Metabolism of Absorbed Aspartate, Asparagine, and Arginine by Rat Small Intestine in Vivo

HERBERT G. WINDMUELLER AND ALBERT E. SPAETH

Laboratory of Nutrition and Endocrinology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

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L-[U-14C]aspartate, L-[U-14C]asparagine, and L-[U-14C]arginine were administered luminally into isolated segments of rat jejunum in situ, and the radioactive products appearing in venous blood from the segment were identified and quantified, in a continuation of similar studies with L-glutamate and L-glutamine (Windmueller, H. G., and Spaeth, A. E. (1975) Arch. Biochem. Biophys. 171, 662-672). Aspartate, administered alone (6 mM) or with 18 other amino acids plus glucose, was absorbed more rapidly than glutamate, but, as with glutamate, less than 1% was recovered intact in intestinal venous blood. More than 80% of aspartate carbon was recovered in CO₂, 24% in organic acids, mostly lactate, 13% in other amino acids (alanine, glutamate, proline, ornithine, and citrulline), and 10% in glucose, apparently the first demonstration of gluconeogenesis by intestine in vivo. In contrast to aspartate and glutamine, nearly all asparagine was absorbed intact, less than 1% being catabolized. About 4% of the absorbed dose was incorporated into the acid-insoluble fraction of intestine, as was the case with all the amino acids studied. In conventional or germ-free rats, only 60% of arginine was absorbed intact, while 33% was hydrolyzed to ornithine and urea. The urea and 38% of the ornithine were released into the blood; the remaining ornithine was metabolized further by intestine to citrulline, proline, glutamate, organic acids, and CO₂. Catabolism of several amino acids from the lumen plus glutamine from arterial blood may provide an important energy source in small intestine.

Intestinal transport has been widely studied, but intestinal metabolism of transported compounds has received little attention. Recently we described a rat intestinal preparation well suited for identifying and quantifying the products released into the portal blood during intestinal transport of compounds from the gut lumen in vivo (1). It was shown with this preparation that virtually all absorbed dietary glutamate and most glutamine were metabolized by intestinal mucosal cells. The carbon from these amino acids appeared in intestinal venous blood in CO₂, lactate, proline, citrulline, and a variety of other minor products.

There is evidence that glutamate and glutamine are not the only dietary amino acids that undergo metabolic transformation in the intestine. Wiseman and co-workers (2-4) and others (5) have shown that absorbed aspartate is transaminated in the intestine and produces an efflux of alanine from the tissue. The fate of the aspartate carbon atoms is uncertain. Incubation studies in vitro have suggested that intestine may also degrade arginine (6, 7), although the extent of arginine catabolism during absorption in the intact animal remains unknown, and the possibility of such catabolism has been only rarely considered (7) in nutritional or transport studies with this amino acid.

In order to obtain more quantitative and detailed information, we have determined the metabolic fate of aspartate, its amide asparagine, and arginine when these amino acids, uniformly labeled with ¹⁴C, are transported from lumen to blood across a segment of rat jejunum in vivo.
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EXPERIMENTAL PROCEDURES

Jejunal segment preparation. Preparation of the rats and isolation and vascular cannulation of the jejunal segment have been described in detail (1). Briefly, the small intestine of an anesthetized rat was exteriorized through an abdominal incision into a 37°C tissue bath containing a physiological salt solution. A 2.5-cm segment of jejunum was isolated by ligatures, and the single vein draining the segment was cannulated. The arterial blood supply to the segment remained intact. All venous blood from the segment was collected in a series of tubes in ice at approximately 8-min intervals. Replacement blood from donor rats was transfused continuously into a saphenous vein at a rate sufficient to maintain arterial blood pressure.

Once the preparation was established, the 14C-labeled test amino acid was introduced into the lumen of the closed segment in 0.3 ml of a physiological salt solution, as indicated for each experiment. Venous blood samples for analysis were collected for 80–85 min. At the end of this time, the intestinal segment was excised, the lumen was flushed with 5 ml of cold 0.15 M NaCl, and the tissue was frozen in Dry Ice and stored at −20°C for later analysis. Each L-[U-14C]labeled amino acid was also added directly to some unlabeled intestinal venous blood; this control sample and blood samples collected during the absorption experiments were handled and analyzed identically. The germ-free intestine-donor rat used in one experiment remained in a sterile isolator until surgery.

In order to inhibit the arginase activity in blood, in the experiments with L-[U-14C]arginine, 0.75 mM sodium borate, pH 7.4 (8, 9), was infused continuously into the intestinal venous cannula at a point about midway between the intestine and the blood collection tubes. The infusion rate was about 10% the rate of venous blood flow. Thus, venous blood was brought immediately to a final borate concentration of approximately 80 mM. Preliminary experiments showed that this concentration of borate was sufficient to inhibit the arginase activity in rat blood by 98%.

Isolation and counting of radioactive products. All determinations of radioactivity were made in a liquid scintillation spectrometer. Corrections for quench were made with internal standard.

Radioactivity in respired 14CO2 in each venous blood sample was determined in a 100-μl aliquot and total blood radioactivity in a 20-μl aliquot (1). Other 14C-labeled metabolites in the blood were determined in neutralized perchloric acid extracts prepared and analyzed as previously described (10), with minor modifications. Portions of the neutralized extracts were applied to columns of Dowex 50 (H+) and eluted with water. Amino acids and urea were eluted with 8 M NH4OH. The water eluates were adjusted to pH 6.5 with NH4OH and applied to columns of Dowex 1 (formate). Glucose and other nonionized compounds were eluted with water, followed by the elution of organic acids with 12 M formic acid. Amino acids, urea, and organic acids were further separated, identified, and counted following thin-layer chromatography of the concentrated column eluates (10). In experiments with labeled arginine, amino acid separations were improved by adjusting the phenol:water (3:1, w/w) solvent to pH 7.0 with 8 M NH4OH. Urea was detected with Ehrlich's reagent (11). [14C]Urea and L-[U-14C]arginine were also determined by an enzymatic procedure, described below, as a check on the chromatographic technique. [14C]Glucose was determined as described below. Radioactivity in the acid-soluble and acid-insoluble fractions of tissue samples were measured as previously described (10).

Enzymatic determination of [14C]urea and L-[U-14C]arginine. The assays were based on the determination of 14CO2 released by urease (EC 3.5.1.5) and arginase (EC 3.5.3.1). In triplicate (A, B, and C), 100-μl aliquots of the neutralized acid extracts of blood were added to 2 ml of buffer (100 mM glycine, 1 mM MnSO4, adjusted to pH 9.0 with NaOH) in the main compartment of an enlarged Warburg-type flask. The center well of each flask contained a counting vial with 0.75 ml each of methanol and phenethylamine, and the tops of the flasks were sealed with a rubber stopper pierced by small-bore tubing through which additions to the main compartment could be made. No further additions were made to flask A; urease, 5 units, was added to flask B; and urease, 5 units, plus arginase, 60 units, were added to flask C. After incubating the flasks for 30 min at 25°C, 3 ml of 170 mM carrier NaHCO3 was added to the main compartment. The mixture was then acidified by tipping in 3 ml of 50% citric acid (w/v) from a side arm. The flasks were gently oscillated for 2 h to effect a quantitative transfer of 14CO2 to the counting vials, which were counted following the addition of 9 ml of Spectrafluor (Amersham/Searle). The difference between 14CO2 in flasks A and B was due to [14C]urea in the samples, and the difference between B and C was due to [14C]arginine. Authentic [14C]urea and L-[U-14C]arginine gave 85 and 93%, respectively, of the expected recovery of 14CO2 in this assay. There was good agreement between the enzymatic and chromatographic analyses for both labeled urea and labeled arginine in the blood extracts (±5%).

Determination of [14C]glucose. The water eluates from the Dowex 1 columns (see above) were evaporated to dryness under vacuum at 30°C and the residue taken up in a small volume of water (concentrated Dowex 1 water eluate).

Portions of the concentrated Dowex 1 eluate, as well as authentic glucose, were chromatographed on
thin-layer MN-300 cellulose plates (250 µm thick, Analtech) in the following solvents: (a) 88% formic acid:methyl ethyl ketone:t-butanol:water (3:6:8:3) (12); and (b) ethyl acetate:pyridine:water (2:1:2) (13). Glucose was visualized with aniline phthalate (14), scraped from the plates, and counted (10).

To confirm the results of chromatography, [14C]glucose was also determined enzymatically in an assay based on the ability of glucose oxidase (EC 1.1.3.4) to convert [14C]glucose, which is not retained by Dowex 1, to [14C]gluconic acid, which is retained. A 10-µl aliquot of the concentrated Dowex 1 water eluate was incubated at 35°C with 47 units of glucose oxidase and 300 units of catalase (EC 1.1.1.6) in 0.8 ml of 50 mM sodium acetate, pH 5.1. A control tube contained the same mixture except with boiled glucose oxidase. Oxygen was bubbled continuously through the solutions during incubation. After 30 min, one drop of phenolphthalein indicator in 95% ethanol was added, followed by the dropwise addition of 0.1 N NaOH until the color remained a deep pink. After standing at room temperature for 15–60 min, to complete the hydrolysis of glucono-δ-lactone to gluconic acid, the reaction mixture was put over a 0.5 x 4-cm column of Dowex 1 (formate) and eluted with 4 ml of water. A 1-ml aliquot of the combined eluate was counted. With authentic [14C]glucose, there was 98.5% retention of radioactivity by Dowex 1 following treatment with glucose oxidase; with neither [14C]glucose nor blood samples was any radioactivity retained when boiled enzyme was used. The retained [14C]gluconic acid could be readily eluted from the columns with formic acid. There was excellent agreement between the chromatographic and enzymatic determinations of [14C]glucose in the concentrated Dowex 1 eluates (± 3%).

**Materials.** All 14C-labeled products were from Amersham/Searle, and the radiochemical purity of each was found to be at least 97% by thin-layer chromatography. The following enzymes were from Sigma Chemical Co.: glucose oxidase, type V, from *Aspergillus niger*; catalase, twice crystallized from bovine liver; urease, type VII, 54 units/mg, from jackbeans; and arginase, 42 units/mg, from bovine liver. Other materials were from the previously indicated sources (10).

**RESULTS AND DISCUSSION**

**Time Course for Absorption**

The rate of appearance of 14C in the blood was similar for the three amino acids studied and also similar to the rate found previously for glutamine (1) (Fig. 1). There was no measurable rate difference for aspartate administered alone or together with glucose and 18 other amino acids. Absorption from the lumen and release of the amino acids and metabolic products into the blood reached 90% of completion in about 20 min under the conditions of these experiments. The rate of glutamate absorption (1) was much slower. Whether or not aspartate and glutamate are actively transported in the intestine has

![Fig. 1. Time course for appearance of radioactivity in intestinal venous blood following the luminal administration of L-14C-labeled amino acids. For each amino acid, a 0.4–2.0-µmol dose was administered into the lumen of an isolated segment of rat jejunum in 0.3 ml of Earle’s balanced salt solution, pH 7.4 (15). Results show cumulative recovery of 14C in venous blood collected from the segment (see Experimental Procedures). Data for asparagine (ASN), aspartate (ASP), and arginine (ARG) are from experiments described in Table 1. Data for glutamate (GLU) and glutamine (GLN) were calculated from previously published results (1). Data are mean values and vertical lines indicate standard errors.](image-url)
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been a subject of controversy. The matter has been reviewed and evidence provided for active transport of both of these dicarboxylic amino acids (17). The large difference in apparent transport rates for the two which we consistently observed (Fig. 1) has, to our knowledge, not been previously reported.

Fate of Absorbed Amino Acids (Table I)

Less than 1% of the aspartate absorbed alone or as part of a simulated meal escaped metabolism by the intestine, consistent with earlier evidence by Wiseman and co-workers (2-4) that absorbed aspartate undergoes transamination and produces an efflux of alanine from the tissue. These results have a bearing on the still-unresolved question of whether some amino acids may be partly absorbed from the lumen via an extracellular pathway, e.g., by way of the tight junctions between cells (18). The finding that virtually all aspartate and glutamate (1) are metabolized shows that for these two amino acids all, or nearly all, transport follows a transcellular pathway.

In contrast to aspartate, there was less than 1% hydrolysis or catabolism of asparagine, confirming the results of Finch and Hird (6) from experiments in vitro with much less sensitive analytical techniques. The results suggest that the active glutaminase (EC 3.5.1.2) in rat intestine does not hydrolyze asparagine. This was confirmed when L-glutamine and L-asparagine were incubated with homogenates of rat intestinal mucosa.

Arginine was substantially metabolized in a germ-free rat as well as in conventional rats. Only 60% of the absorbed arginine appeared intact in the blood, confirming in vivo the loss of arginine observed when this amino acid was incubated with segments of rat (6) and hamster (7) intestine in vitro. In preliminary experiments, when no borate was added to inhibit blood arginase activity, the recovery of intact arginine was only about 40%.

With all three amino acids, the incorporation of carbon into tissue acid-insoluble components accounted for 2-4% of the absorbed dose (Table I), values similar to those observed with L-[U-14C]glutamine and L-[U-14C]glutamate (1). The low 14C recovery in the lumen shows that absorption in each case was virtually complete. Overall recovery of radioactivity was near 100% when the small amounts of 14C in the tissue acid-soluble fraction and in the gut bath fluid are included.

14C-Labeled Products from L-[U-14C]Aspartate and L-[U-14C]Asparagine

Lactate and alanine were the only metabolites of aspartate previously identified, in experiments in which aspartate was incubated with rat small intestine in vitro (20). Table II shows the distribution of aspartate carbon among metabolites released into blood in vivo. Results were similar for aspartate administered alone or administered together with other amino acids and glucose. More than 50% of the carbon appeared in CO2 and 12% in several other amino acids, the same ones synthesized from glutamate and glutamine (1, 10), and 24% appeared in organic acids, mainly lactate. Alanine efflux from the tissue can apparently account for most of the aspartate nitrogen taken up (5); however, the released alanine contained less than 10% of the aspartate carbon. An additional source previously identified for the carbon of the alanine is plasma glucose (10).

A somewhat surprising finding was the appearance of substantial amounts of aspartate carbon in glucose. The [14C]glucose was identified by its reactivity with glucose oxidase from Aspergillus niger. This enzyme is not absolutely specific for glucose, but other sugars, when reactive at all, are oxidized at a much slower rate than glucose (21). This fact, plus the similar mobility of the labeled product and glucose in two thin-layer chromatography solvents, leaves little doubt about its iden-
TABLE I

FATE OF AMINO ACIDS ABSORBED BY JEJUNUM*

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Fraction</th>
<th>Percentage of total 14C dose</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-[U-14C]aspartate* (85 min, n = 5)</td>
<td>L-[U-14C]asparagine* (85 min, n = 2)</td>
<td>L-[U-14C]arginine* (80 min, n = 4)</td>
<td></td>
</tr>
<tr>
<td>Venous blood†</td>
<td>Metabolic products†</td>
<td>92.1 ± 1.1*</td>
<td>0.6, 0.7</td>
<td>33.1 ± 2.9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unchanged amino acid</td>
<td>0.4 ± 0.1</td>
<td>91.0, 92.0</td>
<td>62.2 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td>Acid-insoluble</td>
<td>2.6 ± 0.4</td>
<td>3.2, 4.4</td>
<td>3.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acid-soluble</td>
<td>1.3 ± 0.0</td>
<td>0.7, 1.5</td>
<td>0.9 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Gut bath</td>
<td></td>
<td>0.4 ± 0.0</td>
<td>0.2, 0.3</td>
<td>0.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Lumen</td>
<td></td>
<td>0.5 ± 0.0</td>
<td>1.8, 1.0</td>
<td>0.9 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>97.3 ± 0.9</td>
<td>97.5, 99.9</td>
<td>101.0 ± 1.8</td>
<td></td>
</tr>
</tbody>
</table>

* The indicated dose of labeled amino acid (20 μCi) was luminally administered into closed jejunal segments in situ in 0.3 ml of Earle's balanced salt solution, pH 7.4 (15). All venous blood from the segments was collected in ice in a series of samples at 8-min intervals for 80-85 min. At the end of this time, the segment was excised and the lumen rinsed with 5 ml of cold 0.15 M NaCl. The segment was then frozen in Dry Ice and stored at -20°C for later tissue analysis. Venous blood samples and tissue were analyzed as described in Experimental Procedures. Radioactivity was also determined in the lumen rinse and in gut bath fluid sampled at the end of the experiment.

† In two experiments the dose contained 2 μmol of L-[U-14C]aspartate only. In three other experiments, the dose contained 0.4 μmol of L-[U-14C]aspartate, 20 μmol of glucose, plus 0.2-1.6 μmol each of 18 other L-amino acids in the proportions found in the jejunal lumen of human subjects 4 h after ingesting a high protein meal (16).

‡ The dose contained 2 μmol of L-[U-14C]asparagine.

§ The dose contained 2 μmol of L-[U-14C]arginine. In one of the four experiments the intestine was from a germfree rat. Sodium borate was added to the venous blood samples to a final concentration of 80 mM in order to inhibit arginase activity (see Experimental Procedures).

 Values show total recovery in all 10-11 venous samples collected.

 Values are means ± SE.

The small extent of arginine metabolism precluded a detailed examination of products. Trace amounts of radioactivity accounted for approximately 90% of the radioactivity in the Dowex 1 water eluate fraction of the blood samples. Radioactive product(s), not further identified, were also recovered in this fraction in earlier experiments where blood was from intestine absorbing L-[U-14C]glutamate or L-[U-14C]glutamine (1), suggesting that these amino acids may also give rise to glucose. The conversion of aspartate carbon to glucose was reduced when the amino acid was absorbed as part of a simulated meal (Table II). Anderson has reported the levels of several key gluconeogenic enzymes in rat (22) and guinea pig intestine (23). He also demonstrated net glucose production when homogenates of intestinal mucosa from guinea pigs fasted for 72 h were incubated with inosine diphosphate plus either aspartate, malate, lactate, or pyruvate (23), although some of the glucose may have been derived from the added nucleotide (24). The present studies appear to be the first to demonstrate gluconeogenesis by intestine in vivo.

It is highly unlikely that lumen microorganisms were responsible for the observed aspartate metabolism: (a) The distribution of 14C among the labeled metabolites was very similar in five separate experiments; (b) the number of microorganisms in the upper small intestine is relatively small; (c) metabolism of the administered dose was nearly complete within 25 min; and (d) most of the aspartate metabolites were also produced with L-[U-14C]arginine (see below) and L-[U-14C]glutamine (10) in studies with germ-free rats.

The small extent of arginine metabolism precluded a detailed examination of products. Trace amounts of radioactivity ap-
TABLE II

**Distribution of \(^{14}C\) Among Products Released into Blood During Absorption of L-[U-\(^{14}C\)]Aspartate by Jejunum**

<table>
<thead>
<tr>
<th>Product</th>
<th>Percentage of total (^{14}C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aspartate administered alone</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>52.4, 48.9</td>
</tr>
<tr>
<td>Glucose</td>
<td>9.4, 11.5</td>
</tr>
<tr>
<td>Alanine</td>
<td>9.6, 6.4</td>
</tr>
<tr>
<td>Proline</td>
<td>1.9, 1.4</td>
</tr>
<tr>
<td>Citrulline</td>
<td>1.2, 0.9</td>
</tr>
<tr>
<td>Glutamate</td>
<td>1.2, 1.1</td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.5, 0.3</td>
</tr>
<tr>
<td>Lactate</td>
<td>16.6, 23.4</td>
</tr>
<tr>
<td>Citrate</td>
<td>1.9, 1.8</td>
</tr>
<tr>
<td>Malate</td>
<td>1.2, 0.8</td>
</tr>
<tr>
<td>Other organic acids(^d)</td>
<td>0.9, 1.2</td>
</tr>
<tr>
<td>Others(^d)</td>
<td>3.2, 2.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.0, 100.0</strong></td>
</tr>
</tbody>
</table>

\(^a\) Results are from the experiments described in Table I.

\(^b\) Mean ± SE, n = 3.

\(^c\) α-Ketoglutarate, succinate, fumarate, pyruvate, and 5-oxoproline.

\(^d\) Traces of radioactivity were recovered in serine and glycine and in several minor unidentified products.

The labeled products were similar in conventional and germ-free rats (Table III). The quantitative results in Tables I and III, taken together, show that about one-third of the arginine dose was hydrolyzed to ornithine and urea by an arginase in the tissue (25, 26), that the urea and 38% of the ornithine were released into the blood, and that the remaining 62% of the ornithine was further metabolized to citrulline, proline, glutamate, CO\(_2\), and the other minor products observed. Evidence for the presence of the required enzymes in the intestine has been summarized previously (10). The 16.9% recovery of \(^{14}C\) in \[^{14}C\]urea is very near the 16.7% theoretically expected assuming the absence of urease activity (27).

**Physiological Relevance**

The amino acid concentration in the administered dose, 1.3-6.7 mM, was of the same order as the concentration of free amino acids found in the intestinal lumen of human subjects 3 h after a high protein meal (16). It can be reasonably expected, therefore, that the fate of aspartate and arginine observed in the present studies will reflect the fate of the free amino acids produced by the intraluminal hydrolysis of dietary proteins. There is growing evidence that a large fraction of dietary amino acids are absorbed from the lumen as di- and tripeptides, the products of incomplete protein hydrolysis (28). These peptides are, however, almost completely hydrolyzed at the cell surface during absorption from the lumen or within the absorptive cells, where the free amino acids then become available for metabolism. Burston et al. (29) have shown that when a variety of dipeptides containing aspartate is transported into rings of rat ileum, the aspartate is transaminated producing alanine, the same result obtained with free aspartate. With dipeptides containing aspartate and...
arginine, free arginine was always recovered in the tissue. In studies in the intact animal, the portal blood of dogs fed a horsemeat diet contained only a small fraction of the aspartate in the meal (30). Available data therefore suggest that the fate observed for the amino acids under the conditions of our experiments may be a true reflection of metabolic events in the intestine during dietary protein digestion and absorption in vivo. Intestinal degradation of absorbed arginine has a particular significance in young animals, where dietary arginine is required to achieve maximum rates of growth (31). Furthermore, the extensive metabolism of dietary aspartate, glutamate, glutamine, and arginine, plus the metabolism of glutamine from the circulation (10), may together make a significant contribution to the energy requirements of the intestine.

REFERENCES
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