrated in Figure 29.

Earlier, we reported a method for predicting maximal blood methanol concentrations from a given aspartame dose in adults (99). These calculations suggest that a mean peak blood methanol concentration of 0.42 mg/dl might be expected to result at an aspartame intake of 34 mg/kg body weight. Although this concentration is at the outside limits of the assay sensitivity, it should be possible to detect. However, the equation developed for calculating the theoretical peak blood methanol concentration was developed using data obtained after loading with high doses of aspartame. Methanol may be cleared from the blood more rapidly at the levels produced by smaller doses of aspartame than was noted after ingestion of larger doses. This would lead to a lower blood methanol level than that predicted by our method. This factor probably accounts for our failure to detect methanol at an aspartame intake of 34 mg/kg body weight.

The projected methanol load resulting from the consumption of an aspartame-sweetened beverage might be less than that resulting from the consumption of an equivalent quantity of fruit juice. For example, an aspartame-sweetened beverage would have an aspartame content of about 555 mg/liter if sweetened at a typical soft drink level (10% sucrose wt/vol; aspartame sweetness 180 times that of

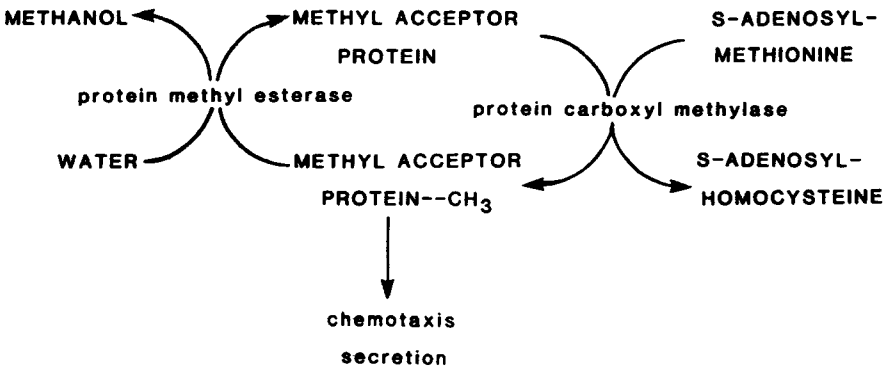


Figure 29 Synthesis of methanol in mammilian tissues. (From Refs. 107, 113.)

sucrose). In terms of aspartame's methanol content, this would be equivalent to 60 mg of methanol per liter—considerably less than the amount listed by Francot and Geoffroy (108) as the average methanol content of fruit juice (140 mg/liter). Other authors have reported similar levels of methanol in fruit juices (109-112).

Studies in Infants

Olney suggested that human infants metabolize the amino acids of aspartame less well than adults (6-9). While testing that hypothesis in infants and adults, blood methanol levels were also measured (113). Those data, summarized by Filer et al. in Chap. 29 (55), indicate similar blood methanol concentrations in infants and adults administered equivalent doses of aspartame.

REFERENCES

1. Oppermann, J. A., Muldoon, E., and Ranney, R. E. (1973). Metabolism of aspartame in monkeys. *J. Nutr.* 103, 1454-1459.
2. Ranney, R. E., Oppermann, J. A., Muldoon, E., and McMahon, F. G. (1976). The comparative metabolism of aspartame in experimental animals and man. *J. Toxicol. Environ. Health* 2, 441-451.
3. Ranney, R. E., and Oppermann, J. A. (1979). A review of metabolism of the aspartyl moiety of aspartame in experimental animals and man. *J. Environ. Pathol. Toxicol.* 2, 979-985.
4. Addison, J. M., Burston, D., Dalrymple, J. A., Matthews, D. M., Payne, J. W., Sleisenger, M. H., and Wilkinson, S. (1975). A common mechanism for transport of di- and tri-peptides by hamster jejunum in vitro. *Clin. Sci. Mol. Med.* 49, 313-322.
5. Matthews, D. M. (1984). Absorption of peptides, amino acids and their methylated derivatives. In *Aspartame: Physiology and Biochemistry*. (Stegink, L. D., and Filer, L. J., Jr., eds.), Marcel Dekker, New York, Chap. 3.
6. Olney, J. W. (1975). Another view of aspartame. In *Sweeteners, Issues and Uncertainties*, National Academy of Sciences, Washington, D.C. pp. 189-195.
7. Olney, J. W. (1976). L-Glutamic and L-aspartic acids—A question of hazard? *Food Cosmet. Toxicol.* 13, 595-596.
8. Olney, J. W. (1980). Testimony. In *Aspartame: Public Board of Inquiry, Transcript of Proceedings*, Vol. 3, Department of Health, Education and Welfare, Food & Drug Administration, Rockville, Md., pp. 117-118.
9. Olney, J. W. (1981). Excitatory neurotoxins as food additives: An evaluation of risk. *Neurotoxicology* 2, 163-192.
10. Reif-Lehrer, L. (1976). Possible significance of adverse reactions to glutamate in humans. *Fed. Proc.* 35, 2205-2211.
11. Stegink, L. D., Shepherd, J. A., Brummel, M. C., and Murray, L. M. (1974). Toxicity of protein hydrolysate solutions: Correlation of glutamate dose and neuronal necrosis to plasma amino acid levels in young mice. *Toxicology* 2, 285-299.

29

Aspartame Ingestion by Human Infants

L. J. Filer, Jr., George L. Baker, and Lewis D. Stegink*
University of Iowa College of Medicine, Iowa City, Iowa

INTRODUCTION

The safety of aspartame has been questioned on the basis of its constituent amino acids, aspartate and phenylalanine (1-5). Others have expressed concern that the metabolism of aspartame releases methanol. High blood levels of each of these compounds are associated with toxicity. Administration of high doses of aspartate to neonatal mice and rats results in elevated plasma aspartate concentrations and hypothalamic neuronal necrosis (6-14). Infant mice given large doses of aspartame develop hypothalamic neuronal necrosis (15,16), presumably from elevated blood aspartate concentrations. However, administration of aspartame at 2 g/kg body weight to infant nonhuman primates with and without added monosodium L-glutamate (1 g/kg) does not result in neuronal necrosis, even though plasma aspartate and glutamate levels are elevated (15,17). Grossly elevated blood phenylalanine concentrations, such as those found in children with classic phenylketonuria, are associated with mental retardation (18-20). This led to the suggestion that aspartame ingestion by children might increase dietary intake of phenylalanine enough to produce elevated plasma phenylalanine concentrations. The ingestion of large quantities of methanol results in elevated blood methanol and formate concentrations and leads to a variety of adverse effects, including metabolic acidosis and blindness (21-24).

Infant mice and monkeys metabolize glutamate, a dicarboxylic amino acid similar to aspartate, more slowly than adult animals (25). On the basis of these

*Present affiliation: Mead Johnson & Company, Evansville, Indiana

studies, Olney (3) suggested that human infants metabolize glutamate and aspartate less rapidly than adults. He predicted that a fixed aspartame dose would produce higher plasma aspartate concentrations in infants as compared to adults and associated this hypothetically increased plasma dicarboxylic amino acid concentration with increased risk. Similarly, Turner (5) suggested that aspartame's phenylalanine content might greatly increase plasma phenylalanine concentrations with potentially deleterious effects.

Because of these concerns, we investigated the ability of 1-year-old infants to metabolize aspartame (26,27). Previously we had studied the effects of aspartame loading at 34, 50, 100, 150, and 200 mg/kg body weight upon plasma amino acid concentrations in adult subjects (28-30). In a follow-up study we investigated the effect of aspartame loading at 34, 50, and 100 mg/kg body weight upon the plasma amino acid concentrations and blood methanol concentrations in 1-year-old infants to determine whether infants metabolize aspartame as well as adults.

MATERIALS AND METHODS

A total of 24 infants aged 8-14 months were studied following an overnight fast. The proposed study was fully explained to at least one of the child's parents, and informed, written consent was obtained. The protocol of the study was reviewed and approved by the Committee on Research Involving Human Subjects of the University of Iowa.

A physical examination, urinalysis, and complete blood count were carried out on each infant prior to entry into the study. Values for all infants were within normal limits for the laboratory.

This investigation was carried out in three stages. In the first study 10 infants were administered 34 mg/kg body weight aspartame dissolved in 180 ml of cherry-flavored beverage mix. In the second study six infants were administered 50 mg/kg body weight aspartame dissolved in the flavored beverage mix. In the third study eight infants were administered 100 mg/kg body weight aspartame dissolved in the flavored beverage mix. The first study was completed and results evaluated prior to starting the second study. Similarly, the second study was completed and results evaluated prior to starting the final study. The details of these studies have been published elsewhere (26,27).

A carbohydrate-free cherry-flavored beverage was used as the vehicle for aspartame administration. The beverage was prepared from dry powder according to package instructions. Aspartame was dissolved in 180 ml (6 oz) of beverage and fed to the fasted infant from a bottle or cup.

RESULTS

Amino Acid Studies

Aspartame Ingestion at 34 mg/kg Body Weight

Plasma aspartate and phenylalanine concentrations in infants administered 34 mg/kg body weight aspartame are shown in Figure 1. These data are compared

Erythrocyte aspartate and phenylalanine concentrations are shown in Figure 4. No significant changes in erythrocyte aspartate concentration over baseline were noted. Erythrocyte phenylalanine concentration showed a similar but reduced rise to that noted in plasma after aspartame loading in both groups.

Blood Methanol Response

Blood methanol concentrations were at the limits of detection in the 10 infants administered aspartame at 34 mg/kg body weight. Analysis of blood for low concentrations of methanol is complicated by the presence of small quantities of material that elutes at the methanol position. This material is also present in baseline samples. This made it difficult to detect low concentrations of methanol. The "apparent" blood methanol concentrations in the infants, corrected for this interfering substance, were below the limits of detection for methanol (0.35 mg/dl). The infant blood methanol response was similar to that noted in 12 normal adults administered an equivalent dose of aspartame.

Similar problems with methanol analyses were noted in infants and adults administered aspartame at 50 mg/kg body weight. In some subjects, at some time points, the blood methanol level did not rise to detectable levels, while in other subjects at other times blood methanol was detectable. In infants the mean (±SEM) peak blood methanol level was 0.30 ± 0.10 mg/dl 30-90 min after loading. In normal adults mean peak blood methanol level was 0.34 ± 0.12 mg/dl.

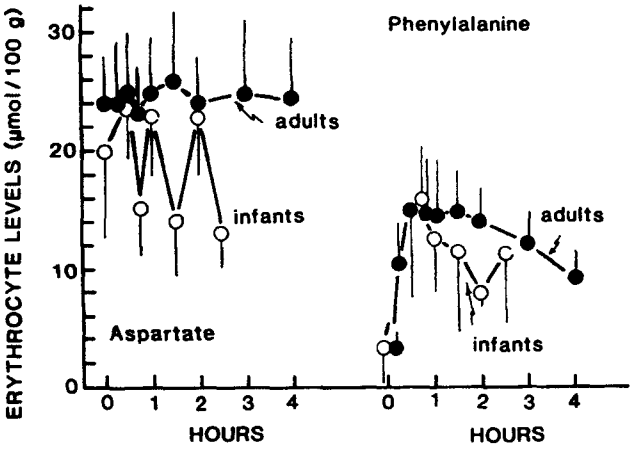


Figure 4 Mean (±SD) erythrocyte aspartate and phenylalanine concentrations in 6 normal adults (●—●) and eight 1-year-old infants (○—○) administered aspartame at 100 mg/kg body weight. Variation in infant erythrocyte aspartate concentration at alternative time points reflects the division of infants into two groups for blood sampling. One group was sampled at 0, 30, 60, and 120 min, the other at 0, 45, 90, and 150 min. No significant change in erythrocyte aspartate concentration occurred relative to base-line levels in either group, base-line erythrocyte aspartate levels differed between groups. This leads to the up-and-down appearance of the graph. From Ref. 26.

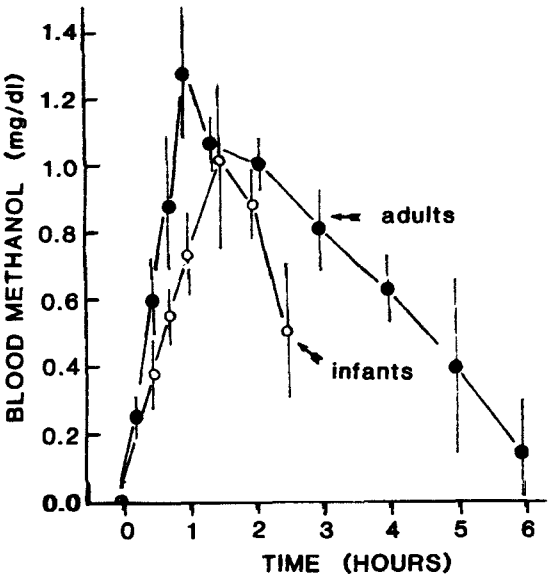


Figure 5 Mean (± SEM) blood methanol concentrations in normal adults (●) and 1-year-old infants (○) administered aspartame at 100 mg/kg body weight. From Ref. 27.

Blood methanol levels in the eight infants administered aspartame at 100 mg/kg body weight are shown in Figure 5. Blood methanol concentrations increased from base-line values at zero time to a mean (±SEM) peak value of 1.02 ± 0.28 mg/dl 90 min postdosing. This response was similar to that observed in six normal adults administered aspartame at 100 mg/kg body weight (38).

These data demonstrate that the infant metabolizes aspartame as well as a normal adult at these levels of ingestion.

DISCUSSION

In earlier studies (25) we demonstrated that infant mice and monkeys metabolize glutamate less rapidly than adults, particularly when very large doses are given. Olney (3) extended these data to suggest that the human infant metabolizes glutamate and aspartate less rapidly than an adult at levels likely to be provided by diets containing added monosodium L-glutamate and aspartame. He associated this hypothetical decrease in metabolic capacity with potential for increased plasma glutamate and/or aspartate concentrations and neuronal damage if compounds containing these amino acids are ingested.

Despite Olney's suggestion, we had little reason to expect that infants would metabolize the aspartate present in aspartame poorly at expected use levels of

the product. Available data indicate adequate metabolism of ingested or infused aspartate by 1-year-old infants, term infants, and low birth weight infants at levels of intake comparable to those present in foods or parenteral solutions. These data are summarized in the following paragraphs.

One-Year-Old Infants

Although we had not tested aspartate metabolism in infants per se, we had compared protein digestion and metabolism in 6 normal adults, 16 normal 1-year-olds, and 8 normal 2-year-olds fed a milk-egg custard meal providing 1 g of protein per kilogram body weight (31,39). Infants fed custard consumed 66 mg of phenylalanine and 80 mg of aspartate per kilogram body weight. This intake compares with the 50 mg/kg body weight phenylalanine and 40 mg/kg body weight aspartate provided by a 100-mg/kg body weight dose of aspartame. The results of the custard study indicated that infants metabolized peptide-bound amino acids, including aspartate and phenylalanine, as well as adults (37,39).

Orally Fed Term and Low Birth Weight Infants

Postprandial plasma amino acid levels were measured in term and low birth weight infants fed either conventional formulas with little free glutamate and aspartate, or a casein hydrolysate formula containing large quantities of these amino acids (31,37,40,41). Plasma glutamate and aspartate levels were within normal ranges for both groups, indicating adequate handling of free glutamate and aspartate at these levels of intake.

Parenterally Fed Term and Premature Infants

Stegink and Baker (36) measured plasma amino acid levels in infants fed totally by vein using casein or fibrin hydrolysate-based preparations. The casein hydrolysate provided 1.2 mmol glutamate and 0.3 mmol aspartate per kilogram per day, while the fibrin hydrolysate provided 0.2 mmol glutamate and 0.4 mmol aspartate per kilogram per day. Plasma glutamate and aspartate levels in these infants were normal. Filer et al. (31) reported plasma amino acid levels in two low birth weight infants (1.38 and 1.51 kg body weight) administered a casein hydrolysate-based parenteral solution. These infants received 1.1-1.4 mmol glutamate and 0.3-0.4 mmol aspartate per kilogram body weight per day. Plasma glutamate and aspartate levels were normal in each case, indicating adequate metabolism of these amino acids. Recently Bell et al. (42) infused young infants with an amino acid-based parenteral regimen providing 1.5 mmol/kg body weight glutamate and 1.0 mmol/kg body weight aspartate per day. Normal plasma glutamate and aspartate concentrations were observed.

These summarized data suggest little risk to normal infants fed moderate quantities of glutamate or aspartate. Since the studies of parenterally and enterally