

The Metabolism of the Aspartyl Moiety of Aspartame

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A handwritten signature in cursive script, reading "R. E. Ranney", is written over a horizontal line. A large, stylized flourish extends from the bottom of the signature.

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The Metabolism of the Aspartyl Moiety of Aspartame

I. Abstract

Aspartame (3-amino-N-(α -carboxyphenethyl)succinamic acid, methyl ester; the methyl ester of aspartylphenylalanine, SC-18862) is hydrolyzed in the gut to yield aspartic acid, phenylalanine and methanol. This review of the literature describes the metabolic paths followed by aspartate in its conversion to CO_2 or its incorporation into body constituents. About 70% of ^{14}C from [asp- ^{14}C]-aspartame is converted in the monkey to $^{14}\text{CO}_2$. Some of the aspartate is converted at the intestinal mucosal level to alanine by decarboxylation. This amino acid may be oxidized to CO_2 by entering the tricarboxylic acid cycle via pyruvate and acetyl CoA. In addition, transamination of aspartate to oxaloacetate permits this product also to enter the tricarboxylic acid cycle. Aspartate may also be incorporated into body constituents such as other amino acids, proteins, pyrimidines, asparagine and N-acetylaspartic acid.

The Metabolism of the Aspartyl Moiety of Aspartame

II. Introduction

Aspartame (3-amino-N-(α -carboxyphenethyl)succinamic acid N-methyl ester; the methyl ester of aspartylphenylalanine; SC-18862) is a sweetening agent that organoleptically has about 180 times the sweetness of sugar (1,2). In considering the metabolism of this dipeptide, it is evident that extensive degradation of the compound may occur after it enters the digestive tract. The compound is stable in an acid medium and would be expected to be little changed by the gastric juice. However, in the small intestine chymotrypsin would be expected to hydrolyze the methyl group (3), and the peptide hydrolases of the microvillar membrane would cleave the dipeptide to its constituent amino acids (4). If this were the case, then all moieties of the compound, methanol, phenylalanine and aspartic acid, should be handled by the body as natural constituents of the diet.

In a previous report, Oppermann et al (5) have compared in monkeys the overall disposition of ^{14}C from free [^{14}C]-aspartic acid with the disposition of ^{14}C from [asp- ^{14}C]-aspartame. The kinetics of the biotransformation of aspartate to CO_2 as well as plasma and excretion ^{14}C kinetics were determined in these studies.

A recent review which has considered different parts of the metabolic pathways followed by aspartic acid has been that of Lowenstein (7) on the tricarboxylic acid cycle. Spencer (8) and Gray and

Cooper (9) have reviewed protein digestion and the intestinal absorption of amino acids.

III. The Absorption and Disposition of ^{14}C from [Asp- ^{14}C]-Aspartame

Oppermann et al (5,10) have compared in rats and monkeys the disposition of ^{14}C in plasma, urine, feces and expired air after intragastric administration of either [U- ^{14}C]-aspartic acid or [U-asp- ^{14}C]-aspartame. Their data showed that after administration of equimolar amounts of either labeled compound to monkeys the conversion of administered ^{14}C to respiratory $^{14}\text{CO}_2$ occurred to the same extent with both compounds (Figure 1). This suggests that the aspartyl moiety was rapidly and completely cleaved from aspartame. Furthermore, it appears that this aspartic acid group of the sweetener was metabolized in much the same manner as free aspartate.

In Figure 2 are seen the plasma ^{14}C levels of monkeys given [^{14}C]-aspartate or [asp- ^{14}C]-aspartame. Early in time, individual variations caused an apparent difference in the ^{14}C in plasma in the two groups of animals, but after four hours the ^{14}C concentrations were essentially the same. There were also differences in the early excretion of $^{14}\text{CO}_2$ and these variations are attributed to the small number of animals in each group (four) and to their normal physiological differences.

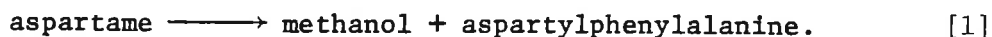
Oppermann (11) has also studied the rates of absorption and disposition of increasing doses of L-aspartic acid in infant and adult mice. Plasma concentrations of aspartic acid were elevated 30 minutes

after oral doses of 1000 mg/kg aspartate were given to 15 day-old or adult mice (Figures 3 and 4). Thereafter, plasma concentrations rapidly declined with a first order half-life of approximately 0.2 hours in both age groups. Aspartic acid plasma concentrations were not appreciably altered after the oral administration of 10 or 100 mg/kg aspartate to either age group.

Peak plasma concentrations after the oral administration of 1000 mg/kg aspartate were greater in 15 day-old mice than in the adult mice. This difference may be a consequence of age dependent differences in the rates of metabolism of aspartic acid in the gut, or in the rates of biotransformation of aspartic acid.

The rates of $^{14}\text{CO}_2$ excretion (% administered ^{14}C appearing as $^{14}\text{CO}_2$ /hour) were also determined after the oral administration of [^{14}C]-aspartate to 15 day-old or adult mice. In adult mice rates of $^{14}\text{CO}_2$ excretion after the oral administration of 10 or 100 mg/kg [^{14}C]-aspartate were similar. However, at 1000 mg/kg the rate of $^{14}\text{CO}_2$ excretion was depressed at 30 minutes after treatment. In infant mice, a dose dependent decrease in the conversion of [^{14}C]-aspartate to $^{14}\text{CO}_2$ was seen. The inability to metabolize the high dose of aspartate at the same rate as the lower doses presumably contributed to the elevated plasma concentrations of aspartic acid in the infant animals given the 1000 mg/kg dose.

The initial hydrolytic reaction which occurred when aspartame entered the small intestine is assumed to be:



The major enzyme which participates in this reaction is presumably chymotrypsin (EC 3.4.4.5), a very active esterase as well as proteolytic enzyme (3).

The aspartylphenylalanine formed in Equation [1] would next be cleaved by dipeptide hydrolases (EC 3.4.3), at the brush border of the intestinal mucosal cells or by an intracellular dipeptidase (9):

aspartylphenylalanine \longrightarrow aspartic acid + phenylalanine. [2]

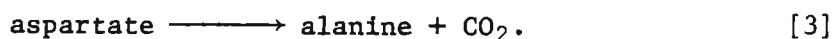
In addition to this pathway of absorption, it is possible that some of the unchanged dipeptide would be transported into the portal circulation. Bouldin et al (12) have described the appearance of intact dipeptides in the mesenteric vein blood of experimental animals. The extent of such absorption seems small since in metabolic studies in several species Oppermann and Ranney (10) were not able to identify unchanged aspartylphenylalanine in plasma samples..

The absorption of aspartate from the intestinal lumen appears to be a carrier-mediated process. This is also true of glutamate transport. Although Parsons and Volman-Mitchell (13) concluded from transamination studies, that the absorption of both aspartate and glutamate were independent, this seemed to have been refuted by Schultz et al (14). The latter workers, using ^{14}C labeled amino acids in vitro, showed conclusively that aspartate and glutamate were absorbed through the action of saturatable, carrier-mediated processes. Each amino acid was a competitive inhibitor of the transport of the other compound.

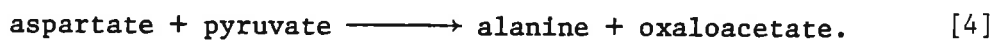
After the absorption of aspartate, the major products which reach the portal circulation are the result of oxidative and transamination

reactions in the mucosal cells. This has been shown by Neame and Wiseman (15) in vivo in the dog as well as by Parsons and Volman-Mitchell (13) in vitro in rats, guinea pigs and chickens. After aspartate was presented either in vivo or in vitro to the absorbing surface, alanine was found to be the major amino acid appearing in the mesenteric vein blood or the serosal surface of an intestinal sac, in each experimental condition.

Of the enzymatic reactions which may be active in the mucosal cells, two systems seem most probable to be effecting these transformations. One of these is aspartic acid-4-decarboxylase (EC 4.1.1.12):



In addition to this reaction, a transamination enzyme must be involved. This is alanine:2 oxoacid aminotransferase (EC 2.6.1.12):



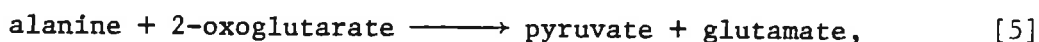
Through these reactions, Parsons and Volman-Mitchell (12) estimated that 85% of the administered aspartate was converted to alanine in their rat in vitro preparations. The conversion to alanine was proportionately less as aspartate concentrations were increased so that more unchanged aspartate entered the blood. The elevated levels of aspartic acid, which appear in the blood of mice after high doses of the compound (Figures 3 and 4), presumable are the result of saturation of the enzymatic reactions described in Equations 3 and 4.

IV. Metabolic Reactions of Aspartate and Alanine

A. Oxidation to CO₂: Oppermann and Ranney (10) found more than

70% of the aspartic acid from aspartame was converted to CO₂ (Figure 1). Although some of this would arise from the decarboxylation of aspartate (Equation 3), the major fraction would result from the oxidation of alanine from Equations 3 and 4 and of oxaloacetate from Equation 4 through the tricarboxylic acid (TCA) cycle.

Alanine enters the TCA cycle via pyruvate and acetyl CoA through the mediation of the following enzyme systems: alanine:2-oxoacid aminotransferase (EC 2.6.1.12):



and the complex enzyme system of pyruvate dehydrogenase (EC 1.2.4.1) and lipoate acetyl transferase (EC 2.3.1.12). The overall reaction products in this system are:



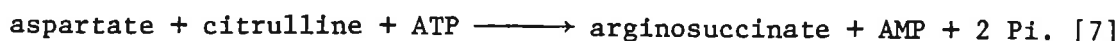
Acetyl CoA then enters the TCA cycle by condensations with oxaloacetate to form citrate (Scheme 1).

Aspartate which has been absorbed from the gut unchanged may enter directly into the TCA cycle by transamination (Scheme 2).

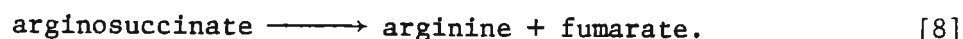
B. Synthetic Reactions Involving Aspartate: After an oral dose of [asp-¹⁴C]-aspartame, about 25% of the label was incorporated into body constituents and 5% was excreted in the feces (5). The long half-life of ¹⁴C in the plasma of monkeys given [asp-¹⁴C]-aspartame (Figure 2) is evidence for such incorporation into plasma protein.

One of the major synthetic reactions in which aspartate takes part is in the formation of arginine. This reaction of aspartate

and citrulline is catalyzed by arginosuccinate synthetase (EC 6.3.4.5):



The next step involves arginosuccinate lyase (EC 4.3.2.1):



Aspartate also is the starting substrate for asparagine formation. This reaction is mediated by asparagine synthetase (EC 6.3.1.1):



Other reactions in which aspartate may participate are the formation of lysine, an 8 step synthesis; the synthesis of uridine monophosphate in 5 steps; the one step synthesis of N-acetylaspartate in brain; and its incorporation into protein.

V. Nutritional Aspects of Aspartate

Since the digestion of aspartame adds a certain amount of aspartic acid to the dietary intake of this amino acid, it is important to determine how significant this addition is. The average dietary intake of protein in the U.S.A. is about 90 g/day. Of this, aspartic acid makes up about 8.8% of the protein or an intake of 7.9 g/day. It has been estimated that an instant-mix soft drink adequately sweetened with aspartame would contain 0.4 g/liter. Ingestion of this liter of soft drink would yield 0.18 g of aspartic acid or 2.3% of the daily intake of this amino acid. The protein content of the normal diet probably varies greater than 2% from day to day, so that this addition to it would be negligible. Even a several-fold greater ingestion of aspartame on a daily basis would appear

to have a negligible effect on the overall amino acid metabolism. Harper et al (16) have reviewed research on amino acid imbalances, and they concluded:

"From the accumulated observations on animals, it seems unlikely that man would ingest excesses of individual amino acids in sufficient quantity to produce adverse effects when subsisting on a diet composed of natural foods. Quantities of individual amino acids sufficient to result in adverse effects would not be used as supplements to foodstuffs. It would require 10-fold the normal supplementary level or more to approach the amounts shown to cause adverse effects in animals. Only if large doses of individual amino acids were administered regularly for some special reason would there be much likelihood of approaching toxic levels. Even then, unless the protein intake of the subject was low, it seems unlikely that quantities sufficient to produce more than mild adverse effects would be ingested."

VI. Conclusions

The available evidence from studies in experimental animals leads to the conclusion that the aspartate moiety of aspartame is metabolized in a manner similar to that of dietary aspartic acid. The major fraction of this moiety is utilized for energy through

oxidation in the tricarboxylic acid cycle. Incorporation into protein, other amino acids, and nucleotides are lesser pathways followed by this amino acid.

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VIII. Figures and Schemes

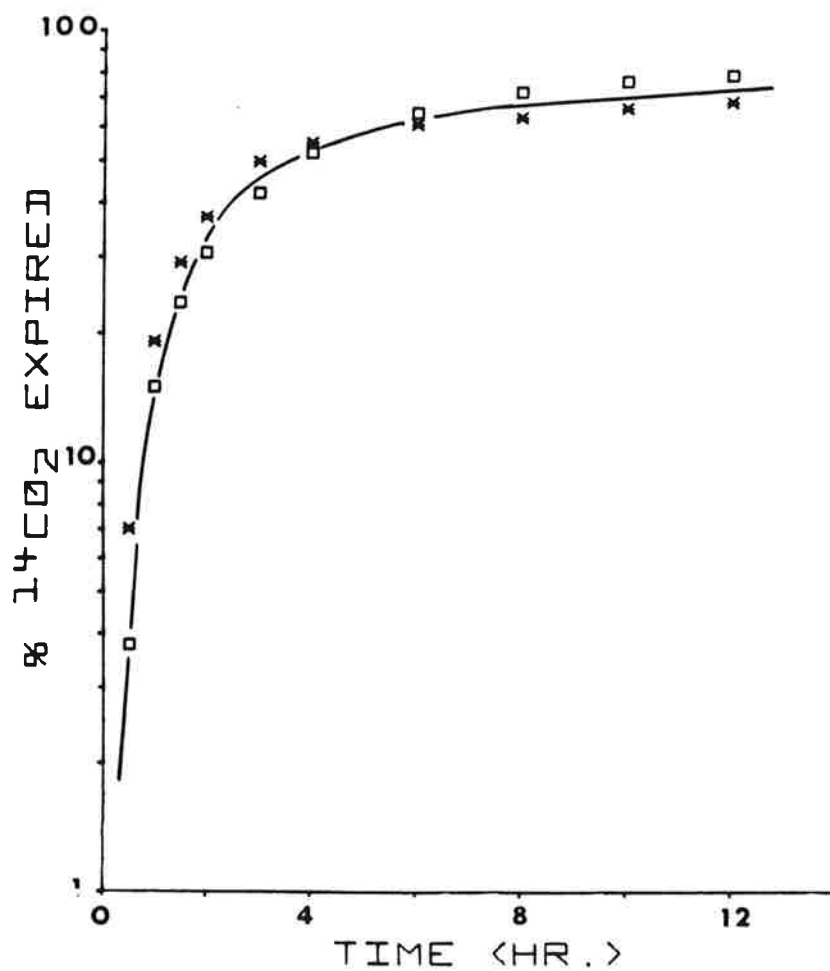
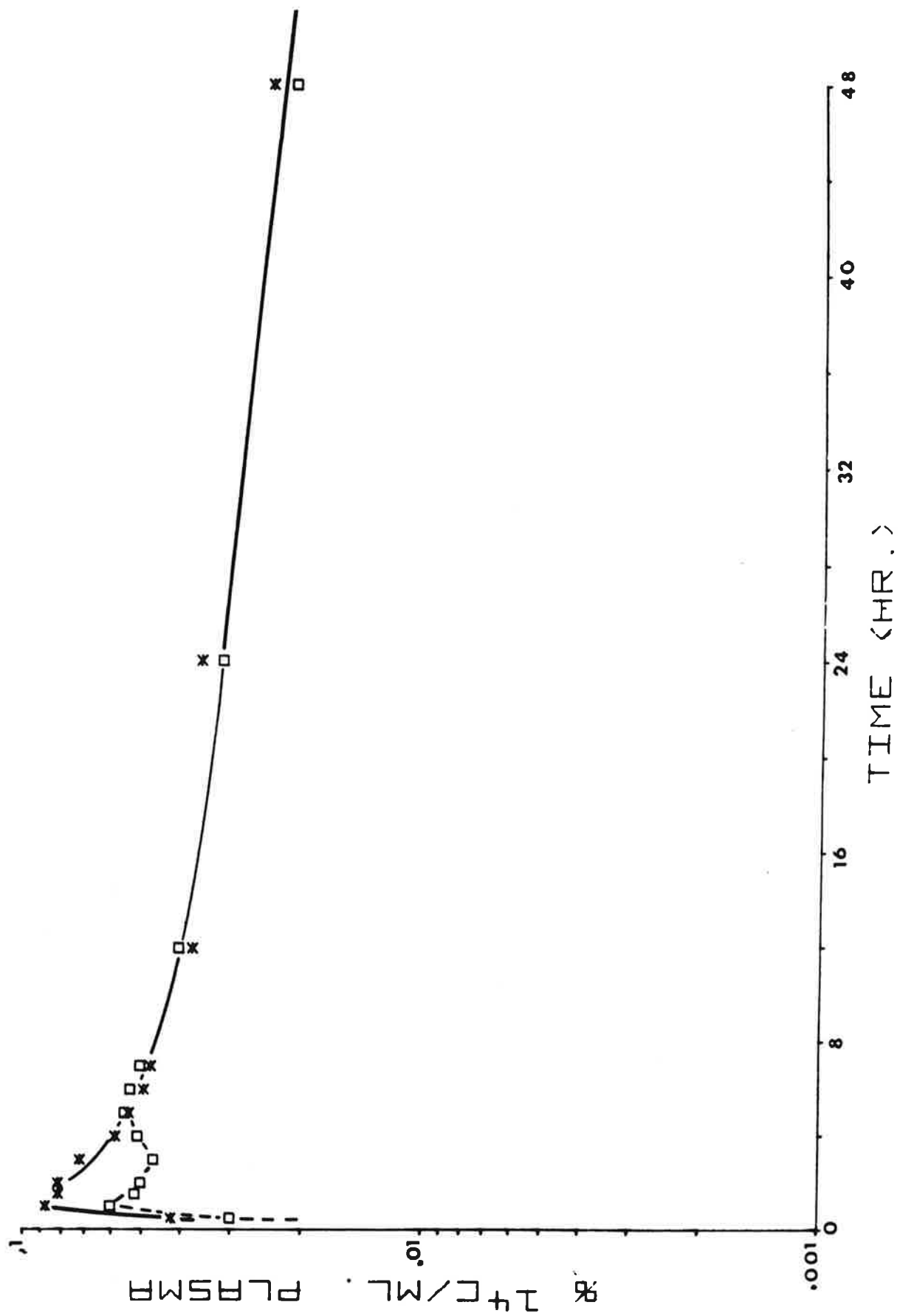


Figure 1. Cumulative $^{14}\text{CO}_2$ excretion by monkeys given [^{14}C]-aspartic acid (*) or [asp- ^{14}C]-aspartame (□). Ordinate: cumulative % administered ^{14}C in expired air; abscissa: hours after oral administration of the compounds.



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Figure 2. Plasma ^{14}C levels in monkeys given $[^{14}\text{C}]$ -aspartic acid (*) or $[\text{asp-}^{14}\text{C}]$ -aspartame (□). Ordinate: % administered ^{14}C /ml plasma; abscissa: hours after oral administration of the compound.

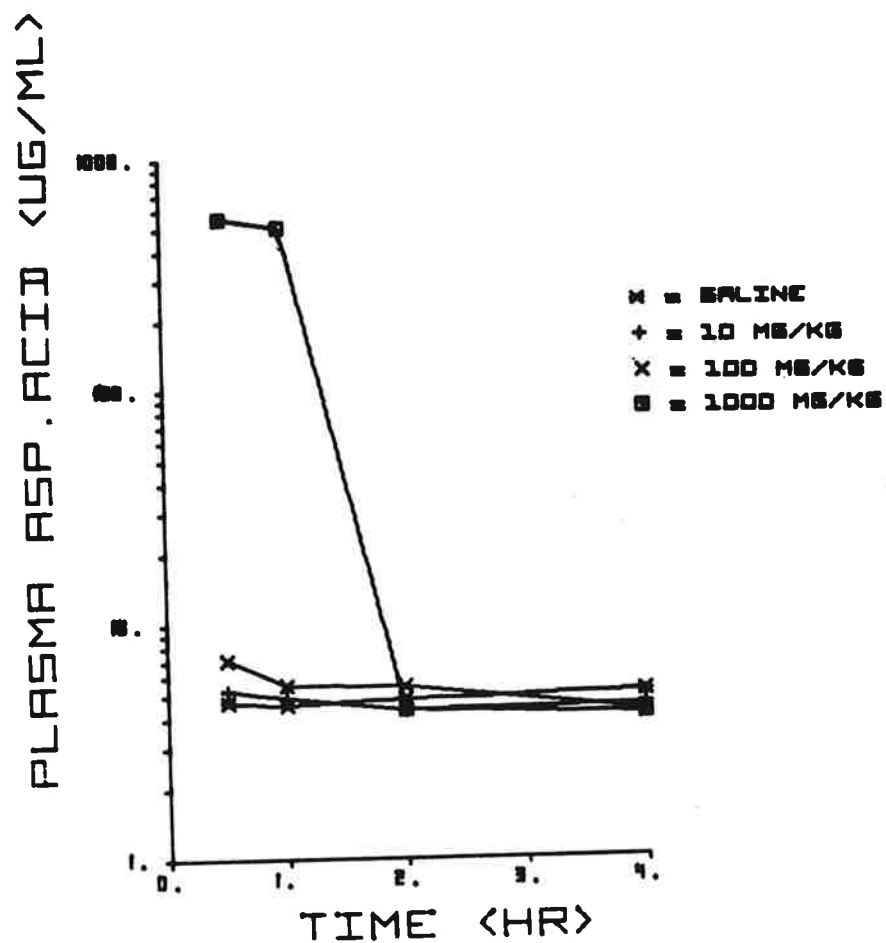


Figure 3. Plasma concentrations of aspartic acid after the oral administration of L-aspartate to 15 day-old mice. Units: ordinate: µg aspartic acid/ml of plasma; abscissa: hours after administration of aspartate. Each point is the pooled plasma sample obtained from 10-12 mice.

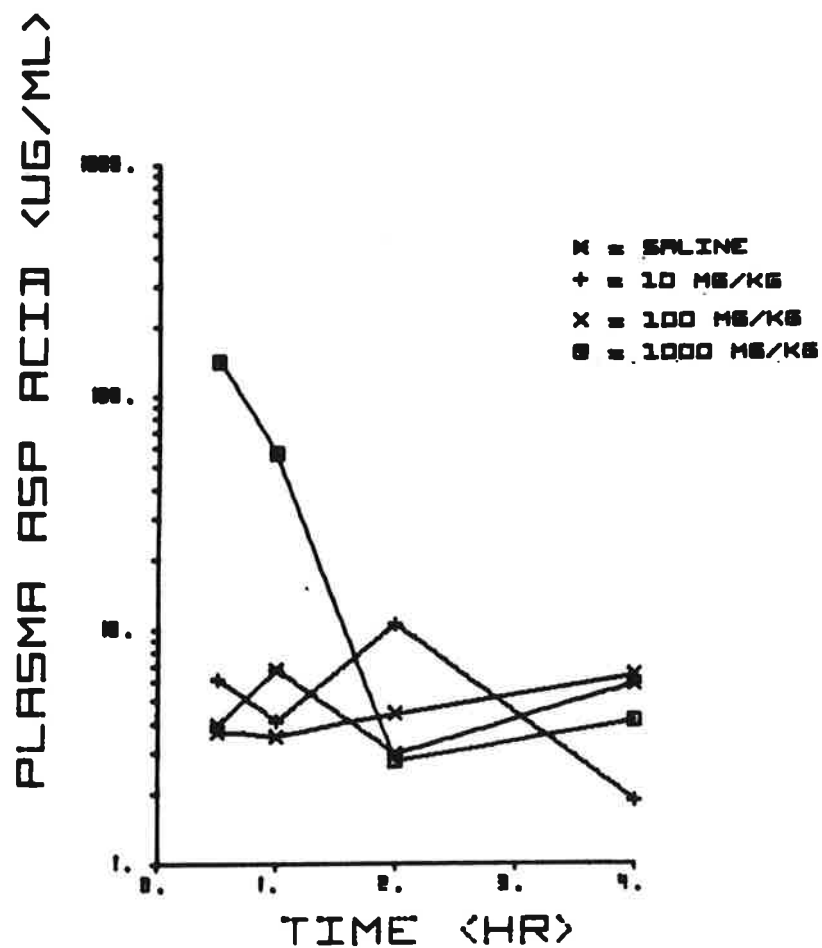
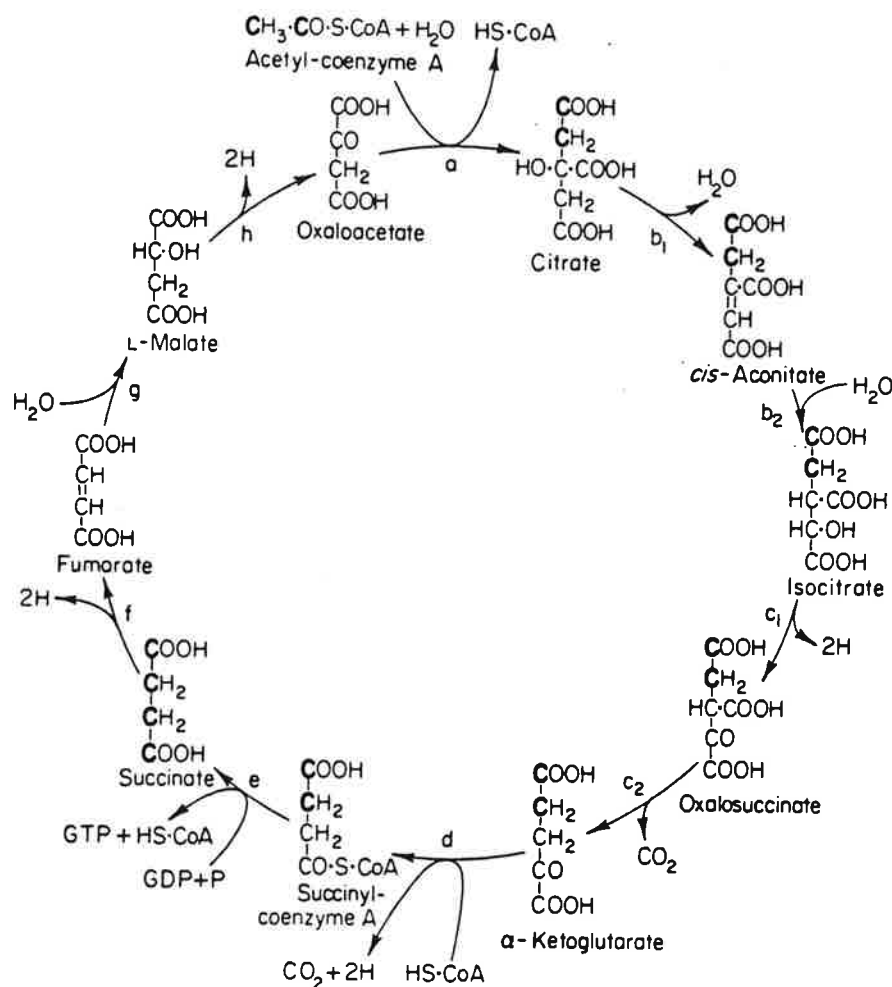


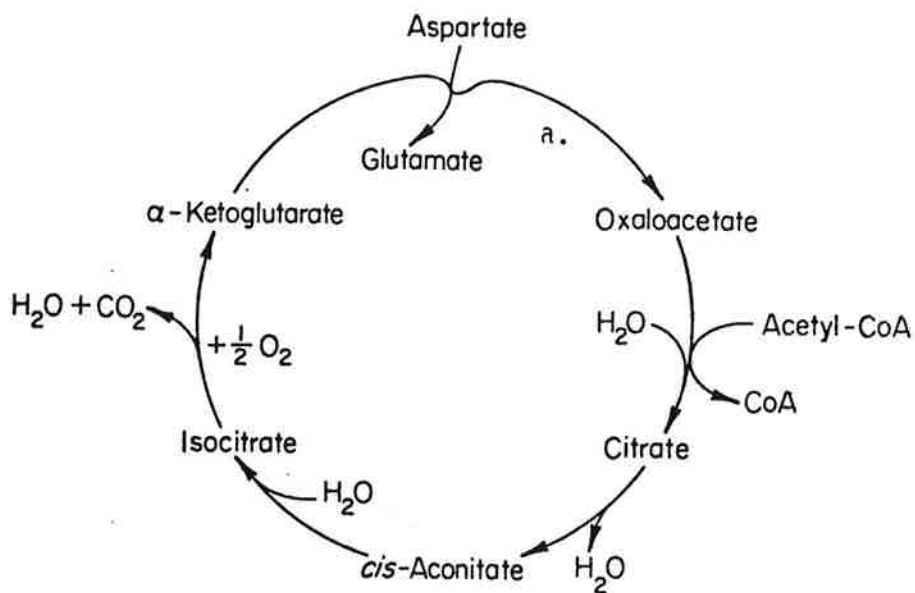
Figure 4. Plasma concentrations of aspartic acid after the oral administration of L-aspartate to adult mice. Units: ordinate: μg aspartic acid/ml of plasma; abscissa: hours after administration of aspartate. Each point is the pooled plasma sample obtained from 10-12 mice.



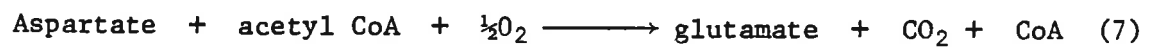
Scheme 1. The tricarboxylic acid cycle. The diagram illustrates the complete combustion of one acetic acid equivalent. The end products shown are CO_2 , water and hydrogen. The four pairs of hydrogen atoms are transferred to coenzymes, and subsequently react through the electron carrier chain with molecular oxygen to form water. The two carbons of acetate are fed into the cycle in the form of the acetyl group of acetyl-coenzyme A. The fate of these carbons is shown in heavy outline to the point where they reach succinate. The carbon atoms of an acetyl group fed into the cycle remain in the compounds of the cycle during the first turn. Reversibility of the reactions is not indicated. Under physiological conditions, the compounds that participate in the cycle exist to a large extent as anions, and in the scheme are named as such. However, for the sake of clarity, the formulas shown are those of the free acids.

The enzymes catalyzing the reactions are: a, condensing enzyme (EC 4.1.3.7); b (1 and 2), aconitase (EC 4.2.1.3); c (1 and 2), isocitrate dehydrogenase (EC 1.1.1.42); d, oxoglutarate dehydrogenase (EC 1.2.4.2); e, succinate thiokinase (EC 6.2.1.4); f, succinate dehydrogenase (EC 1.3.99.1); g, fumarase (EC 4.2.1.2); and h, malate dehydrogenase (EC 1.1.1.37).

Note the following: (1) citrate can also yield isocitrate without cis-aconitate occurring as a free intermediate, (2) oxalosuccinate probably occurs only as an enzyme-bound intermediate, (3) in brain α -ketoglutarate can also yield succinate via γ -aminobutyrate, and (4) succinyl-coenzyme A can also yield succinate via reactions catalyzed by succinyl-coenzyme A deacylase or succinyl-coenzyme A transferase. This Scheme is reproduced from reference 7.



Scheme 2: The aspartate-glutamate cycle. The net effect of one turn of the cycle is:



The enzymes involved in this sequence are:

a, glutamate-oxaloacetate transaminase (EC 2.6.11) and those listed in Scheme 1. This scheme is reproduced from reference 7.