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IN VITRO ACCUMULATION OF AMINO ACIDS BY INTESTINAL STRIPS FROM NORMAL AND ESSENTIAL FATTY ACID-DEFICIENT RATS

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SUMMARY

1. Steady-state accumulation of phenylalanine, leucine, glutamic acid and lysine by the small intestine from rats fed corn oil (control) or hydrogenated coconut oil (essential fatty acid-deficient) was studied by an *in vitro* technique.

2. On a unit length basis, the mid-to-distal sections of the intestine were most active in accumulating the amino acids. Accumulation of phenylalanine by the middle and distal sections, and of leucine by all three sections of small intestine, was significantly greater in control than in deficient rats. No significant dietary effects were noted in the uptake of glutamic acid or lysine per unit length of rat small intestine.

3. When data were expressed on a tissue-dry weight basis, bimodal uptake patterns were evident with maxima usually in the proximal and distal regions of the intestine. Accumulation of phenylalanine, leucine and lysine was significantly greater in some intestinal sections from deficient rats on this basis. These data reflect variations in dry weight per unit length throughout the intestine and the lower weight per unit length observed in the intestine of the deficient animals.

INTRODUCTION

In a previous communication[1], the extent to which a diet deficient in essential fatty acids modified the fatty acid composition of rat mucosal lipids was reported. Since the intestinal epithelium is membranous in nature and the essential fatty acids have been implicated in the maintenance of the integrity of such structures, it is reasonable to hypothesize that changes will occur in the properties of the intestinal mucosa as a result of a deficiency of these acids. SNIPES[2] has reported that essential fatty acid deficiency resulted in morphological changes in rat jejunal epithelium and that such changes were accompanied by a decrease in the animals' ability to absorb fat. Moreover, this deficiency syndrome is also characterized by changes in mitochondrial function[3,4]. Since many transport processes are energy dependent, such impairment of mitochondrial function could adversely affect nutrient absorption by the intestine. The present study was undertaken in order to test the hypothesis that essential fatty acid deficiency will result in modification of the ability of the rat

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intestine to absorb or accumulate amino acids. Four acids were chosen, leucine, phenylalanine, glutamic acid and lysine, representing compounds with different chemical and nutritional characteristics and being either actively or passively transported by rat intestine.

MATERIALS AND METHODS

Male weanling rats of the Wistar strain were obtained from Woodlyn Farms, Guelph, Ontario. L-Glutamic acid, L-leucine, L-lysine monohydrochloride and L-phenylalanine, analytical grade, were purchased from Calbiochem, Los Angeles, Calif. Their uniformly ^{14}C-labeled counterparts were obtained from Amersham-Searle Corp., Des Plaines, Ill., as were the NCS-solubilizer and Spectrafluor solution (50 g 2,5-diphenyloxazole and 0.625 g 1,4-bis-2-(5-phenyloxazolyl)benzene in 500 ml toluene) employed in the assay of ^{14}C in the tissues. All other chemicals were of reagent grade quality.

Male weanling rats, weighing 40–60 g, were maintained on the diets previously described[5] and containing either 10% corn oil or 10% hydrogenated coconut oil (kindly donated by Canada Packers Ltd., Toronto, Canada). The diets were fed *ad libitum* and the animals were allowed free access to water and were weighed periodically. The *in vitro* studies commenced 16 weeks after the initiation of the experiment and continued for 4 weeks. Animals were randomly selected from the two dietary groups for these studies.

After being deprived of food for 18 h, the rats were anesthetized with diethyl ether and the abdomen was opened by a mid-line incision. The intestine was rapidly stripped of mesentery, severed at the pylorus and the ileo–cecal junction and gently flushed with 0.9% NaCl. It was subsequently everted by a modification of the procedure of WILSON AND WISEMAN[6] using a plastic coated wire with a hook at one end.

In order to reduce the free amino acid content of the tissue, the everted intestine was incubated for 20 min at 37° in 100 ml of Krebs–Ringer bicarbonate (pH 7.4) containing 0.25% glucose and continuously flushed with CO_2– O_2 (5:95, by vol.). It was then transferred to 100 ml of Krebs–Ringer bicarbonate (pH 7.4) containing 0.25% glucose and 2 mmoles of amino acid labeled with 2.5 μC of ^{14}C. The solution was maintained at 37°, flushed with CO_2– O_2 (5:95, by vol.) and continuously shaken during the 1-h incubation period. At the end of this period, the intestine was removed, rinsed 3 times in cold Krebs–Ringer solution to remove adhering amino acid solution and gently blotted dry. The duodenum was removed and discarded. The remainder of the intestine was measured and divided into 20 equal segments, each of which was transferred to a tared scintillation vial and weighed. 3 ml of NCS solubilizer was added to each vial and the tissues were digested at 50° for 2 h. After cooling, 10 ml of 6% Spectrafluor in toluene were added to each vial and the contents were vigorously mixed. The vials were placed in a liquid scintillation counter (Nuclear-Chicago Mark I) and held at 5° for 45 min prior to counting. The degree of quenching was determined by the channels-ratio technique and the steady-state accumulation of amino acid was calculated from the corrected ^{14}C activity and expressed in terms of the unit length or unit dry weight of intestine. The moisture content of the tissue was determined in a separate experiment by drying to constant weight at 110°.

Amino acid accumulation was plotted as a function of the relative distance of the intestinal segment along the intestine (duodenum = 0, cecum = 1.0). Data were subjected to an analysis of variance[7]. The data from the first and twentieth segments were discarded (because of their proximity to the duodenum and cecum) and the data for the remaining 18 segments were pooled in three equal groups representing the proximal, middle and distal intestinal sections, and were compared by means of the F-test[7].

RESULTS

Preliminary experiments were carried out to determine the time course of accumulation of each amino acid by the different sections of the intestine. Maximum accumulation was achieved in 30–40 min with little change noted after 60 min incubation. The latter period was chosen to represent steady-state accumulation.

Figs. 1 and 2 present the distribution of amino acid accumulation per unit length of small intestine. Maximal accumulations were usually noted in the mid to distal regions of the intestine but the maximum was broad and poorly defined in

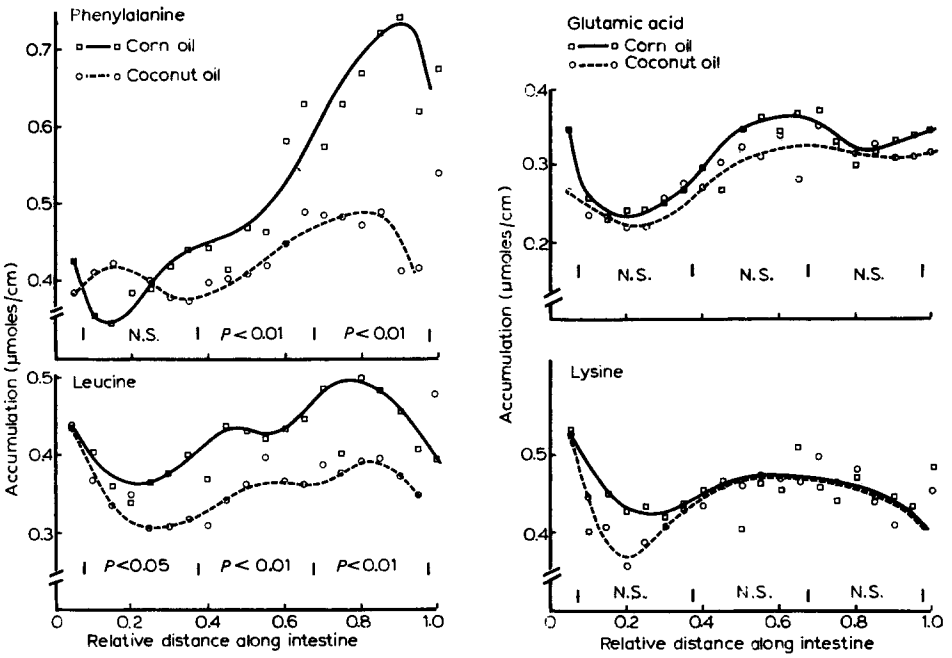
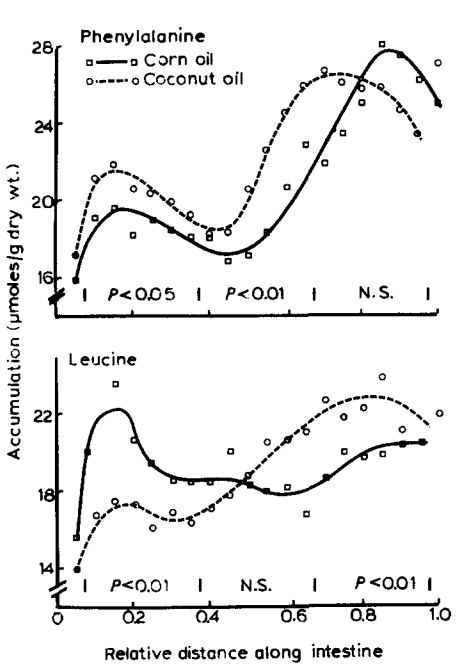


Fig. 1. Distribution of phenylalanine and leucine accumulation per cm of small intestine from rats receiving corn oil and hydrogenated coconut oil. Each point is the mean of values from 4 animals. Significant differences between the two dietary groups are denoted by the value of P for the proximal, middle and distal sections of the intestine. N.S. (not significant) indicates that $P > 0.05$. Incubation conditions: 60 min in 100 ml Krebs–Ringer bicarbonate (pH 7.4), containing 0.25% glucose and 2 mmoles of amino acid labeled with 2.5 μC of ^{14}C, and flushed with CO_2–O_2 (5:95, by vol.).

Fig. 2. Distribution of glutamic and lysine accumulation per cm of small intestine from rats receiving corn oil and hydrogenated coconut oil. Conditions as in Fig. 1.

the case of lysine. The segments adjacent to the duodenum also exhibited a marked degree of accumulation of leucine, glutamic acid and lysine, but not of phenylalanine. Accumulation of phenylalanine was significantly lower in the mid and distal sections from rats fed coconut oil than in the corresponding sections from the control animals. The essential fatty acid deficiency resulted in significantly lower uptake of leucine by all three intestinal sections but had no effect on glutamic acid and lysine accumulation.



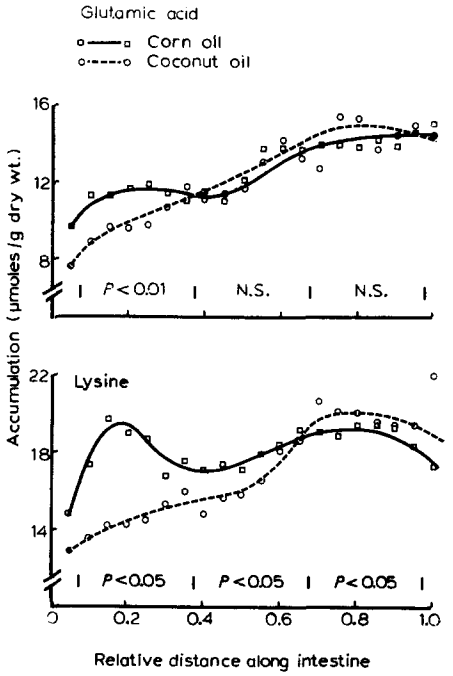


Fig. 3. Distribution of phenylalanine and leucine accumulation per g dry weight of small intestine from rats receiving corn oil and hydrogenated coconut oil. Conditions as in Fig. 1.

Fig. 4. Distribution of glutamic acid and lysine accumulation per g dry weight of small intestine from rats receiving corn oil and hydrogenated coconut oil. Conditions as in Fig. 1.

A different picture emerged when data were plotted on a unit weight basis (Figs. 3 and 4). Bimodal accumulation patterns were evident, well defined for phenylalanine but less so for leucine. Similar somewhat poorly defined bimodal patterns were also obtained for glutamic acid and lysine (Fig. 4). On a unit weight basis, accumulation of phenylalanine was significantly greater in the proximal and middle intestinal sections from the deficient rats. Leucine accumulation was lower in the proximal section and higher in the distal section of the deficient animals, a cross-over in the uptake patterns of the two groups being evident. Similar cross-overs were noted for glutamic acid accumulation, which was significantly lower in the proximal intestinal section from the deficient rats, and for lysine accumulation, which was lower in the proximal section but higher in the distal section from the deficient animals. These results are in marked contrast to those obtained by plotting the data on a unit length basis.

DISCUSSION

Two major differences resulted from plotting the data on a unit weight rather than a unit length basis, the translation of the minimum accumulation per unit length to a maximum accumulation per unit weight in the proximal intestine, and the occurrence of a cross-over in the uptake patterns of the two groups when the unit weight basis was employed. These differences resulted from variations in the dry weight of tissue along the length of the intestine (Fig. 5), variations which did not result from differences in the water content of the intestinal segments since this was reasonably constant. The dry weight per unit length of the mid and distal portions of the intestine from the deficient rats was lower than that for the control group, resulting in the cross-over in the amino acid accumulation profiles. The lower weight per unit length of intestine from the animals fed coconut oil may have been a direct consequence of the lower body weight of these animals (232 g *vs.* 360 g for the controls, $P < 0.001$).

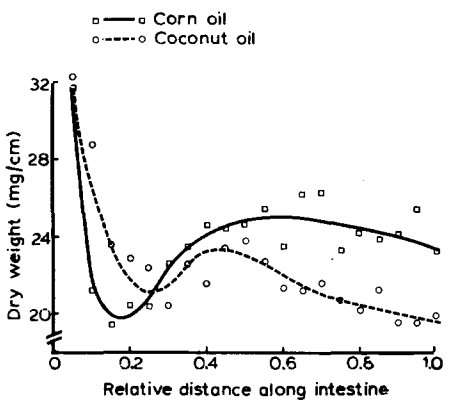


Fig. 5. Effect of location within the intestine on the weight per unit length of the intestinal segments from rats receiving corn oil and hydrogenated coconut oil. Each point is the mean of values obtained from 16 animals.

In general, previous studies on amino acid absorption *in vitro* have employed a dry weight basis for comparing amino acid uptake by different segments of the intestine. Comparisons must therefore be made with the data presented in Figs. 3 and 4. It must be noted, however, that in the present study, steady state accumulation of amino acids was measured, whereas many of the reports in the literature deal with the rate of uptake of amino acids by the intestine. In a comprehensive study of [^{14}C]amino acid uptake by different sections of rat intestine, RAMASWAMY AND RADHAKRISHNAN[8] noted a bimodal pattern of uptake for several amino acids including phenylalanine, leucine and glutamic acid. These peaks were usually in the proximal and distal regions of the intestine. However, other workers[9,10] have reported that the mid-section of rat gut is most active in accumulating leucine. In contrast to the present study, RAMASWAMY AND RADHAKRISHNAN[8] reported that lysine uptake was uniform throughtout the small intestine.

The major effect of essential fatty acid deficiency was the reduction in the amount of amino acid absorbed by the intestine. Similar studies on amino acid uptake

have not been conducted but IMAMI *et al.*[11] did examine valine transport by intestinal sacs from normal and essential fatty acid-deficient rats. They found an impairment in the transport of this acid into the serosal fluid in sacs from the deficient animals.

Essential fatty acid deficiency could affect absorption of amino acids either by modifying the lipophilic barrier presented to the acid or by impairing the energy-yielding processes in the cell. Essential fatty acid deficiency reduced the uptake per cm of intestine of phenylalanine and leucine, both of which are actively transported[12,13], but had no effect on the uptake of glutamic acid, which is not actively transported[14,15], or of lysine, which only undergoes active transport at concentrations much lower than that employed in the present study[16]. Thus it is possible that the major effect of essential fatty acid deficiency was exerted through its effect on the energy-yielding capacity of the mitochondrion[3,4]. However, caution must be exercised in interpreting the current data in terms of active transport since relatively high concentrations of amino acids were employed and it is doubtful that transport against a concentration gradient was occurring. Moreover, steady state accumulation is a complex function of several parameters including both active and passive transport[10].

Changes in the membrane of the mucosal cell could account for the data presented in this paper. The importance of the lipophilic side chain of the amino acid to its concentration by the intestinal cell has been stressed[17]; the more lipophilic this side chain, the more readily is it postulated to dissolve in the lipid-rich mucosal cell membrane[18]. The effects of essential fatty acid deficiency were more pronounced for amino acids having side chains with substantial lipophilic character, namely phenylalanine and leucine (*cf.* Figs. 1 and 2). Previously reported changes in the lipid constituents of the mucosal cell[1] may have substantially decreased their potential for interaction with the aromatic and aliphatic side chains of phenylalanine and leucine.

It is evident from the data presented above that essential fatty acid deficiency does exhibit a marked effect on the ability of rat intestine to accumulate amino acids under steady-state conditions. The nature of this effect depends on the acid in question and on the mode of expression the results. At the present time it is not possible to draw any conclusions regarding a simple correlation between the lipid components of the various intestinal sections and their ability to accumulate amino acids. Indeed, such a simple correlation may not exist.

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