Metabolism of Aspartame in Monkeys

J. A. OPPERMANN, E. MULDOON AND R. E. RANNEY
Department of Biochemistry, Searle Laboratories, Division of G. D. Searle and Company, Box 5110, Chicago, Illinois 60680

ABSTRACT Aspartame (SC-18862, 3-amino-N-(α-carboxyphenethyl)succinamic acid, methyl ester; the methyl ester of aspartyl-phenylalanine) is a sweetening agent that organoleptically has about 180 times the sweetness of sugar. Because it so closely resembles naturally occurring dipeptides, it was believed that it would be digested in a similar manner. To show this, the metabolism of [^{14}C]aspartame labeled separately in the methyl, aspartyl and phenylalanine moieties was compared with the metabolism of ^{14}C-labeled methanol, aspartic acid, and phenylalanine. The metabolism of each moiety of aspartame was found to be the same as its free counterpart. Parameters measured were: conversion to $^{14}CO_2$, incorporation of ^{14}C into plasma proteins, and urinary and fecal excretion of the label. It was concluded that aspartame was digested to its three constituents that were then absorbed as natural constituents of the diet. J. Nutr. *103:* 1454–1459, 1973.

INDEXING KEY WORDS aspartame · monkeys · metabolism · sweetener · dipeptide

Aspartame (SC-18862, 3-amino-N-(α-carboxyphenethyl)succinamic acid, methyl ester; the methyl ester of aspartyl-phenylalanine) is a sweetening agent that organoleptically has about 180 times the sweetness of sugar (1). 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 (2), and the peptide hydrolases of the microvillar membrane would cleave the dipeptide to its constituent amino acids (3). 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.

Dietary methanol is derived in large part from fresh fruits and vegetables. It occurs as the free alcohol or esterified with fatty acids, or as the product resulting from the hydrolysis of methoxy groups of polysaccharides such as pectin (4–7). In fruit juices the methanol content may range from 12 to 640 mg/liter with an average of 140 mg/liter (7). Dietary methanol is metabolized in the body primarily to CO_2 in the one-carbon metabolic pool (8, 9), although some may be excreted unchanged in the breath (10).

Dietary phenylalanine is derived from ingested protein. Absorbed phenylalanine not only is incorporated directly into protein, but also is metabolized via tyrosine, homogentisate, and fumaryl acetoacetate to CO_2 via the tricarboxylic acid cycle (11).

The aspartic acid in the diet is also derived from ingested protein. Aspartate is rapidly converted to CO_2 after deamination and entrance into the tricarboxylic cycle via oxaloacetate. It is incorporated into protein, and it also enters into purine and pyrimidine biosynthesis via carbamyl aspartate (11).

To verify the hypothetical metabolism of aspartame proposed above, studies of its metabolism were begun by investigating the pharmacokinetics of the separate moieties labeled with ^{14}C in order to see how the monkey handled methanol, aspartic acid, and phenylalanine. A comparison of the metabolism of [^{14}C]methyl, [^{14}C]phe-

Received for publication April 9, 1973.

nylalanine, and [^{14}C]aspartyl labeled aspartame with these base lines demonstrated the correctness of our hypothesis. These labeled moieties of the sweetener were metabolized in the same fashion and to the same extent as the free compounds. The only differences noted were the rates of metabolism, and these were attributed to the finite time needed for gastric emptying, cleavage of the ester bond, and hydrolysis of the dipeptide to its constituent amino acids.

METHODS

Treatment of animals. Young female rhesus monkeys (3 to 7 kg) fed ad libitum [1] were utilized in these studies. Labeled compounds were administered orally at the dose of 0.068 mmoles/kg (10 to 50 μCi/dose). Animals given [asp-^{14}C]aspartame or [^{14}C]aspartic acid were pretreated for 5 days with unlabeled compounds. Before treatment, each animal was sedated with 1 mg/kg phencyclidine hydrochloride. A Teflon catheter [2] (18G, 2.5 inches) was inserted into a saphenous vein. After catheterization each animal was placed in a primate chair, and [^{14}C]aspartame or a ^{14}C-labeled compound was administered by oral intubation. Immediately after administration of the labeled compound, a plastic helmet was placed over each monkey's head; and, by means of a vacuum, room air (approximately 15 liters/minute) was drawn through the helmet and through three consecutive gas washers each containing 100 ml of ethanolamine:methylcellusolve (1:2). After 7 to 10 hours of $^{14}CO_2$ collection, the animals were removed from the primate chairs and were transferred to stainless steel metabolism cages from which urine and feces were collected. The exact times of collection in each study are given in the figure legends.

Plasma ^{14}C. Blood samples were obtained at each time interval by withdrawal through the catheter and were placed in test tubes containing a small quantity of heparin. The catheter was maintained patent by periodically flushing it with a small quantity of heparin solution (1 mg/ml). Blood samples collected after 12 hours were obtained by venous puncture.

After centrifugation of the whole blood, plasma was assayed for radioactivity by adding 0.1-ml or 0.2-ml aliquots to 15 ml of Bray's scintillation fluid (12), and the ^{14}C was measured in a liquid scintillation spectrometer.[3]

$^{14}CO_2$. At each time interval the contents of three gas washers were pooled, the volume was determined, a 0.5-ml aliquot was added to 20 ml of scintillation fluid [6.4 g of 2,5-diphenyloxazole, 0.16 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene, 0.564 liter of ethanol, 1 liter of toluene], and the radioactivity was determined.

Urinary ^{14}C. Urinary radioactivity was determined by adding 0.5 ml of undiluted urine to 20 ml of Bray's scintillation mixture (12) for liquid scintillation spectrometry.

Fecal ^{14}C. Fecal samples were homogenized in water and lyophilized. An aliquot of this dry powder was combusted to CO_2 in a sample oxidizer.[4] The CO_2 trapping–scintillation mixture was composed of 5 ml of ethanolamine, 9 ml of methanol, and 5 ml of scintillation solution [15 g of 2,5-diphenyloxazole, 1 g of *p*-bis-(*O*-methylstynyl)-benzene; dissolved in 1 liter of toluene]. All radioactivity measurements were counted to $\pm 1\%$ accuracy and were corrected for background and counting efficiency by an external standard method.

RESULTS

Methanol metabolism. In figure 1 the cumulative expired $^{14}CO_2$ is plotted after oral administration of equimolar amounts of either [^{14}C]methanol or [Me-^{14}C]aspartame. It can be seen that the conversion of administered ^{14}C to respiratory $^{14}CO_2$ occurred to the same extent with both compounds. This suggests that the methyl moiety was rapidly and completely cleaved from aspartame. Furthermore, it appears that the methyl group of aspartame was oxidized to the same extent as methanol. However, during the first hour after aspartame administration, there was little $^{14}CO_2$ expired, whereas $^{14}CO_2$ output after methanol treatment was very rapid. This difference may be explained by ready absorption of methanol from the stomach, while aspartame must have had to pass into the

[1] Teklad primate diet.
[2] Longdwel, Becton-Dickinson and Co., Rutherford, N. J.
[3] Mark II, Nuclear-Chicago Corp., Des Plaines, Ill.
[4] Model 305 Tri-Carb, Packard Instrument Co., Downers Grove, Ill.

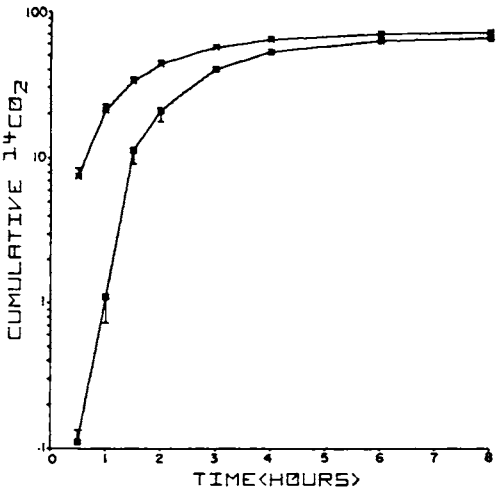

Fig. 1 Mean cumulative excretion of ^{14}C in expired air of monkeys given [^{14}C]methanol (*) or [Me-^{14}C]aspartame (□). Ordinate, % administered ^{14}C recovered in expired air; abscissa, hours after treatment. Collections were made for 8 hours. Each value is the mean of studies of three to four animals. Vertical bars indicate SEM. Differences in values at 8 hours are not statistically significant ($P > 0.05$).

small intestine before hydrolysis and absorption occurred.

In figure 2 the plasma levels of ^{14}C following [^{14}C]methanol or [Me-^{14}C]aspartame are shown. It can be seen that with both compounds the plasma levels of ^{14}C were low and that the disappearance of radioactivity was slow. Furthermore, with aspartame the rate of decline of radioactivity appeared to change with time. This may be due to the incorporation of the label into two or more metabolic pools, each of which had a different turnover time. Figure 2 also illustrates the delay in the absorption of the label after administration of aspartame.

In the 8-hour period that followed the administration of [^{14}C]methanol, 73.0 ± 3.1% (mean ± SEM) of the ^{14}C was excreted in the expired air, 3.17 ± 0.31% was excreted in the urine, and there was little radioactivity in the feces. During the same period after [Me-^{14}C]aspartame treatment, 67.1 ± 2.1% of the ^{14}C was found in the expired air, 1.57 ± 0.32% was in the urine, and the ^{14}C in the feces was negligible.

Phenylalanine metabolism. In figure 3 are shown the cumulative expired $^{14}CO_2$ values after oral administration of [^{14}C]phenylalanine and [Phe-^{14}C]aspartame. The excretion of $^{14}CO_2$ by the [^{14}C]aspartame-treated animals was the same as that from those given [^{14}C]phenylalanine.

The plasma levels of ^{14}C after oral administration of [Phe-^{14}C] aspartame of [^{14}C]phenylalanine are shown in figure 4. The peak plasma level of radioactivity occurred after 5 hours. After 48 hours the disappearance of radioactivity was slow suggesting that the label had been incor-

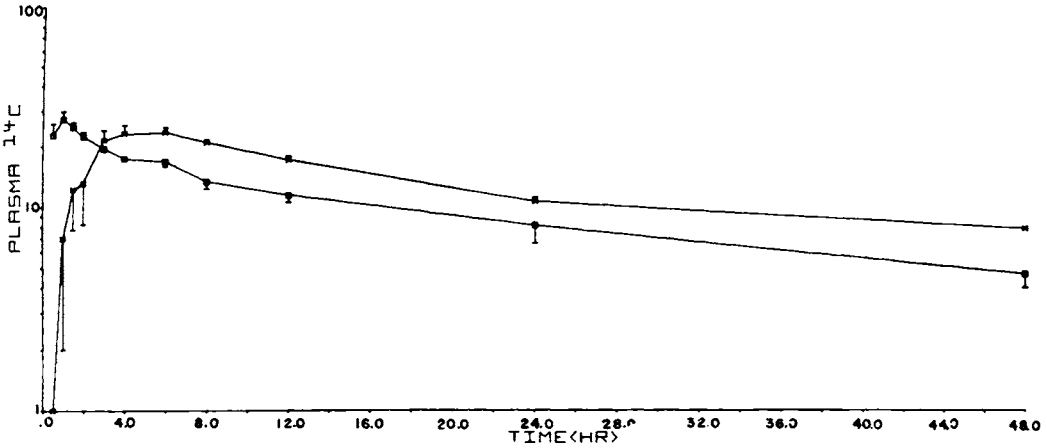

Fig. 2 Mean plasma ^{14}C of monkeys given [^{14}C]methanol (*) or [Me-^{14}C]aspartame (□) intragastrically. Ordinate, % administered ^{14}C per liter plasma; abscissa, hours after treatment. Each value is the mean of studies of three to four animals. Vertical bars indicate SEM.

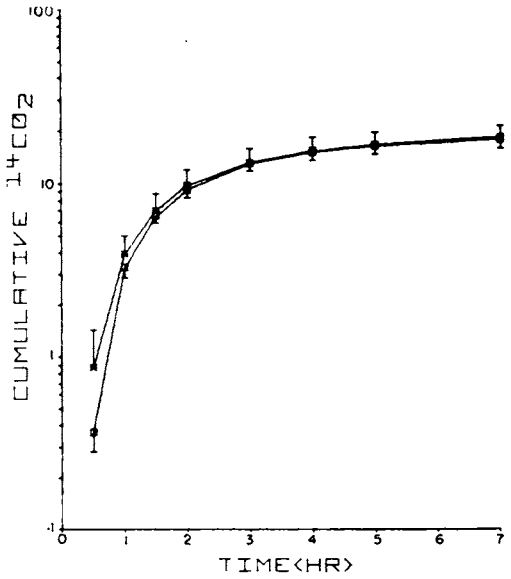

Fig. 3 Mean cumulative excretion of ^{14}C in expired air of monkeys given [^{14}C]phenylalanine (*) or [Phe-^{14}C]aspartame (□). Ordinate, % administered ^{14}C recovered in expired air; abscissa, hours after treatment. Collections were made for 7 hours. Each value is the mean of studies of four animals. Vertical bars indicate SEM. Differences in values at 7 hours are not statistically significant ($P > 0.05$).

porated into a metabolic pool with a slow turnover time. This, therefore, could represent the incorporation of the labeled phenylalanine moiety into plasma proteins and other natural body constituents.

In the 7-hour period that followed the administration of [^{14}C]phenylalanine, 17.9 ± 3.0% of the label was excreted as $^{14}CO_2$. In a 48-hour collection period 3.10 ± 0.75% appeared in the urine, and 4.71 ± 1.3% was in the feces. During the same periods after treatment with [Phe-^{14}C]aspartame, 17.4 ± 2.1% of the ^{14}C was found in the expired air, 2.79 ± 0.41% was in the urine, and 1.57 ± 0.71% was in the feces.

Aspartic acid metabolism. In figure 5 are shown the cumulative expired $^{14}CO_2$ values after oral administration of [^{14}C]aspartic acid or [Asp-^{14}C]aspartame. The [Asp-^{14}C]aspartame values at early times were less than those for the free amino acid, but the totals of ^{14}C excreted were similar for each group of animals.

In figure 6 mean plasma ^{14}C levels after the administration of [Asp-^{14}C]aspartame and [^{14}C]aspartic acid are shown. Considerable individual variations in the plasma radioactivity levels were observed among animals in each treatment group at early time points. This was also evident in the $^{14}CO_2$ excretion and probably reflects differences among the overall physiological states of the animals.

The mean plasma ^{14}C curves appeared to be biphasic with both compounds. In addition, the terminal portions of the curves

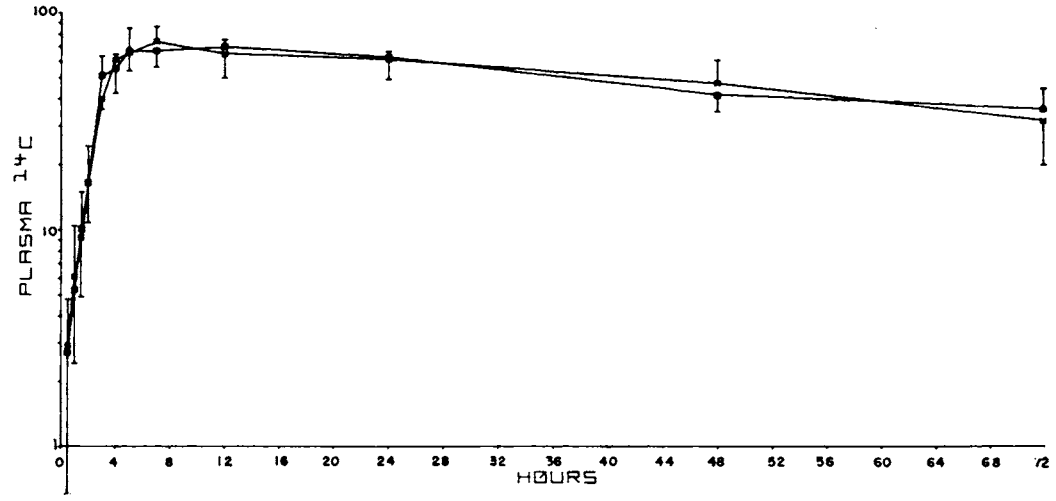

Fig. 4 Mean plasma ^{14}C of monkeys given [^{14}C]phenylalanine (*) or [Phe-^{14}C]aspartame (□). Ordinate, % administered ^{14}C per liter plasma; abscissa, hours after treatment. Each value is the mean of studies of four animals. Vertical bars indicate SEM.

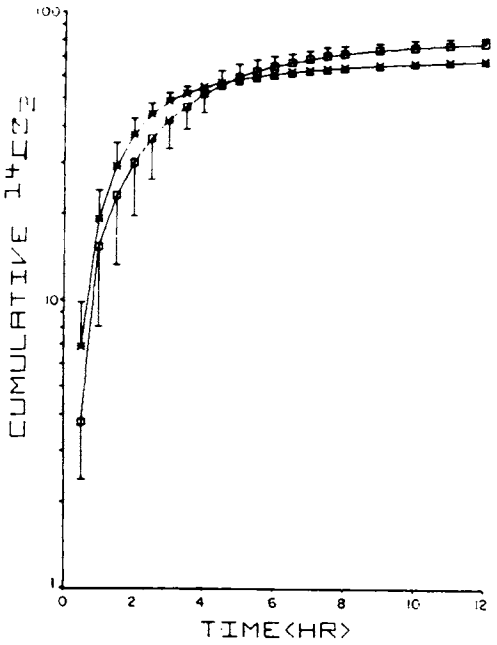

Fig. 5 Mean cumulative excretion of ^{14}C in expired air of monkeys given [^{14}C]aspartic acid (*) or [Asp-^{14}C]aspartame (□). Ordinate, % administered ^{14}C recovered in expired air; abscissa, hours after treatment. Collections were made for 12 hours. Each value is the mean of studies of three to four animals. Vertical bars indicate SEM. Differences in values at any time period are not statistically significant ($P > 0.05$).

were nonlinear. Incorporation of aspartic acid into plasma proteins may account for both the biphasic phenomena as well as the nonlinearity of the curves.

In the 10-hour period that followed the administration of [Asp-^{14}C]aspartame, 74.7 ± 3.9% of the ^{14}C was excreted as $^{14}CO_2$; and in the 4 days following this treatment, 2.12 ± 0.38% was excreted in the urine, and 1.63 ± 0.36% was detected in the feces. After [^{14}C]aspartic acid was given, 65.8 ± 1.9% of the label was found in $^{14}CO_2$; and during 4 days, 3.80 ± 0.12% was excreted in the urine, and 1.51 ± 0.32% was in the feces.

DISCUSSION

These data provide evidence that supports the hypothesis that aspartame was hydrolyzed in the gut of the monkey to release its separate moieties for metabolism by normal metabolic pathways. Irrespective of its source as free [^{14}C]methanol or [Me-^{14}C]aspartame, the label was rapidly absorbed, oxidized, and excreted in the expired air as $^{14}CO_2$. That fraction not so excreted (about 30%) was converted to body constituents through the one-carbon metabolic pool. This interpretation is supported by the slow disappearance of ^{14}C from the plasma. In this case the label had

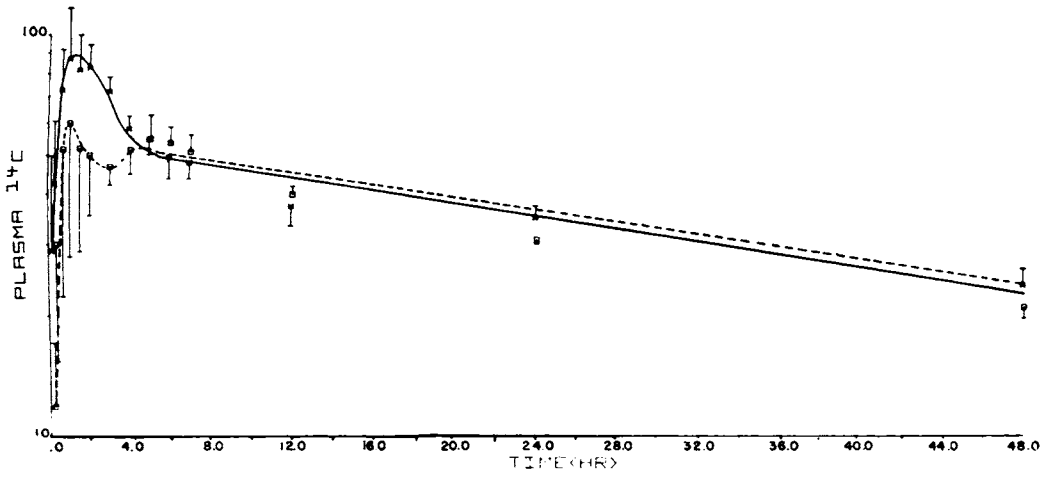

Fig. 6 The mean plasma ^{14}C levels of monkeys given orally [Asp-^{14}C]aspartame (□) or [^{14}C]aspartic acid (*). The values are expressed as % administered ^{14}C per liter plasma. Each value is the mean of four animals. Those portions of the curves from 5 to 48 hours are the regression lines calculated from the indicated points. Vertical bars indicate SEM.

apparently been incorporated into plasma proteins.

The phenylalanine moiety of aspartame followed the same metabolic pathways as the free amino acid. Studies in the dog [5] have demonstrated that unhydrolyzed aspartame cannot be detected in the plasma. The formation of $^{14}CO_2$ from [Phe-^{14}C]aspartame was the same as that from [^{14}C] phenylalanine and appeared to occur after obligate hydrolysis of the dipeptide before absorption. The amounts of radioactivity incorporated into plasma protein were the same when either labeled compound was administered.

The metabolic transformations of aspartic acid are complex; however, the major pathway for free aspartic acid is the conversion to oxaloacetic acid via transamination (11). The entrance of this latter compound (uniformly labeled in this case) into the tricarboxylic acid cycle results in 50% of the label being lost as $^{14}CO_2$ in one complete pass through the cycle. The similarity in the rates and amounts of $^{14}CO_2$ excretion following [Asp-^{14}C]aspartame or [^{14}C]aspartic acid administration suggests that free aspartic acid was rapidly produced from the ingested aspartame. Equal amounts of ^{14}C from the two labeled compounds were incorporated into plasma proteins.

Since the phenylalanine derived from aspartame was incorporated into body protein to a great extent, the following study (13) was carried out to determine if continued ingestion of aspartame had any effect on phenylalanine metabolic parameters.

ACKNOWLEDGMENT

The authors gratefully acknowledge the able assistance of Mrs. Virginia Bonnie in preparing the manuscript and Mr. Thomas Krown in the collection of data. The synthesis of [methyl-^{14}C]aspartame, [Phe(U)-^{14}C]aspartame, and [Asp(U)-^{14}C]aspartame was carried out by Mr. J. M. Schlatter of the Department of Chemical Research, Searle Laboratories.[6]

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[5] Oppermann, J. A., manuscript in preparation.

[6] [^{14}C]Methanol, [(U)^{14}C]L-phenylalanine and [(U)^{14}C]L-aspartic acid were obtained from Amersham/Searle Corp., Arlington Heights, Ill.