In Vitro Studies on Protein Digestion, Amino Acid Absorption Interactions[1] (34212)

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L-Isomers of amino acids are absorbed by a number of transport mechanisms in the gut (1, 2). Although each amino acid has a characteristic rate of uptake, in the presence of other amino acids uptake rate (1) and entry site competition within groups of amino acids has been reported (1–4). Delhumeau *et al.* (5) investigated rates of uptake of amino acids from equimolar amino acid mixtures or amino acid mixtures simulating proteins in the rat. They observed that the percentage uptake from the initial concentration of each amino acid was not changed by different molar ratios but that amino acid mixtures simulating proteins can affect the overall rate of amino acid uptake. Further, enzymic liberation of amino acids in the stomach and intestine from proteins may modify amino acid uptake rates. To extend the above observations, we studied the effect of protein amino acid composition patterns and pepsin amino acid liberation patterns on uptake of histidine and methionine by rat jejunal rings *in vitro*.

Materials and Methods. Whole egg protein, rumen protozoal protein and rumen bacterial protein preparations (6) were subjected to *in vitro* pepsin digestion for up to 150 min. Subsamples of the digests were taken at 0 (Blank), 30, 60, 150 min and analyzed for free amino acid content (8). The following amino acid patterns were then constructed for each protein from the digest results and previous amino acid analysis of the proteins (6): (i) Amino acid mixtures in proportions similar to that in either egg protein, protozoal protein, or bacterial protein were designated as EC, PC, and BC, respectively. (ii) Amino acid mixtures in proportion similar to the 30, 60, 150 min pepsin amino acid liberation patterns from egg protein, protozoal protein, and bacterial protein were designated as E30, E60, E150; P30, P60, P150; and B30, B60, B150, respectively.

In vitro uptake of histidine ^{14}C or methionine ^{14}C was studied with rat jejunal rings using procedures outlined previously (4). The jejunal rings were extracted with 0.1 *N* HNO_3 for 3 hr and an aliquot of the 0.1 *N* HNO_3 extract was used for radioactivity measurement with a liquid scintillation spectrometer. Nonspecific ^{14}C adsorption by jejunal rings was determined in all experiments. All histidine or methionine transport experiments were conducted separately. The effect of amino acid patterns on histidine or methionine uptake was assessed by comparing maximal methionine or histidine uptake with methionine or histidine uptake in the presence of the amino acid patterns. To determine the effect of amino acid patterns on methionine or histidine uptake, experimental buffers (Table I) were prepared in such a manner that 1-mg total amino acid of each mixture was dissolved in 1 ml of Krebs–Ringer–bicarbonate buffer.

For maximal histidine or methionine uptake control buffers were prepared which contained only methionine or histidine (Table I), but at concentrations (per ml of KRB buffer) identical to the histidine or methionine concentrations in the experimental buffers. For example, for the EC pattern, the control methionine buffer contained 35 μg of methionine/ml while the experimental buffer contained 1 mg of total amino acid/ml in-

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TABLE I. Composition of Buffers for Amino Acid Uptake Studies.

Protein source pattern[a]	Buffers[b]			
	Control		Experimental	
	Methionine	Histidine	Methionine	Histidine
EC	32[c]	21[c]	32[d]	21[d]
E30	8	14	8	14
E60	5	21	5	21
E150	10	15	10	15
PC	25	24	25	24
P30	20	18	20	18
P60	21	8	21	8
P150	18	7	18	7
BC	36	24	36	24
B30	20	7	20	7
B60	16	7	16	7
B150	16	10	16	10

[a] See text for code definitions.

[b] Krebs–Ringer–bicarbonate, pH 7.4.

[c] (μg/ml of KRB buffer).

[d] One mg total amino acid including micrograms of methionine or histidine as indicated per milliliter of KRB buffer.

cluding 32 μg of methionine. Jejunal rings were incubated with the KRB buffers (Table I) under an O_2/CO_2 (95%/5%) atmosphere. Each incubation medium contained 1 μCi of either methionine-^{14}C or histidine-^{14}C. After the incubations were completed, total ^{14}C activity in the ring extracts and incubation buffers was determined. Since the buffers contained varying concentrations of histidine or methionine specific activities (dpm/μmole of histidine or methionine) were calculated for each incubation medium and amino acid uptake expressed as micromoles per 100 mg of wet jejunal ring. Student's t test was used for statistical evaluation of the amino acid uptake studies.

Results. Egg protein, rumen protozoal and bacterial protein were subjected to pepsin digestion for 30, 60, and 150 min. Since time of pepsin exposure did not markedly influence the liberation patterns, Fig. 1 gives a representative pattern for each protein. Results are expressed as a ratio, by individual amino acids, of the amino acid liberation pattern to the original amino acid composition times 100. Thus, any ratio greater than 100 indicates preferential pepsin liberation of a given amino acid compared to the amino acid concentrations in the protein, whereas, any ratio below 100 indicates less preferential pepsin liberation of a given amino acid. In all cases, proteolytic degradation by pepsin resulted in liberation of free amino acid mixtures whose composition differed markedly from the amino acid composition of the intact protein sources (Fig. 1). The extent of proteolysis was proportional to duration of pepsin digestion with a maximum digestion at 150 min of 19, 59, and 44% for the egg, protozoal, and bacterial protein, respectively.

Methionine or histidine uptake into rat jejunal rings was determined in the absence (control) and presence of the various amino acid mixtures (Table I). Uptakes of methionine or histidine in the presence of amino acid mixtures are expressed as a percentage of the control uptake (Fig. 2). Histidine or methionine uptake in the presence of each of the various amino acid mixtures were lower ($p<.01$) than the controls. The results show that the presence of each amino acid mixture depressed histidine or methionine uptake.

Type of amino acid mixture, however, modified uptake depressions of histidine or methionine (Table II). This table gives the

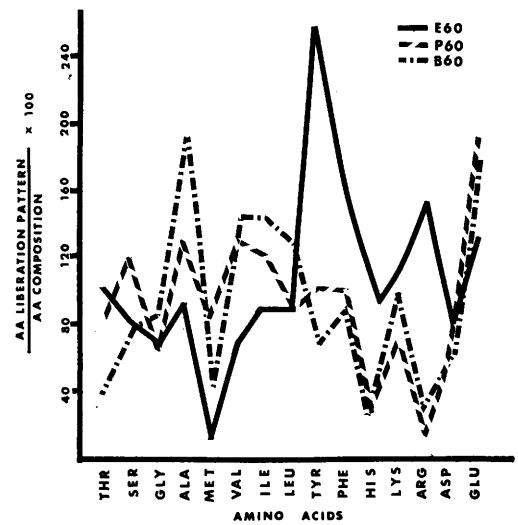

FIG. 1. Relative compositions of amino acids liberated by pepsin.

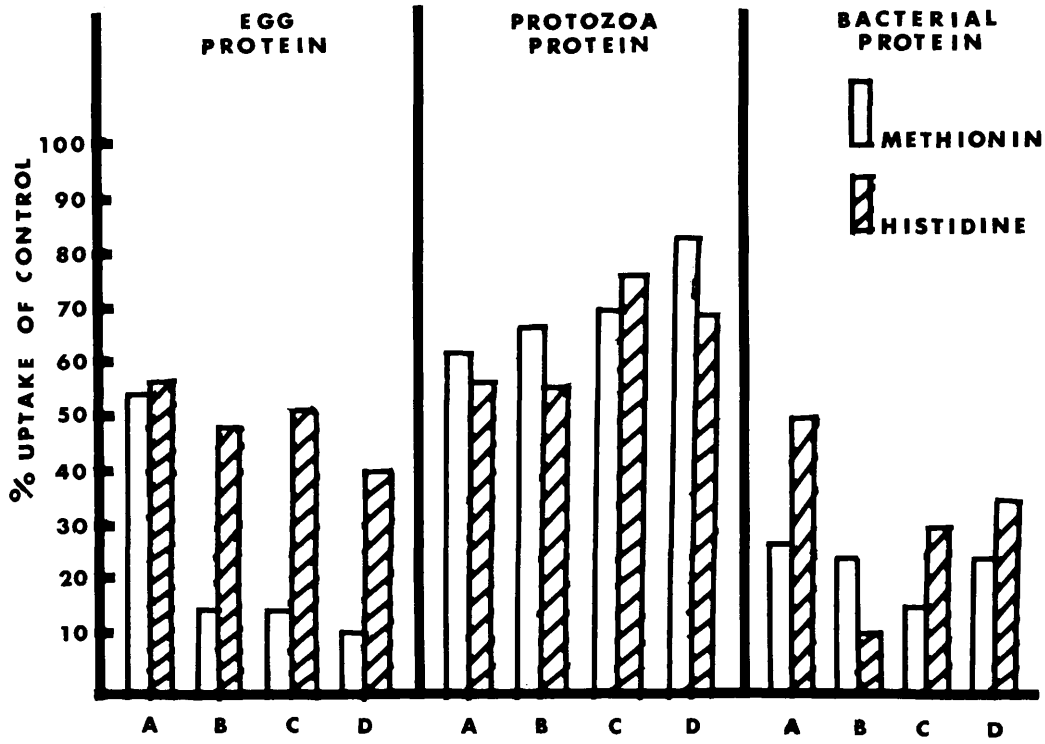

FIG. 2. Effect of amino acid patterns on methionine or histidine uptake into rat jejunal rings *in vitro*: (A) protein composition amino acid pattern; (B) 30-min pepsin amino acid liberation pattern; (C). 60-min pepsin amino acid liberation pattern; and (D) 150-min pepsin amino acid liberation pattern.

levels of significance of differences in histidine or methionine uptake depressions between the proteins' amino acid composition patterns and the three pepsin amino acid liberation patterns. Thus for egg protein, methionine uptake was significantly lower for E30, E60, and E150 ($p<.01$) respectively, and histidine uptake was significantly lower for E30, E60, and E150 ($p<.05$, $<.10$, $<.01$), respectively. With protozoal protein, the uptake of methionine was significantly higher ($p<.01$) in the presence of P150; histidine uptake was significantly lower ($p<.05$) with P30 but was significantly higher ($p<.05$) with P60. The experiments with bacterial protein showed significantly ($p<.01$) lower histidine uptake in the presence of each of the three pepsin amino acid liberation patterns.

Figure 3 shows the relationship between absolute histidine or methionine concentration in the incubation buffers and uptake depressions for the 4 amino acid patterns (Table I) for each of the three protein sources. Uptake by rat jejunal rings was related to absolute histidine or methionine concentra-

TABLE II. Levels of Significance in Amino Acid Uptake Depressions between Protein Amino Acid and Pepsin Amino Acid Liberation Patterns.

Pepsin amino acid liberation pattern	Probabilities for	
	Methionine	Histidine
E30[a]	<.01	<.05
E60	<.01	<.01
E150	<.01	<.01
P30	NS	<.05
P60	NS	<.05
P150	<.01	NS
B30	NS	<.01
B60	NS	<.01
B150	NS	<.01

[a] EC was compared with E30, E60, E150; PC was compared with P30, P60, P150; and BC was compared with B30, B60, B150.

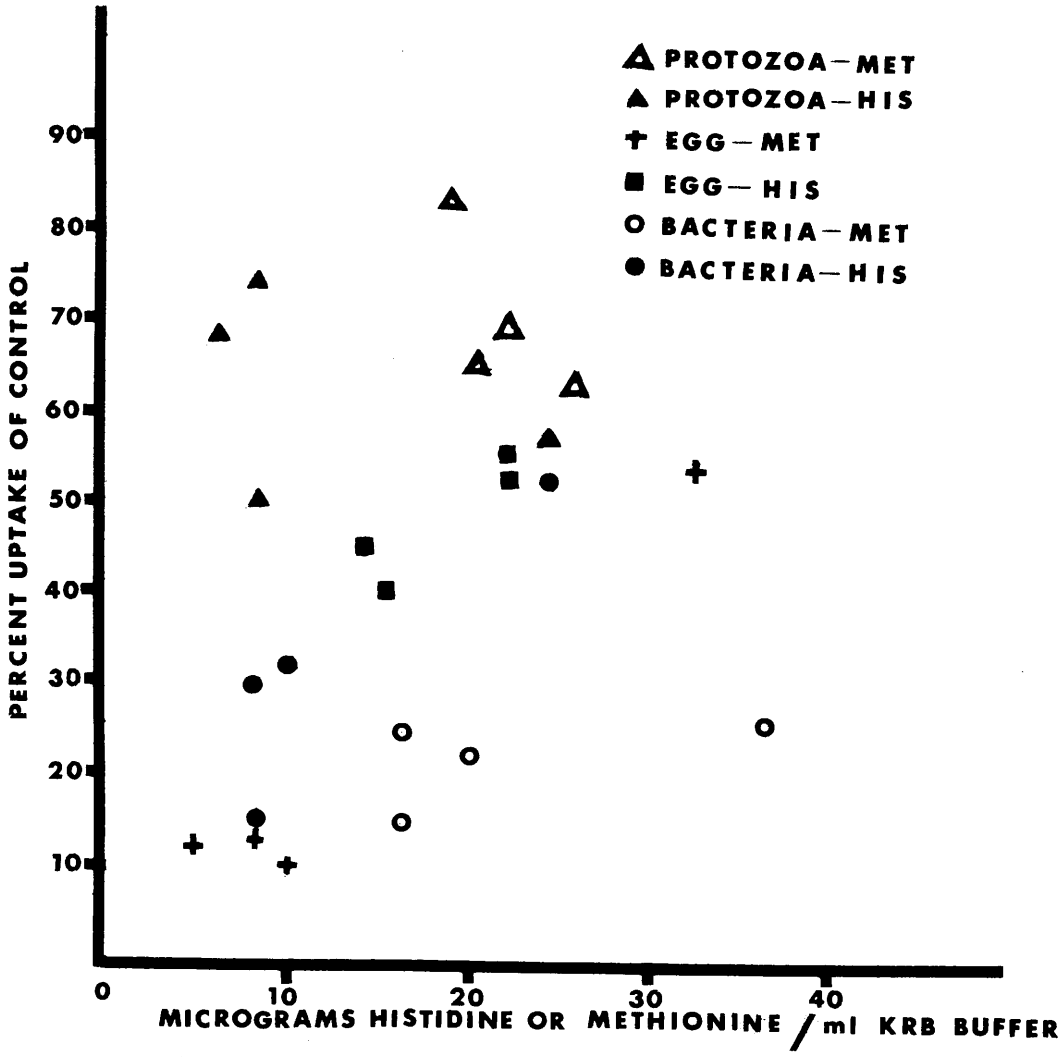

FIG. 3. Relationship between absolute histidine or methionine concentration in KRB buffer and uptake in the presence of amino acid patterns (4 observations/point).

tion but as methionine or histidine concentration increased in the buffer uptake for the egg and bacterial protein amino acid mixtures increased while uptake for the protozoal protein amino acid mixtures decreased.

Discussion. The purpose of this study was to determine if the pattern of amino acids liberated from proteins by pepsin modified histidine or methionine uptake by rat jejunal rings compared to uptake of these amino acids in the presence of the proteins' composition patterns. Sheffner *et al.* (9) and Bergen *et al.* (8) reported that the composition of amino acids mixtures liberated by *in vitro* proteolysis can differ from the amino acid composition of the proteins; similar differences were obtained with pepsin digestion of proteins in this study. Delhumeau *et al.* (5) suggested that amino acid uptake in the presence of amino acid mixtures liberated by enzymic degradation may differ from amino acid uptake in the presence of amino acid mixtures simulating dietary proteins. In agreement, the present data indicate that histidine or methionine uptake in the presence of pepsin amino acid liberation patterns differ from that with amino acid mixtures simulating the amino acid content of

the proteins used. The data in Fig. 3 suggest that uptake of histidine or methionine in the presence of the various amino acid mixtures was related to their concentrations in KRB buffer. *In vitro* (1) and *in vivo* (10) work demonstrated that gut transport of a single amino acid increased with concentration until entry sites were saturated. Therefore, with amino acid mixtures from egg or bacterial protein, uptake increased as histidine or methionine concentration increased. The opposite results were obtained with amino acid mixtures from protozoal protein. A number of amino acids have been implicated as competitors of methionine or histidine transport (1), but the degree of competition differs with amino acids. In the case of complete amino acid mixtures as used in this study the situation is extremely complex and incompletely understood. These divergent results may be explained by the following postulates. As histidine or methionine concentrations increased in the egg or bacterial amino acid mixtures, the effective level of transport competitors stayed constant and hence uptake increased. For protozoal protein, however, the ratio of effective transport competitors to histidine or methionine increased as the methionine or histidine levels increased in the KRB buffer, hence uptake of histidine or methionine were depressed.

Summary. The compositions of amino acids liberated by pepsin digestion from egg, rumen protozoal and rumen bacterial protein differed markedly from the amino acid composition of the three protein sources. Total histidine and methionine uptake by rat jejunal rings *in vitro* in the presence of amino acid mixtures simulating the pepsin liberation patterns differed from histidine and methionine uptake in the presence of amino acid mixtures simulating the three protein sources. The data suggested that *in vitro* histidine or methionine uptake was primarily dependent on the concentration of these amino acids in the incubation buffer but extent of uptake was modified by the amino acid patterns.

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