Transport of Amino Acids via the Mesenteric Lymph Duct in Rats* (33636)

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It is known that: (i) amino acids absorbed from the intestinal tract are transported to the liver *via* the portal system; (ii) significant quantities of water are transported by the mesenteric lymph; (iii) long chain fatty acid lipids as chylomicrons are transported in lymph; and (iv) mesenteric lymph is mixed with hepatic and systemic lymph as it appears in the thoracic duct before entering the blood stream. In order to investigate amino acid transport from the intestinal lumen to lymph coming directly from the small intestine, free from the hepatic and systemic lymph, it was necessary to cannulate the mesenteric lymph duct. Bollman *et al.* (1) and Lambert (2) reported procedures for the cannulation of various lymphatic ducts in rats; we used a surgical procedure developed from modifications of their work (3).

Rats with cannulas implanted into the mesenteric lymph duct were fed radioactive amino acids by stomach tube. The radioactivity was then followed into this lymph both as the free amino acid and protein fractions.

Methods and Materials. Male rats of Sprague-Dawley origin, weighing between 250-400 g were used in these investigations. They were fed 1–2 ml of corn oil by stomach tube 30–45 min before surgery. Animals were anesthesized by an intraperitoneal injection of 2% sodium pentobarbital (50 mg/kg). Heparin (0.2 ml of heparin solution, 1000 USP units/ml, kindly furnished by Eli Lilly and Co.) was injected intraperitoneally to prevent clotting of the lymph.

Briefly, the cannulation procedure (3) is as follows: The mesenteric lymph duct, located in the lower portion of the hepatoduodeno ligament, runs parallel to the mesenteric artery, in the same sheath, as the artery enters the intestinal mesentery. The duct is disected free of the artery; a ligature of fine silk is placed beneath the duct and a small incision is made in the wall of the duct. Fine polyethylene tubing is passed into the lumen of the duct 3–6 mm. The ligature is then secured around the duct and the cannula. At this stage a steady flow of lymph can be visualized through the cannula. The viscera are then replaced and the cavity is closed surgically.

Feeding experiments. After cannulation the rat was placed into a special cage which was designed in such a manner as to allow the animal comfortable conditions for surgical recovery, was easily kept clean, and protected the implanted cannula. The animal was fed Purina laboratory chow, and 0.85% saline *ad libitum*. Such a preparation allowed for the collection of mesenteric lymph by gravity flow into calibrated collecting tubes.

In a series of experiments, three different radioactive amino acids were used and their incorporation into lymph amino acid and protein fractions was followed. All amino acids used were of the L-configuration. Radioactive amino acids were uniformly labeled (New England Nuclear Corp., Boston, Mass.) Rats were taken off food several hours before the radioactive amino acids were fed by stomach tube. Experimental conditions were as follows: (i) Threonine-^{14}C was fed to a cannulated rat 2 hr after surgery; 15 μCi (sp act., 100 μCi/74 μg), mixed in 2 ml of bovine cream (12.5% butterfat). (ii) Leucine-^{14}C was fed 5 hr after surgery at a level of 20 μCi (sp. act., 100 μCi/47.5 μg) in 2 ml of bovine cream mixture containing 30 μmoles each of nonradioactive leucine and valine. (iii) Valine-^{14}C was fed to a cannulated rat 20 hr after surgery at a level of 10 μCi (sp act., 100 μCi/60.2 μg) in 2 ml of bovine cream

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mixture containing 30 μmoles each of nonradioactive valine and leucine. After the animals were fed in this manner, they were returned to their cages and allowed laboratory chow and saline *ad libitum*. Under these experimental conditions they produced between 1–3 ml of lymph/hr, which was collected as 0.5-ml fractions (0.01 ml of heparin had been added to each collection tube).

Chemical and radioactivity measurements. Lymph protein concentration was determined by the method of Lowry *et al.* (4).

The method employed for amino acid and protein fractionation of lymph was adapted from Awapara's ethanol extraction procedure (5). After the addition of 95% ethanol, the sample was centrifuged at 2000 rpm for 15 min in an International model HN Centrifuge. The free amino acids in the supernatant fraction were decanted into a 5-ml volumetric flask. The protein precipitate was washed twice with 80% ethanol and the three supernatant fractions were pooled and taken up to a constant volume of 5 ml with 80% ethanol. One ml was then added to a counting vial and 10 ml of dioxane scintillation solution[1] were added.

To the protein precipitate was added 1.0 ml of Nuclear Chicago Solubilizer (NCS) Reagent which dissolved the protein. Ten ml of toluene scintillation solvent[2] were then added and the contents were transferred quantitatively to a counting vial. All vials were counted in a Packard Tri-Carb scintillation spectrometer (series 314 E, Packard Instrument Co., Inc., Downers Grove, Ill.).

Lymph amino acid analyses were made by automatic column chromatography (6) using a Beckman/Spinco amino acid analyzer with simultaneous scintillation counting of the effluent for levels of radioactivity in a manner similar to that employed by Elwyn (7). Lymph samples were prepared for amino acid analysis in a manner similar to that proposed by Stein *et al.* (8). Analytical grade anion exchange resin AG1-X8, 200–400 mesh (Bio-Rad Laboratories, Richmond, Calif.) was used to free the extract of picric acid.

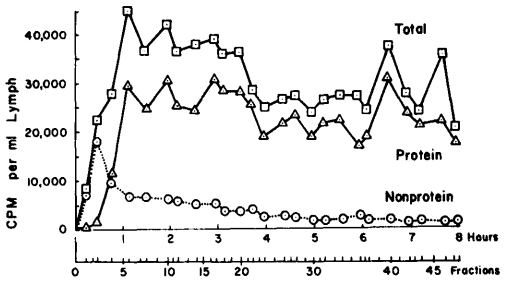

FIG. 1. Distribution of radioactivity in lymph after feeding leucine-^{14}C in cream at time zero. One-half ml fractions of lymph were collected over an 8-hr period at the intervals indicated as *fractions*. Total lymph radioactivity (□) protein activity (△), and nonprotein activity (○) (the amino acid fraction) are indicated.

Results and Discussion. The distribution of radioactivity over an 8-hr period after the feeding of leucine-^{14}C is shown in Fig. 1. As shown, the free-amino acid was released into the lymph quite soon after the material was placed into the intestinal tract, and the fed amino acid entered into the synthesis of radioactive protein released into the lymph. Similar distribution patterns were obtained on animals fed valine-^{14}C and threonine-^{14}C.

Chromatographic analyses of the nonprotein fractions monitored for radioactivity are shown in Fig. 2. The amino acid fed is the major radioactive component found in this fraction. This is very apparent for the early fractions, 1–4, but not for that collected 5–6 hr after feeding. It is also apparent that a fast-moving metabolite, which appears just ahead of taurine and urea is formed from the amino acid and is transported into lymph. Similar peaks appear with each amino acid tested; these have not been identified conclu-

[1] Dioxane scintillation solution: Into 100 ml of *p*-dioxane, 65 g of naphthalene, 7.0 g of 2,-5-diphenyloxazol (PPO), and 50.0 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) were dissolved, and mixed with 100 ml of ethanol (95%) and 29.5 ml of water. This scintillation preparation will hold salts and amino acids in solution.

[2] Toluene scintillation solution: PPO (4.0 g) and POPOP (100 mg) were dissolved in 1000 ml of toluene.

[3] The data presented in Figs. 1–3 are from typical experiments, since confirmation experiments present data which can be superimposed upon those presented.

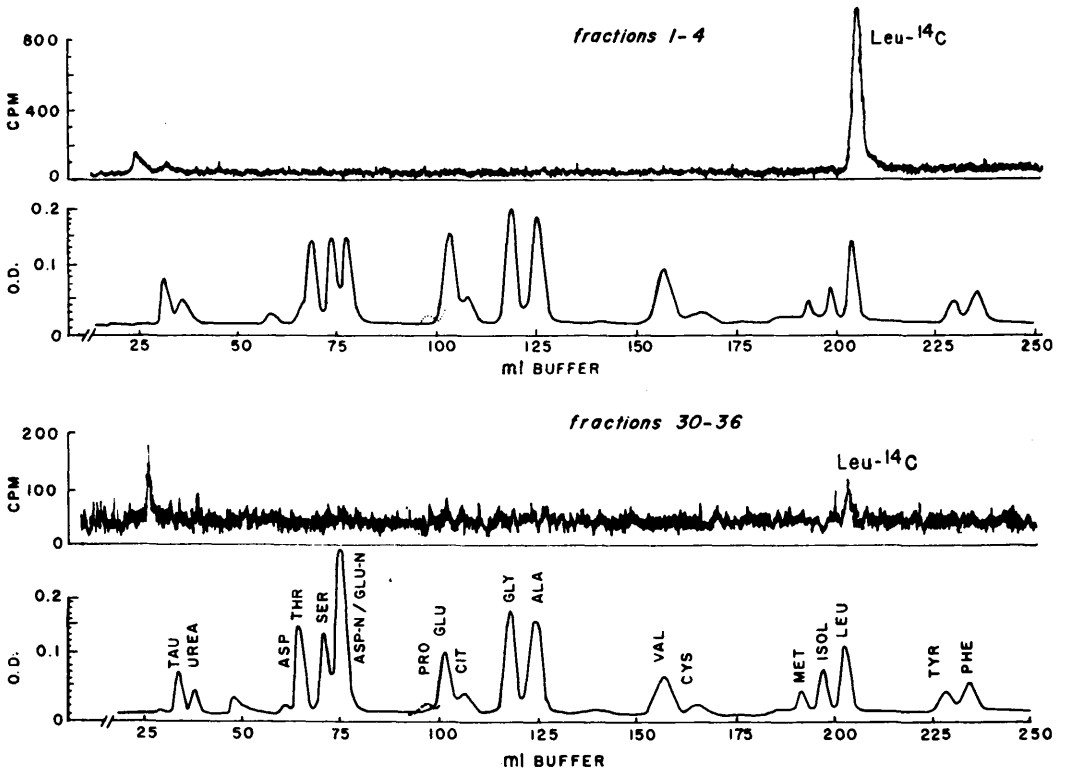

FIG. 2. Column chromatograph monitored for radioactivity of lymph amino acid fraction after feeding, leucine-^{14}C in cream (test meal). Lymph fractions 1–4 were collected within 1 hr and fractions 30–36 were collected 5–6 hr after feeding the test meal (cf. Fig. 1 for fraction, volume, and time relationship). Basic amino acids are not shown since there was no radioactivity in that group.

sively, but behave as though they are keto-derivatives of the deaminated radioactive amino acids. There was no radioactivity associated with any other amino acids. However, threonine-^{14}C did have a greater proportion of its radioactivity in a metabolic breakdown product than did leucine or valine. Leucine-^{14}C was used in subsequent experiments, since it has fewer known metabolic pathways than threonine. Also, Hatch *et al.* (9) observed a predominence of nonpolar amino acids in the lowest density lipoprotein fraction from a homogenate of rat intestinal mucosa. Leucine had the highest molar ratio in this fraction.

The protein concentration of the mesenteric lymph was 1.1% (0.7–1.7 range). However, the protein concentration was dependent on the lymph flow, which was variable, and followed the food and drink pattern of the animal. When an animal drinks a saline solution the lymph flow increases strikingly, as first observed by Koler and Mann (10), and later studied by others (11, 12). Rats in our experiments were maintained on saline and had a lymph flow-rate ranging between 1–3 ml/hr.

It has also been observed that when an animal drinks a saline solution, which raises considerably the lymph flow there is also an increase in the rate of protein elimination in the lymph (13). A saline solution increased the loss of protein in the lymph since the increase in flow was not accompanied by a decrease in protein concentration. However, in the present experiments, saline and chow were available to the animal *ad libitum*, and the lymph protein concentration varied randomly.

While the lymph flow and protein concentration vary, the specific activity of the lymph protein was relatively smooth (Fig.

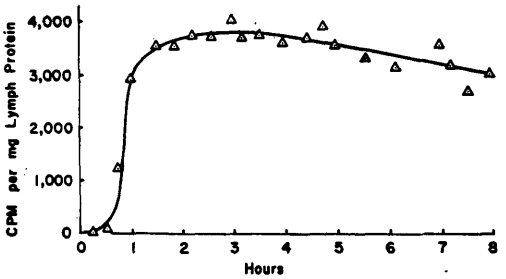

FIG. 3. Specific activity of radioactive lymph protein after feeding leucine-^{14}C in cream (cf. Fig. 1 for radioactivity distribution in the lymph).

3). The specific activity reached a peak about 1.5 hr after feeding the radioactive test meal and appeared to decline slowly over the 8-hr period.

The radioactivity of the protein fraction of the lymph was due to the incorporation of the fed radioactive amino acid. This was verified by monitored column chromatography of acid hydrolysates of the washed protein fractions. The only radioactivity found in these hydrolysates was that of the radioactive amino acid fed, namely, leucine, threonine, or valine, which had been incorporated into protein and released into the lymph.

Lymph amino acid concentrations were compared with concentrations found in the plasma (14). Whereas the protein concentrations in mesenteric lymph were one-tenth to one-quarter that of plasma, the total concentration of amino acids in each was similar. However, when the amino acids were compared on a molar basis with leucine the distribution was quite different from that found

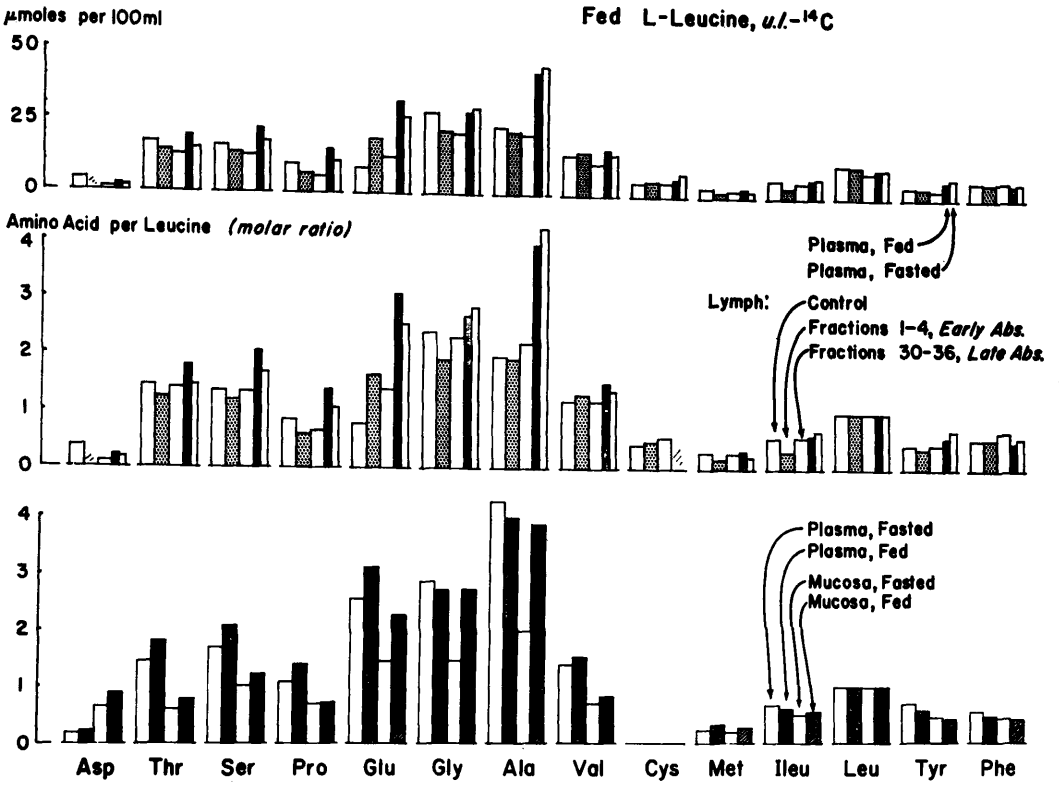

FIG. 4. Amino acid distribution in plasma, intestinal mucosal scrapings and mesenteric lymph of the rat. Lymph fractions are identified in Fig. 1. The upper most group of values are expressed in μmoles per 100 milliliters, the control fraction of lymph was collected before this test meal was fed. The two lower groups of data are expressed in molar ratios referred to leucine. Plasma data were obtained from rats fed Purine laboratory chow up to the time of sacrifice; fasted rats were without food for 18 hr.

in fed or fasted rat plasma or the intestinal mucosa.

The addition of nonradioactive leucine and valine (30 μmoles each) to the test meal had an effect on the amino acid ratios in the first 45 min after the oral feeding. When compared with leucine at a molar ratio of one, there was a depression in the early absorption phase (1–45 min) in nearly all the amino acids except valine, glutamic acid, and cystine. This occurred at the time when the specific activity of the newly formed protein was appearing in the lymph. Most amino acid ratios returned to the control level in the late absorption phase (lymph collected 3 hr or later after feeding the test meal). The amino acids fed were not in sufficient quantity to have any great effect upon the pool size of the intact rat.

A number of sources can contribute amino acids to the mesenteric lymph; (a) diet, (b) plasma, (c) and the amino acid pool from the mucosal cells (15). The data in Fig. 4 indicate that there are discrete differences in the distribution profile of the lymph amino acids compared to plasma or the mucosal pool (fed or fasted). What is more, the concentration of mucosal amino acids is about 10 times that found in blood plasma, yet lymph amino acids follow more closely to the concentrations found in blood.

Summary. It was demonstrated that dietary amino acids appear in the mesenteric lymph and are transported from the intestinal lumen both as free amino acids and newly synthesized protein via the lymphatic system. The amino acid profiles in intestinal lymph are compared to those in plasma and the intestinal mucosa. There seems to be a depression of lymph amino acid levels during the early phase of intestinal absorption which returns to normal in the later phase. This depression occurs at the same time the newly synthesized protein appears in the lymph.

1. Bollman, J. L., Cain, J. C., and Grindley, J. H., J. Lab. Clin. Med. **33**, 1349 (1948).

2. Lambert, R., "Surgery of the Digestive System of the Rat," p. 175 ff. Thomas, Springfield, Illinois (1965).

3. Largis, E. E., M.S. Thesis, 1967, Univ. of North Dakota, Grand Forks, North Dakota.

4. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

5. Awapara, J., Arch. Biochem. **19**, 172 (1948).

6. Spackman, D. H., Stein, W. H., and Moore, S., Anal. Chem. **30**, 411 (1958).

7. Elwyn, D. H., Atomlight **37**, 5 (1964).

8. Stein, W. H. and Moore, S., J. Biol. Chem. **211**, 915 (1954).

9. Hatch, F. T., Yoshiro, A., Hagopian, L. M., and Rubenstein, J. J., J. Biol. Chem. **241**, 1655 (1966).

10. Koler, R. D. and Mann, J. D., Proc. Soc. Exptl. Biol. Med. **76**, 221 (1951).

11. Shrewsburg, M. M. and Reinhardt, W. O., Am. J. Physiol. **168**, 366 (1952).

12. Kim, K. S. and Bollman, J. L., Am. J. Physiol. **179**, 273 (1954).

13. Borgstrom, B. and Laurell, C. B., Acta Physiol. Scand. **29**, 264 (1953).

14. Jacobs, F. A. and Lang, A. H., Proc. Soc. Exptl. Biol. Med. **118**, 772 (1965).

15. Jacobs, F. A., Federation Proc. **24**, 946 (1965).

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