Absorption and Metabolism of Lysine Maillard Products in Relation to Utilization of L-Lysine

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An in vivo study was conducted to investigate to what extent the lysine Maillard products are absorbed and metabolized and how they affect the absorption and utilization of L-lysine in protein synthesis. The lysine Maillard products, mono- (MFL) and difructose-L-lysine (DFL), were synthesized from a mixture of glucose and [^{14}C]lysine (6:1). A portal vein catheterization technique with duodenal infusion and a jugular vein administration was employed for the study of absorption and metabolism, respectively. MFL was absorbed in a significant amount probably by active transport and incorporated into the liver microsome, whereas DFL was absorbed in a negligible amount probably through diffusion. Compared to 22% of lysine, 72% of the absorbed MFL was excreted. MFL retarded competitively the absorption of lysine, while DFL reduced the absorption of lysine by blocking the absorption site.

The Maillard reaction (nonenzymatic browning) plays an important role in food processing, since it occurs under conditions of both heat treatment and storage at room temperature. Nutritionally detrimental effects include (1) loss of nutritional value as a result of the unavailability of the components, reducing sugars and amino acids; and (2) antinutritive properties of the resultant products. Demonstrated anitnutritive properties include in vitro reduced digestibility (Sgarbieri et al., 1973), inhibition of mucosal disaccharidase activity (Lee et al., 1977a), decreased rate of absorption (Sgarbieri et al., 1973; Lee et al., 1977b), and modification in the mechanism and site of absorption (Mori and Nakatsuji, 1977; Tanaka et al., 1975). Urinary excretion of amino acids and peptides increases after feeding heat-damaged proteins (Ford and Shorrock, 1971).

Heat sterilization of parenteral solutions containing protein hydrolysates along with glucose results in the formation of glucose–amino acid complexes. The intravenous administration of heat-sterilized parenteral nutrition formulas has been associated with mild dehydration in infants (Stegink et al., 1974) and with excessive urinary zinc excretion in both infants and adults (Freeman et al., 1975; Stegink et al., 1975). When these formulae were administered to the pregnant rhesus monkey, the sugar–amino acid complexes accumulated in the maternal plasma and were transported to the fetal circulation. Although all of the compounds studied crossed the placenta, fetal levels were consistently lower than maternal levels (Stegink et al., 1977).

Protein metabolism is altered in the presence of the Maillard product. In vitro incorporation of amino acids into protein was stimulated by the addition of the sugar–amino acid complexes (Amadori compounds) along with iron (Borsook et al., 1955). Incorporation of the Maillard product of glucose and leucine into rat liver was much less (1.5%) than incorporation of the free amino acid (Sgarbieri et al., 1973). In vivo incorporation of the amino acid into liver protein of a chick fed fructose-L-phenylalanine containing the same amino acid (phenylalanine) was reduced in comparison with those not fed this complex (Johnson et al., 1977).

Losses of lysine in proteinaceous products result mainly from the formation of sugar–lysine complexes (Adrian, 1967; Finot et al., 1979; Hurrell, 1980). Lysine was chosen as the test amino acid because of its significance nutritionally as the limiting amino acid in cereal diets (Clark et al., 1977) and because of its susceptibility to the Maillard reaction at the ϵ-amino group on its side chain (Erbersdobler, 1977). The present study was designed to study the kinetics of absorption and metabolic fate of the lysine Maillard products in relation to the absorption and metabolism of lysine in the rat by in vivo techniques. An in vitro absorption study based on the everted sac method (Alfke, 1974) has been published. The author observed a small portion of lysine Maillard products was absorbed but speculated a passive absorption by diffusion. This report was further substantiated by an in vivo digestion study on the rat where N^{ϵ}-fructose-L-lysine (lysine Maillard product) was partly absorbed by the intestine (20–60%) and was not utilized by the rat (Finot et al., 1977).

METHODS AND MATERIALS

Synthesis of Glucose–Lysine Amadori Compounds. Amadori compounds formed during Maillard browning of a glucose–lysine mixture, mono- (MFL) and difructoselysine (DFL), were synthesized by the method of Finot and Mauron (1969) with some modification (Lee et al., 1984). A 15-g portion of glucose and 2.5 g of lysine were dissolved in 250 mL of methanol, and the resultant mixture was refluxed for $4^1/_2$ h. The reaction mixture was concentrated and fractionated on a Dowex 50 W-X4 column ($1^1/_2$ in. × 18 in.; 200-mesh resin) with a 0.2 M pyridine–acetic acid buffer at pH 4.25. MFL and DFL were identified on a TLC plate coated with cellulose (Avicel; 250-μm layer; Analtech, Inc., Newark, DE) along with the original refluxed sample as reference, following the reported chromatogram (Finot and Mauron, 1969). No attempts were made to further fractionate MFL into α and ϵ forms. Therefore, the MFL prepared in this study contained both α and ϵ forms. According to the chromatogram reported by Finot and Mauron (1969), the ϵ form appeared to be present in a greater amount than the α form in the MFL fraction.

In Vivo Absorption Study. The absorption study was carried out following the technique developed by Gallo-Torres and Ludorf (1974) with some modification. Two female Sprague–Dawley rats (ACE Animals, Inc., Boyertown, PA) weighing 150–200 g were used for the absorption study of each product sample. The animals were starved overnight and were anesthetized the next morning by inhalation of methoxyfluorane (Metofane; Pitman-Moore, Inc., Washington Crossing, NJ) prior to the operation. The animal was placed on an operation board, and both fore and hind feet were tied with great care to avoid restraining peripheral blood circulation. Anesthesia was continued until the operation was completed while overinhalation was avoided. The liver was exposed, and the portal and splenic

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veins were located. A cannula (Intramedic polyethylene tube, PE50; Ace Scientific Supply Co., Linden, NJ) was bent 1 cm from the tip at a 70° angle. Its tip was cut in such a way that the opening of the bevel was exposed to the operator. The cannula was then heparinized overnight. Before the operation the tip was frozen in dry ice to harden. The heparinized cannula was inserted into the portal vein in such a way that its tip rested approximately 5 mm below the splenic vein and secured with adhesive (Eastman 910; Eastman Chemical Products, Inc., Kingport, TN) and a small plastic disk. A needle (Gauge No. 17) was inserted through the abdominal wall, the end of the cannula placed in it, and the cannula pulled to the outside of the rat's body. The duodenum was then exposed. A cannula was inserted into it and secured with thread (surgical silk size 5–0; Ethicon, Inc., Somerville, NJ). It was also drawn to the outside in the same manner as the portal vein cannula. The incision was sutured and the rat allowed to recover in a restraining chamber. The restraining chamber consisted of a base and two plastic ends with holes for the rat's nose and tail, respectively. The ends were connected by adjustable steel rods that were used to restrain the animal.

Products to be infused were dissolved in a Kreb–Henseleit solution (pH 7.4) (Krebs and Henseleit, 1932) at a concentration of 50 mM. Upon recovery, 0.5-mL samples with an average radioactivity of 0.36 μCi (equivalent to 8×10^5 dpm) were infused into the duodenal cannula, and the sample solution remaining in the cannula was flushed in with a Kreb–Henseleit solution. Blood samples were taken into heparinized capillary tubes (B53; Arthur H. Thomas Co., Philadelphia, PA) from the portal vein cannula at about 5-min intervals in an amount of 65 μL/collection. Blood sample collection continued for 60–70 min or until the radioactivity returned to the base line. Samples infused were as follows: [^{14}C]lysine, [^{14}C]MFL, [^{14}C]DFL, [^{14}C]lysine plus MFL, and [^{14}C]lysine plus DFL.

Blood samples were centrifuged in a hematocrit centrifuge (International Equipment Co., Needham, MA) to obtain the plasma. The volume of the plasma was measured, and plasma was mixed with 15 mL of Aquasol (New England Nuclear, Boston, MA), a xylene-based scintillation solution containing all the necessary fluors. Samples were shaken and counted in a liquid scintillation counter (Beckman DPM-100; Beckman Instruments, Inc., Fullerton, CA) for 20 min (to minimize error). A quenching curve was prepared by use of a series of standard quenching cocktails (Quenched Carbon-14 Standards Set; Beckman Instruments) and this curve was used to convert counts per minute (cpm) to disintegrations per minute (dpm).

From the volume of the plasma samples, all results were converted to dpm in 0.1 mL of plasma. Aliquots of the infused samples were counted to determine the total radioactivity infused. Dpm in 0.1 mL of plasma/total radioactivity infused was plotted against time after infusion in order to observe the kinetics of absorption of the various substances.

Protein Synthesis Study. Female Sprague–Dawley rats fed laboratory chow (Ralston Purina Co., St. Louis, MO) and weighing approximately 200 g were used for the protein synthesis study. Through the jugular vein, 0.5 mL of 50 mM radiolabeled samples with an average activity of 0.0059 μCi (equivalent to 1.3×10^4 dpm) was infused: either lysine, MFL, DFL, or lysine plus unlabeled MFL or DFL. Rats were placed in metabolic cages with access to water only, and urine samples were collected. They were sacrificed 5–6 h later; livers were excised, patted dry, and immediately placed in 0.25 M sucrose at 0 °C. Livers were weighed by displacement, and the microsome fraction was isolated by the method of Kamath and Rubin (1972) as follows: Liver tissues were homogenized in six volumes of 0.25 M sucrose/2 mM Tricine [*N*-tris(hydroxymethyl)methylglycine]/1 mM EDTA at pH 7.6 with a motor-drive homogenizer in ice. They were then centrifuged at 12000 *g* and at 0 °C for 10 min in a Beckman centifuge (J-21; Beckman Instruments, Inc., Palo Alto, CA). The supernatant was diluted six times with 0.125 M sucrose containing 8 mM $CaCl_2$ and centrifuged again at 30*g* at room temperature for 10 min. On an International centrifuge (Model UV, International Equipment), the sediment was freeze-dried for later analysis. Analysis was carried out by dissolving the tissue fraction in Beckman tissue solubilizer (0.5 N quaternary ammonium hydroxide in toluene) using 1 mL/200 mg of dried microsomal tissue. One-fifth of the solution (about 1 mL) was then mixed with Aquasol, giving a total volume of 15 mL, and counted in the liquid scintillation counter. The volume of urine collected was recorded, and these samples were also counted after mixing the total with Aquasol to bring the total volume to 15 mL. Aliquots of the infused samples were counted to determine the radioactivity infused.

Incorporation of radioactivity into the liver microsome was expressed as percent of infused counts in total liver microsome and as percent of control ([^{14}C]lysine). Urinary excretion was also expressed in the same manner.

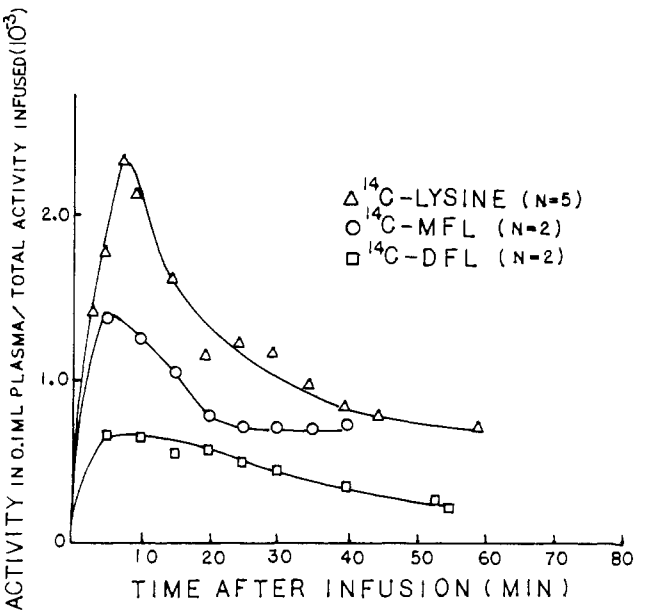

Figure 1. Absorption kinetics of the Maillard products from a glucose–lysine reaction system. A duodenal infusion was made with 0.5 mL of 50 mM solutions having a radioactivity of 0.36 μCi. *n* represents the number of replications.

RESULTS AND DISCUSSION

A reaction time of $4^1/_2$ h yielded sugar–amino acid complexes (MFL and DFL) to the extent of 83% of the reaction mixture.

The kinetics of the in vivo absorption study for MFL and DFL are shown in Figure 1. Absorption of these products took place to a lesser extent than that of lysine, with MFL being absorbed faster and to a greater extent than DFL. The more rapid absorption of MFL may be because in MFL one of the amino groups remained free in either α or ε form and is still recognized by the carrier for active transport. On the other hand, in DFL both α- and ε-NH_2 are blocked and not recognized by carriers. Therefore, its absorption, if there is any, can be explained by diffusion.

Table I. Microsomal Incorporation of MFL and DFL after Jugular Vein Administration[a]

product infused	% microsomal incorpn (% infused/g microsome)	% Lys (incorpn)
[^{14}C]lysine	0.131 ± 0.0028	100
[^{14}C]MFL	0.073 ± 0.0127	56
[^{14}C]DFL	0.079 ± 0.0098	60
MFL + [^{14}C]lysine	0.189 ± 0.0010	144
DFL + [^{14}C]lysine	0.180 ± 0.0001	137

[a] Two replicate runs were made for infusion of each product. The average body and liver weights (g) were 204.7 ± 18.22 and 9.44 ± 0.835, respectively.

The absorption rate for lysine dropped sharply following its maximum peak, whereas the rate of change of absorption in the other two curves was slower. No delay in absorption peak for any of them was observed. Each curve on this graph represents a series of means for two rats studied. Because of the complexity of the experimental procedure, a small number of animals were used. Since the main focus of the present absorption study was on comparison of absorption kinetics, no statistical analysis was attempted.

The absorption of lysine in the presence of the sugar–amino acid complexes is shown in Figure 2. When MFL and DFL were given along with lysine, the absorption of lysine was inhibited. In the presence of MFL, the absorption peak was delayed, being reached at 20 min in comparison with 5–7 min for the other two curves.

These results for absorption of lysine reflect those for Lee et al. (1977b) with tryptophan. The greater absorption of MFL compared to DFL and its absorption-retarding effect may indicate that MFL competes with lysine for the absorption carriers. On the other hand, the slower absorption of DFL and its failure to delay effect on lysine absorption may suggest that DFL simply blocks the absorption site rather than competing with lysine for absorption carriers. The greater absorption of MFL compared to DFL may be due to its uptake by either an amino acid carrier or a peptide carrier at the brush border of the mucosal cells, since the free amino group and positive charge act to increase its affinity for the carrier (Adibi and Gray, 1967). Compared to lysine, the absorption of the sugar–amino acid complexes is retarded as evidenced by the slow decline in the absorption rate for both MFL and DFL.

The extent of incorporation of radioactivity into the microsomal fractions is given in Table I. Both MFL and DFL were incorporated to a much lesser extent than lysine. Strikingly, more lysine was incorporated in the presence of products, namely 144% and 137% incorporation in the presence of MFL and DFL, respectively. A similar observation was previously reported by Borsook et al. (1955) in the in vitro liver homogenate system. However, these findings appear to be in contradiction to that of Johnson et al. (1973b) who reported an inhibited incorporation of

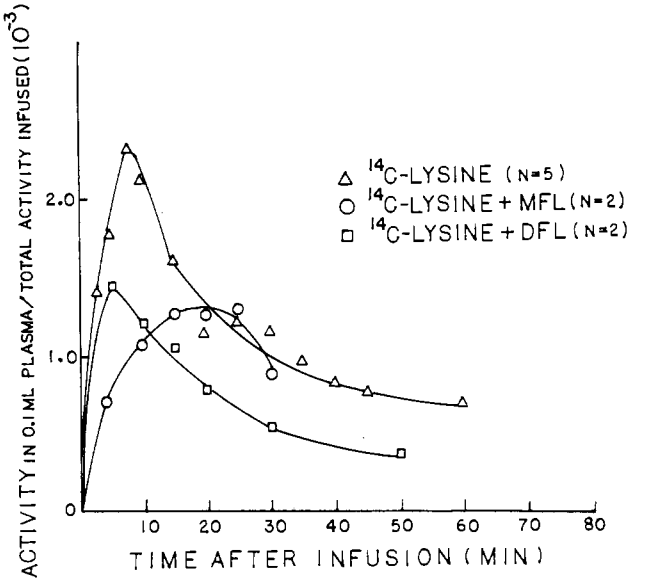

Figure 2. Interfered absorption of lysine by the Maillard reaction products. *n* represents the number of replications. A duodenal infusion was made with 0.5 mL of 50 mM solutions having a radioactivity of 0.36 μCi, where the concentrations of added MFL and DFL were both 50 mM, same as that of lysine.

phenylalanine in the presence of fructose-L-phenylalanine when fed to checks. These contradictory findings need to be clarified as to the amino acid specificity, the manner of administration, and the location of incorporation. The percent of incorporation for MFL and DFL was 56% and 60%, respectively, of the amount for lysine.

The incorporation of radiolabeled MFL and DFL into liver microsomes contrasts sharply with the result of Sgarbieri et al. (1973), who found fructose-L-leucine to be incorporated into the liver at a rate 1.5% that of the free leucine when oral intubation was used. Since a discrepancy in the liver incorporation between the Maillard lysine and leucine products cannot be explained by a lack of the metabolism of branched-chain amino acids such as leucine in the liver (Shinnick and Harper, 1976) since the rate of incorporation was based on the free amino acid. Perhaps there is another mechanism causing such a difference in the liver incorporation.

Table II presents the results of urinary excretion of products and lysine with and without the Maillard reaction products. Radioactivity in the urine was 15 and 13 times greater for MFL and DFL, respectively, than for lysine. This result 5 h after jugular vein administration is in agreement with that of Ford and Shorrock (1971). From the combined results of microsomal incorporation (Table I) and urinary excretion, it can be seen that DFL was incorporated into microsome more than MFL, but it was also excreted more than MFL. The microsome appears to be merely an acceptor site, and products may have been

Table II. Urinary Excretion of MFL and DFL after Jugular Vein Administration

product infused	% urinary excretion/mL urine[a]	rel excretion	total urine collected for 5 h, mL	% total urinary excretion/total infused act. (urine collected for 16 h, mL)
[^{14}C]lysine	0.17 ± 0.036	1.00	1.45 ± 0.919	21.94 ± 0.77 (24.3 ± 1.15)
[^{14}C]MFL	2.58 ± 0.297	1.52	1.40 ± 0.282	71.79 ± 4.81 (29.5 ± 0.70)
[^{14}C]DFL	2.23 ± 1.450	1.31	1.10 ± 0.848	96.16 ± 6.09 (32.0 ± 9.89)
MFL + [^{14}C]lysine	0.25 ± 0.056	1.47	1.65 ± 0.353	
DFL + [^{14}C]lysine	0.25 ± 0.084	1.47	1.60 ± 0.283	

[a] Measured from the urine collected for 5 h after administration.

attached temporarily to the acceptor site and later freed and excreted through urine. It is interesting to note that, in the presence of products, lysine was excreted in a greater amount than in the absence of products. This may indicate a possible interference with renal reabsorption of lysine by the lysine Maillard products.

Our observation of stimulated liver incorporation of lysine in the presence of MFL and DFL agrees with the results of Borsook et al. (1955). They observed a stimulating effect of commercially dried liver and of boiled plasma filtrate on incorporation of amino acids into proteins in rabbit reticulocytes.

Increased urinary excretion of radioactive compounds was noticed after jugular vein injection of labeled MFL and DFL. Under these conditions, less incorporation into liver microsomes took place and large amounts of the Maillard complexes were excreted into the urine. Increased urinary excretion of labeled lysine in the presence of MFL and DFL was unexpected. It is speculated that although lysine in the presence of these products is incorporated into liver microsomes to a greater extent than for lysine alone, it is less well utilized by other tissues. Perhaps muscle tissue incorporation is reduced in the presence of the Maillard complexes. Waterlow and Stephan (1967) have shown evidence that lysine penetrates tissues more slowly than other amino acids. Inhibition of lysine reabsorption in the kidney tubules in a manner similar to that observed in the absorption study may account for its increased excretion. It would be interesting to see whether this result could be duplicated with other amino acids.

The results indicating incorporation of Maillard products into liver microsomes when the substances are given intravenously should be of concern to those who use total parenteral formulas for nutritional maintenance under conditions of surgery or disease. In these circumstances even a slight toxic effect is of the utmost concern because of the stress associated with the patient's condition. Further study is needed to clarify these phenomena.

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