

The Metabolism of Aspartate in Infant and Adult Mice

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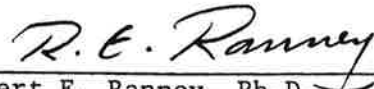
The Metabolism of Aspartate in Infant and Adult Mice

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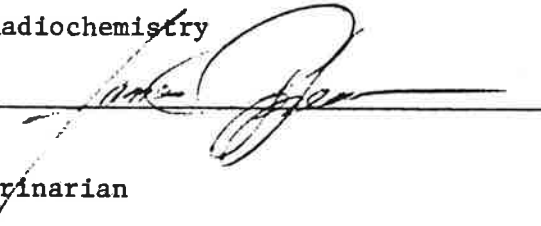
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Table of Contents

	Page
I. Abstract	1
II. Introduction	3
III. Materials and Methods	4
1. L-[U- ¹⁴ C]-Aspartic Acid	4
2. Animal Treatment	4
3. Preparation of Dosing Solutions	4
4. Blood Sampling: Adult Mice	5
5. Blood Sampling: 15 Day-Old Mice	5
6. ¹⁴ CO ₂ Excretion	5
7. ¹⁴ C Measurements	5
8. Aspartic Acid Determination	6
IV. Results	6
1. Plasma Concentrations of Aspartic Acid: 15 Day-Old Mice	6
2. Plasma Concentrations of Aspartic Acid: Adult Mice	8
3. ¹⁴ CO ₂ Excretion	9
V. Discussion	10
VI. References	14
VII. Tables	15
VIII. Figures	18
IX. Appendix Tables	21

The Metabolism of Aspartate in Infant and Adult Mice

I. Abstract

Plasma concentrations of aspartic acid were determined after the oral or intraperitoneal (ip) administration of 0, 10, 100 and 1000 mg/kg L-aspartate to 15 day-old and adult mice. Plasma concentrations of aspartic acid were elevated 30 minutes after 1000 mg/kg L-aspartate (oral or ip) to 15 day-old and adult mice. Thereafter, plasma concentrations rapidly declined exponentially (log concentration versus time) with a half-life of approximately 0.2 hours in both age groups. Aspartic acid plasma concentrations were not appreciably altered after the oral or ip administration of 10 and 100 mg/kg L-aspartate.

After oral administration of 1000 mg/kg L-aspartate the area under the plasma aspartic acid concentration curve was much greater for 15 day-old mice than that for adult mice. Since plasma concentrations of aspartic acid in both age groups declined with similar rates after ip administration of L-aspartate this difference cannot be accounted for by differences in the systemic metabolism of aspartate. However the systemic availability of orally administered aspartic acid may differ with age because of differences in the rates of metabolism of aspartic acid in the gut.

The rates of $^{14}\text{CO}_2$ excretion were also determined after the oral or ip administration of $[^{14}\text{C}]$ -L-aspartate to 15 day-old and adult mice. After the oral or ip administration of 10 and 100 mg/kg $[^{14}\text{C}]$ -L-aspartate, these rates were similar in both 15 day-old and adult mice. However at

MRC-751-0021

1000 mg/kg, the rates of $^{14}\text{CO}_2$ excretion, as percent of dose, were depressed during the first 30 minutes after both oral and ip administration of the compound to both age groups. This inability to metabolize high doses of aspartate at the same rates as lower doses, may contribute to the elevated plasma concentrations of aspartic acid in animals given 1000 mg/kg.

The Metabolism of Aspartate in Infant and Adult Mice

II. Introduction

Administration of a large oral dose of aspartate or glutamate (1000 mg/kg) has been reported to produce hypothalamic damage in infant mice (1). However, conflicting reports have appeared as to whether or not this effect occurs in primates such as the rhesus monkey. Olney et al (2,3) have reported hypothalamic lesions in infant rhesus monkeys given 2700 mg/kg glutamate by subcutaneous injection. In these studies blood glutamic acid concentrations were elevated above normal. Conversely, Newman et al (4) administered glutamate at oral doses of up to 4000 mg/kg to infant rhesus monkeys and did not find evidence of hypothalamic damage. Plasma glutamic acid concentrations were also measured in these studies, and were found to be within the normal range for monkeys of this age. These results suggested that a relationship existed between plasma glutamic acid concentrations and the occurrence of hypothalamic lesions, a point noted by both Olney et al (5) and Stegink et al (6).

Although plasma glutamic acid concentrations in mice after administration of various oral doses of glutamate have been reported, relatively little is known concerning plasma aspartic acid levels after various doses of aspartate. This study describes the absorption and metabolism of aspartic acid after the oral or ip administration of aspartate to 15 day-old and to adult mice.

MRC-751-0021

III. Materials and Methods

1. L-[U-¹⁴C]-Aspartic Acid: L-[U-¹⁴C]-aspartic acid was obtained from Amersham/Searle Corp. (Arlington Heights, Ill.) with a specific activity of 10 mCi/mmol and with a radiochemical purity of 98%. Radiochemical purity was determined in three thin-layer chromatography solvent systems.

2. Animal Treatment: Charles River, CD-1 mice, weighing between 6-9 g (15 day-old mice) and 35-40 g (90 day-old, adult) were used in this study. Nursing pups were removed from their mother 3 hours prior to aspartate administration. Adult mice were fasted overnight. Oral drug administration was accomplished by intubation with an 18 gauge, curved intubation needle (adult mice) or Tygon® tubing (0.64 mm, i.d. x 1.02 mm o.d. - 15 day-old mice). Intraperitoneal (ip) administration was by means of a 26 gauge needle. The volume of the injected amino acid solution was between 0.15 and 0.40 ml in all studies.

3. Preparation of Dosing Solutions: Attempts to dissolve L-aspartic acid in distilled water at the required concentrations were unsuccessful. For this reason, monosodium L-aspartate was prepared by the equimolar addition of L-aspartic acid (Aldrich Chemical Co., Milwaukee, Wis.) and sodium hydroxide. Solutions were prepared in distilled water having final aspartic acid concentrations of: 1-100 mg/ml (adults); or 0.3-33 mg/ml (15 day-old mice).

Radioactive dosing solutions were prepared as above and contained approximately 3 µCi/ml.

MRC-751-0021

4. Blood Sampling: Adult Mice: Adult mice were sacrificed by decapitation and blood was collected from the wound into glass tubes containing heparin. Plasma was obtained by centrifugation and equal quantities of plasma from 5 mice were pooled at each time point. Plasma samples were stored frozen at -20°C until subsequent analysis.

5. Blood Sampling: 15 Day-Old Mice: At each time point 10-12 15 day-old mice were anesthetized by ethyl ether inhalation; each animal was placed on its back and pinned to a cork board. A small pocket of skin was formed in each animal by making a right angle incision in the skin between the head and the left foreleg. The subclavian vein was cut and blood was collected from the pocket into a plastic syringe (1 ml capacity) containing heparin. The hub of the syringe was sealed by heat, and plasma was obtained by centrifugation. The pooled plasma samples were stored frozen at -20°C until subsequent analysis.

6. $^{14}\text{CO}_2$ Excretion: Immediately after [^{14}C]-aspartate administration three mice were placed in a cylindrical glass metabolism cage, and 1.5 liters/minute of room air was drawn in series through the metabolism cage and through three gas washers, each of which contained 20 ml ethanolamine:methyl cellosolve (1:2, v/v). The washers were changed at 30 minute intervals; their contents were pooled, and aliquots of the pooled samples were assayed for radioactivity.

7. ^{14}C Measurements: Aliquots from the CO_2 trapping solutions and the dosing solutions were added to vials containing 15 ml PCSTM liquid scintillation solution (Amersham/Searle Corp.). Vials were assayed for

MRC-751-0021

radioactivity by liquid scintillation spectrometry (Mark II spectrometer, Searle Analytic Inc., Des Plaines, Ill.). All ^{14}C determinations were corrected for background and for ^{14}C quench (external standard channels ratio method).

8. Aspartic Acid Determination: The concentration of aspartic acid in plasma was determined by Analytical Biochemistry Laboratories, Columbia, Mo., utilizing a modification of the method of Stein and Moore (11). Sulfosalicylic acid (1.5 ml, 3%) was added to a test tube containing 0.5 ml of plasma. After thorough mixing and centrifugation, the supernatant fraction was filtered on a 0.2 μ MilliporeTM filter. The filtrate was collected and 1.0 ml was added to a tube which contained 0.75 ml of 0.143 N sodium hydroxide. Aliquots of this solution were placed on a cation exchange column (19 cm x 0.64 cm, Bio Rad BRX-48015) in an autoanalyzer. The column was maintained at 60°C and was eluted with the following buffer solutions: A, 0.20 N sodium citrate, pH 3.25; and B, 3.5 N sodium citrate, pH 5.80. The buffer flow rate was 60 ml/hour and the ninhydrin flow rate was 30 ml/hour. After the amino acid in the sample reacted with ninhydrin the optical density of the solution was converted to concentration units with an Infotronics CRS 110A data acquisition system.

IV. Results

1. Plasma Concentrations of Aspartic Acid: 15 Day-Old Mice:

Figure 1a (Appendix Table 1) gives the plasma concentrations of aspartic

MRC-751-0021

acid in 15 day-old mice after the oral administration of 0, 10, 100 and 1000 mg/kg monosodium aspartate. An elevation in the plasma concentrations was present at 30 minutes and 60 minutes after the 1000 mg/kg dose. However, after a dose of 100 mg/kg only a minimal increase in the plasma concentration was observed. At 10 mg/kg the plasma concentrations at all times were similar to control values.

A similar plasma profile was observed after ip administration of various doses of aspartate to 15 day-old mice (Figure 1b, Appendix Table 2). The plasma concentrations of aspartic acid 30 minutes after the 1000 mg/kg dose were 180 times greater than the plasma aspartic acid concentrations in mice given saline. It was again significant to note that plasma concentrations were only moderately increased at the dose of 100 mg/kg and not altered at all at a dose of 10 mg/kg.

Peak plasma concentrations of aspartic acid after the 1000 mg/kg dose were slightly greater after ip administration (718 $\mu\text{g/ml}$) than after oral administration (554 $\mu\text{g/ml}$). However, the areas under the oral and ip plasma concentration-time curves were 667 $\mu\text{g/ml}\cdot\text{hr}$ and 628 $\mu\text{g/ml}\cdot\text{hr}$, respectively. The similarity in these values suggested that the bioavailability of L-aspartate was similar after both oral and ip administration to 15 day-old mice.

The plasma concentrations of aspartic acid in 15 day-old mice given aspartate intraperitoneally (1000 mg/kg) declined with a half-life of 0.21 hours and this half-life after oral administration of the compound was 0.15 hours. These rapid disappearance rates reflected the

MRC-751-0021

rapid systemic metabolism of aspartic acid and partially accounted for the nearly constant plasma concentrations observed after lower doses of aspartic acid.

2. Plasma Concentrations of Aspartic Acid: Adult Mice: Figures 2a and 2b (Appendix Tables 3 and 4) give the plasma concentration of aspartic acid after the oral or ip administration of various doses of L-aspartate to adult mice. Dose dependent increases in plasma concentrations of aspartic acid were not observed after either oral or ip administration of 10 or 100 mg/kg L-aspartate. However, after both oral and ip doses of 1000 mg/kg L-aspartate, elevated plasma concentrations of aspartic acid occurred.

A considerable amount of variability was observed in plasma aspartic acid concentrations that were below 10 $\mu\text{g/ml}$. This variability may have been a result of the method of blood collection used in these studies since it was noted that these plasma samples were hemolyzed to a greater extent than those obtained from the 15 day-old mice. An intentionally hemolyzed control blood sample contained 2.9 μg aspartic acid/ml of plasma before, and 4 μg aspartic acid/ml of plasma after, hemolysis thereby suggesting that hemolysis may have contributed to the variability observed at low aspartic acid concentrations.

After 1000 mg/kg L-aspartate plasma concentrations of aspartic acid declined with half-lives of 0.19 hours (ip) and 0.26 hours (oral). These rates of disappearance of aspartate approximated the decline observed after ip administration of 1000 mg/kg aspartate to 15 day-old mice (0.21 hours).

MRC-751-0021

The areas under the aspartic acid plasma concentration curves after oral and ip administration of 1000 mg/kg L-aspartate were 122 $\mu\text{g/ml}\cdot\text{hr}$ and 891 $\mu\text{g/ml}\cdot\text{hr}$, respectively. These results suggested that the systemic availability of aspartic acid in adult mice was much less after oral administration than after ip administration. Therefore, this differed from that observed in 15 day-old mice in which the apparent systemic availability of aspartate after either oral or ip administration was similar.

3. $^{14}\text{CO}_2$ Excretion: Table 1 gives the rates of $^{14}\text{CO}_2$ excretion after the oral or ip administration of [^{14}C]-L-aspartate to 15 day-old mice. Only minor differences in the rates of $^{14}\text{CO}_2$ excretion (expressed as percent of administered ^{14}C) were observed in the 10 and 100 mg/kg treatment groups. However, at the 1000 mg/kg dose, the rate of $^{14}\text{CO}_2$ excretion was greatly reduced during the initial 30 minute interval after both oral and ip aspartate administration. These results suggested that the animals' ability to oxidize the exogenous aspartate to CO_2 was exceeded in the group of 15 day-old mice given 1000 mg/kg. After 1 hour the rates of conversion of aspartate to $^{14}\text{CO}_2$ were not appreciably different among the three treatment groups. The decrease in the rate of $^{14}\text{CO}_2$ production after a dose of 1000 mg/kg [^{14}C]-L-aspartate corresponded to the time interval during which greatly elevated plasma concentrations of aspartic acid were observed (Figure 1).

A similar effect was also observed in adult mice (Table 2). A pronounced decrease in the rate of $^{14}\text{CO}_2$ excretion occurred during the

MRC-751-0021

0-30 minute interval after 1000 mg/kg [^{14}C]-L-aspartate as compared to the rates after 10 and 100 mg/kg. This decrease in the metabolism rate may have been partially responsible for the elevated plasma concentrations which also occurred at those times.

V. Discussion

Oral or ip administration of 10 and 100 mg/kg L-aspartate to adult mice did not produce dose related alterations in plasma concentrations of aspartic acid. Similarly, plasma levels of aspartic acid were not increased after either oral or ip administration of 10 mg L-aspartate to 15 day-old mice. At a dose of 100 mg/kg a small increase in plasma aspartate concentrations (less than 4 $\mu\text{g/ml}$) was observed at 30 minutes both after oral and ip administration.

On the other hand, elevated plasma concentrations of aspartic acid occurred after oral and ip administration of 1000 mg/kg L-aspartate to 15 day-old or adult mice. Peak plasma concentrations of aspartic acid as great as 180 times control concentrations were observed after ip administration. The minimal increase in plasma concentrations after 10 and 100 mg/kg L-aspartate and the great increase in plasma concentration after 1000 mg/kg, suggested that the pharmacokinetics of aspartate were altered after very high doses of aspartate. This alteration in the pharmacokinetics may have occurred as a result of saturation of either distributive, metabolic, or excretory processes.

MRC-751-0021

The areas under the plasma aspartic acid concentration-time curves were determined after oral and ip administration of 1000 mg/kg L-aspartate to adult and 15 day-old mice. In 15 day-old mice the areas under the oral and ip curves were similar. These results suggested that the systemic availability of aspartic acid was similar after either route of administration. However, this differed from that observed in adult mice in which the area under the plasma aspartic acid concentration-time curve after ip administration was 7 times greater than the area under the curve after oral administration. It therefore appeared that the systemic availability of L-aspartate in adult mice was much less after oral administration than after ip administration.

Both intraperitoneally and orally administered compounds were absorbed via the portal vein (7). Therefore, the observed difference in the ip and oral systemic availability of aspartic acid was not a result of first pass metabolism by the liver (first pass effect). However, aspartic acid is metabolized by the small intestine during the absorption process (8,9). It therefore appeared that the apparent reduced bioavailability of L-aspartate after oral administration to adult mice resulted from metabolism by the wall of the gastrointestinal tract. Reduced oral bioavailability was not observed in 15 day-old mice and these results suggested that metabolism of aspartic acid by the gastrointestinal tract was not developed to the same degree in younger animals as it was in adults.

The area under the aspartic acid plasma curve and the peak plasma

MRC-751-0021

concentration were much lower after oral administration of 1000 mg/kg L-aspartate to adult mice than those same parameters in 15 day-old mice (141 μ g/ml vs 554 μ g/ml). This was not a result of slower systemic metabolism or excretion in the 15 day-old mice since the decline in the plasma concentration of aspartic acid in these animals was faster (0.15 hr) than that observed in adult mice (0.26 hr). However, since aspartic acid was metabolized in the wall of the gastrointestinal tract during absorption, it is tempting to postulate that the rate of metabolism by the gastrointestinal tract in 15 day-old mice was less than the rate in adult animals, and that this difference resulted in the higher plasma concentrations of aspartic acid in the newborn mice.

It was interesting to note that a similar phenomena occurred after monosodium glutamate administration. After oral administration of 4 g/kg glutamate, peak plasma glutamate concentrations were 2300 μ g/ml in neonatal mice whereas they were 1050 μ g/ml in adult mice (10). Since glutamate was also metabolized in the wall of the gastrointestinal tract (8,9), this difference again may have resulted from age-dependent differences in the activity of these enzymes. However, it was also possible that the concentration differences of aspartate and glutamate occurred as a result of age-dependent differences in the rate of absorption or distribution of aspartic acid.

In the present studies, the rate of $^{14}\text{CO}_2$ excretion, as percent of dose, was decreased after administration of 1000 mg/kg [^{14}C]-L-aspartate

MRC-751-0021

relative to the rates after either oral or ip administration of 10 and 100 mg/kg in both 15 day-old and adult mice. The decrease in the rate occurred at times when the plasma concentration of aspartic acid was greatly elevated, and may have reflected saturation of an enzyme associated with aspartic acid metabolism. This saturation effect may have contributed to the elevated plasma concentration of aspartic acid observed at the high dose level.

Whatever the mechanism, it was clear that after equivalent massive oral doses of either glutamate or aspartate, higher plasma concentrations occurred in newborn mice than in adults. Therefore, this difference may explain the increased susceptibility of infant mice to hypothalamic damage produced by massive oral doses of aspartate or glutamate (12).

VI. References

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MRC-751-0021

VII. Tables

MRC-751-0021

Table 1

The Excretion of $^{14}\text{CO}_2$ After the Oral and Intraperitoneal
Administration of 10, 100 or 1000 mg/kg
[^{14}C]-L-Aspartate to 15 Day-Old Mice

Time after Aspartate Administration (hours)	$^{14}\text{CO}_2$ Excretion*					
	Dose Administered (mg/kg)					
	10		100		1000	
	oral	ip	oral	ip	oral	ip
0.5	34.5	44.9	25.8	33.7	7.2	4.4
1.0	25.2	17.3	17.8	16.3	24.6	18.9
1.5	10.3	7.3	14.3	8.2	12.2	13.7
2.0	5.5	5.8	6.4	5.2	7.2	11.0
2.5	3.9	3.3	2.9	3.8	4.7	6.2
3.0	2.4	2.1	1.8	2.0	3.4	3.8
3.5	1.0	1.3	1.2	1.4	2.6	2.4
4.0	0.7	1.5	1.1	1.1	2.1	1.8
4.5	0.8	1.2	0.7	0.7	1.7	1.7
5.0	0.6	0.9	0.6	0.5	1.4	1.2
Total	84.9	85.6	72.6	72.9	67.1	65.1

* Percent of administered ^{14}C excreted as $^{14}\text{CO}_2$ per 0.5 hours.

Table 2

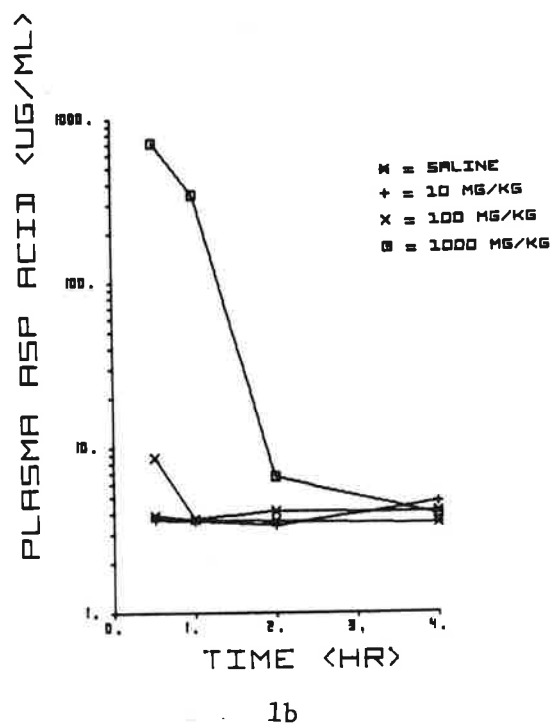
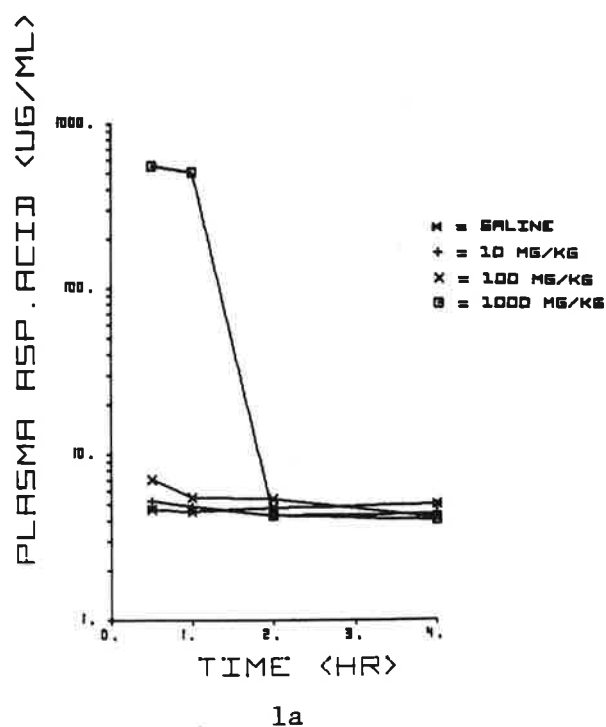
The Excretion of $^{14}\text{CO}_2$ After the Oral and Intraperitoneal
Administration of 10, 100 or 1000 mg/kg
[^{14}C]-L-Aspartate to Adult Mice

Time after Aspartate Administration (hours)	$^{14}\text{CO}_2$ Excretion*					
	Dose Administered (mg/kg)					
	10		100		1000	
	oral	ip	oral	ip	oral	ip
0.5	41.8	36.1	29.2	31.3	15.1	9.7
1.0	10.2	15.4	17.4	17.4	21.4	16.4
1.5	4.2	6.7	8.1	6.9	15.3	12.7
2.0	2.2	3.5	4.4	3.1	8.9	6.9
2.5	1.4	2.5	2.9	2.0	5.2	4.9
3.0	1.2	1.8	1.9	1.4	3.1	2.9
3.5	0.9	1.1	1.5	1.3	2.4	2.1
4.0	0.8	0.9	1.2	0.8	1.6	1.4
4.5	0.6	0.8	1.1	0.6	1.2	1.0
5.0	0.7	0.6	0.9	0.6	1.2	0.7
5.5	0.8	0.5	0.7	0.5	0.8	0.7
6.0	0.5	0.4	0.6	0.4	0.9	0.6
Total	65.3	70.3	69.9	66.3	77.1	60.0

* Percent of administered ^{14}C excreted as $^{14}\text{CO}_2$ per 0.5 hours.

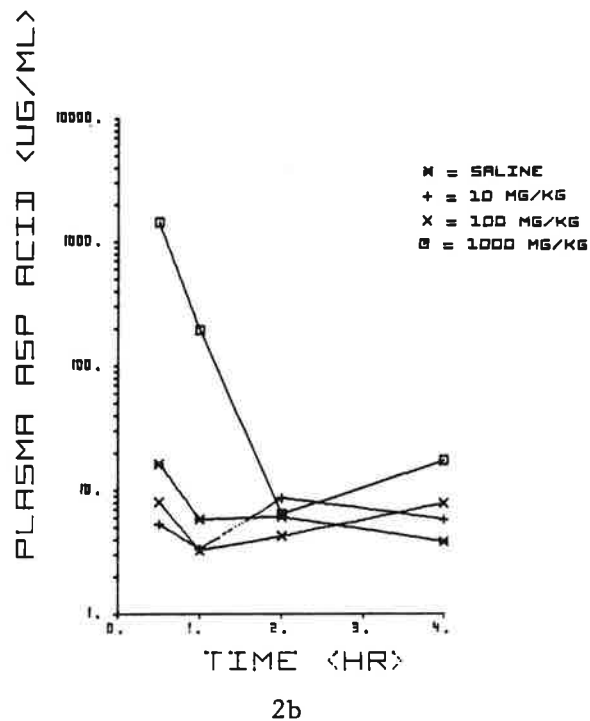
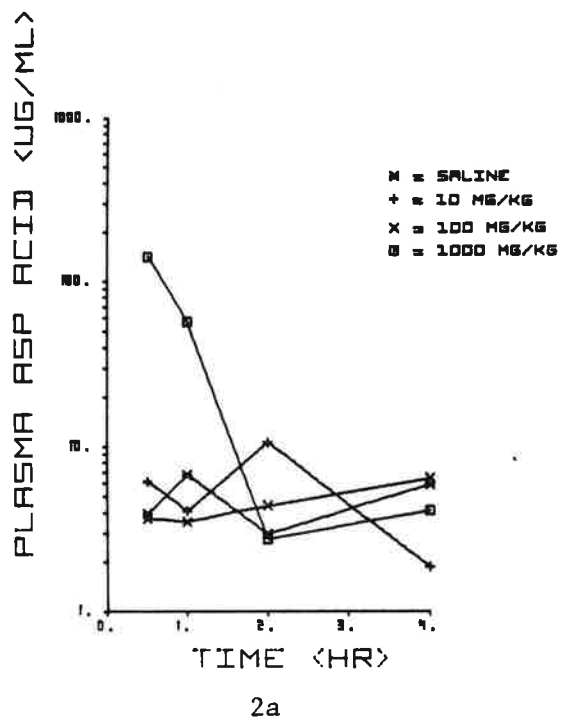
VIII. Figures

MRC-751-0021



Figs. 1a and 1b: Plasma concentrations of aspartic acid after the oral (1a) or intraperitoneal (1b) administration of L-aspartate to 15 day-old mice. Ordinate: μg aspartic acid/ml plasma; abscissa: hours after administration of aspartate. Each point is the pooled plasma sample obtained from 10-12 mice.

MRC-751-0021



Figs. 2a and 2b: Plasma concentrations of aspartic acid after the oral (2a) or intraperitoneal (2b) 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 five mice.

IX. Appendix Tables

MRC-751-0021

Appendix Table 1

Plasma Concentration of Aspartic Acid After the Oral
Administration of 0, 10, 100 or 1000 mg/kg
Monosodium-L-Aspartate to 15 Day-Old Mice

Time after Aspartate Administration (hours)	Plasma Concentration (μ g Aspartic Acid/ml)			
	Dose Administered (mg/kg)			
	0	10	100	1000
0.5	4.7	5.2	7.1	554.
1.0	4.5	4.9	5.5	506.
2.0	4.8	4.3	5.4	4.3
4.0	5.1	4.5	4.2	4.1

MRC-751-0021

Appendix Table 2

Plasma Concentrations of Aspartic Acid After the
Intraperitoneal Administration of 0,10,100 or 1000 mg/kg
Monosodium-L-Aspartate to 15 Day-Old Mice

Time After Aspartate Administration (hours)	Plasma Concentration (μ g Aspartic Acid/ml)			
	Dose Administered (mg/kg)			
	0	10	100	1000
0.5	3.9	3.7	8.7	718.3
1.0	3.7	3.6	3.7	341.5
2.0	3.6	3.4	4.1	6.7
4.0	3.5	4.7	4.1	3.9

MRC-751-0021

Appendix Table 3

Plasma Concentrations of Aspartic Acid After the
Oral Administration of 0, 10, 100 or 1000 mg/kg
Monosodium-L-Aspartate to Adult Mice

Time After Aspartate Administration (hours)	Plasma Concentration (μ g Aspartic Acid/ml)			
	Dose Administered (mg/kg)			
	0	10	100	1000
0.5	4.0	6.2	3.7	141.
1.0	6.8	4.1	3.5	57.0
2.0	3.0	10.6	4.4	2.8
4.0	5.9	1.9	6.4	4.1

MRC-751-0021

Appendix Table 4

Plasma Concentrations of Aspartic Acid After the
Intraperitoneal Administration of 0, 10, 100 or 1000 mg/kg
Monosodium-L-Aspartate to Adult Mice

Time After Aspartate Administration (hours)	Plasma Concentration (μ g Aspartic Acid/ml)			
	Dose Administered (mg/kg)			
	0	10	100	1000
0.5	16.4	5.3	8.0	1435.
1.0	5.8	3.4	3.3	195.
2.0	6.1	8.7	4.3	6.5
4.0	3.9	5.9 ^a	8.0	17.6

^a This value was estimated because of interfering compounds in the sample.