Protein Digestion in Human Intestine as Reflected in Luminal, Mucosal, and Plasma Amino Acid Concentrations after Meals

SIAMAK A. ADIBI and DONALD W. MERCER

From the Gastrointestinal and Nutrition Unit and the Laboratory of Clinical Chemistry of Montefiore Hospital, and the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

ABSTRACT Normal human volunteers were intubated with either aspiration tubes or a biopsy capsule placed in the small intestine. The subjects were then fed a test meal containing 50 g of purified bovine serum albumin which served as the model dietary protein. Electrophoretic analysis of intestinal fluids showed that for at least 4 h the fed albumin was detectable in jejunal and ileal fluids. On separate occasions, subjects were fed the same meal without the protein. No protein was detected in intestinal fluids when the protein-free meal was fed. After the protein-rich meal, total concentrations of measured free and peptide amino acids rose from 3.21 to 29.29, and 15.94 to 117.97 μmol/ml, respectively, (P values < 0.02) in the jejunum. Similarly, total concentrations of measured free and peptide amino acids rose from 5.45 to 19.74, and 13.59 to 65.39, respectively, (P values < 0.05) in the ileum. In contrast, concentrations of free and peptide amino acids in intestinal fluids did not increase after the protein-free meal. While intracellular concentrations of amino acids in the jejunal mucosa did not show significant changes, plasma concentrations of each individual free amino acid were increased after the protein-rich meal and were either decreased or unaltered after the protein-free meal. The amino acid composition of the fed protein was reflected in the increases in intraluminal and plasma concentrations of individual amino acids after the protein-rich meal. It is concluded that after the ingestion of a test meal containing a substantial amount of protein which is within the usual range of dietary intake: (*a*) the exogenous protein is the principal source of the increased free and peptide amino acids in the intraluminal contents and in the plasma; (*b*) there are greater amounts of amino acids present as small peptides than in the free form in the gut lumen; (*c*) the ingested protein can be recovered as late as 4 h both in the jejunum and in the ileum.

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INTRODUCTION

Digestion is the first step necessary for the proper utilization of dietary proteins. Our knowledge concerning the intestinal fate of proteins in man is limited to two published studies (1, 2). Therefore, many aspects of this physiological process still remain unexplored. In these two studies, protein digestion was investigated either by determining the disappearance of radioactivity from the gut lumen after the oral intake of a tracer dose of a labeled protein or by comparing the amino acid composition of intestinal contents with that of meals containing a small amount (15 g) of protein.

The present studies were performed to examine the phenomenon of proteolysis in human intestine after the ingestion of a test meal containing a substantial but physiological amount (50 g) of a purified protein (bovine serum albumin). Bovine serum albumin, easily distinguishable from other proteins by its distinct electrophoretic pattern, is not normally present in the human gastrointestinal tract and is water soluble. Therefore, the use of such a protein as a model dietary protein permitted us to follow the appearance and disappearance of the fed protein in the intestinal fluids after oral intake.

While digestion of the fed protein was being followed, changes in amino acid concentrations of jejunal and ileal contents were also measured at regular intervals. The

amino acid concentrations were fractionated into peptide and free forms to provide a profile of the products of protein digestion in vivo. Additional studies were carried out to examine the importance of exogenous vs. endogenous proteins by determining the concentrations of free and peptide amino acids in the gut lumen when bovine serum albumin was excluded from the test meal. Finally, studies were performed to monitor simultaneously the concentrations of free amino acids in the jejunal mucosal cells and peripheral plasma before and after food ingestion.

METHODS

Healthy male volunteers closely matched in weight (71–78 kg), height (172–181 cm), and age (19–24 yr old) were utilized. All the studies to be presented in this report were approved by the Committee for the Protection of Human Subjects in Experiments. Furthermore, a written consent was obtained from each of the volunteers or his parent.

All subjects were intubated with a double-lumen tube by the method previously described (3). After an overnight fast, one tube was positioned in the upper jejunum (110 cm away from the teeth) and the other tube was positioned in the upper ileum (200 cm away from the teeth). The position of the tube was checked in each person by a radiological method described previously (3). Subsequently, venous blood, and jejunal and ileal aspirates were obtained. Between 8 and 9 a.m., the subjects were fed a test meal containing 50 g of purified bovine serum albumin,[1] 120 g of cornstarch, 40 g of olive oil, 5 ml of lemon juice, 4.5 g of salt, and 500 ml of water (the protein-rich meal). The caloric content (1040 cal) and distribution (19% protein, 46% carbohydrate, 35% fat) of the test meal used in our study closely simulated the nutrient content of a meal that is ordinarily consumed in the United States (4). The customary daily caloric intake of our experimental subjects, who were physically very active, was in the range of 3000–4000 cal/day; a 1000-cal meal approximated a usual meal for these subjects.

At regular intervals, 30 min, 1, 2, 3, and 4 h after the meal, venous blood was collected, and jejunal and ileal contents were aspirated. As soon as an intestinal aspirate was obtained, it was divided into two portions. One portion was immediately frozen at −20°C for the protein analysis, and the other portion, which was used for amino acid analysis, was heated for 15 min at 95°C. The heating was done to permanently deactivate the proteolytic enzymes (5). Venous blood was collected in a heparinized test tube. The plasma was immediately separated from the cells by centrifugation (2000 rpm) in a refrigerated (4°C) centrifuge for 10 min. On separate occasions, instead of the protein-rich meal, the subjects were fed a protein-free meal, and similar studies were performed. The composition of the protein-free meal was identical to the protein-rich meal, except for its lack of bovine serum albumin. The pH of each test meal was adjusted to a near neutral pH by the addition of a small volume of NaOH. The osmolalities of the protein-rich and the protein-free meals were 304 and 296 mosmol/kg respectively. Both meals were ingested in a period of 3–5 min.

To determine the changes in the mucosal-cell concentrations of free amino acids after a protein-containing meal, the jejunum was biopsied with a Crosby capsule before or 1 h after the protein-rich meal. After the biopsy the mucosal tissue was immediately removed from the capsule. The tissue was blotted on a filter paper and frozen in liquid nitrogen. The interval from the time of biopsy to the time of freezing ranged from 50 to 100 s.

Protein analysis. Intestinal aspirates, after being thawed, were centrifuged in a refrigerated centrifuge at 10,000 *g* for 15 min. The protein content of the supernate was analyzed by electrophoresis on polyacrylamide gel using an analytical polyacrylamide vertical gel apparatus[2] by the method previously described by Davis (6). After electrophoresis, gels were stained for protein with naphthalene black and then destained to remove excess dye with an electrolytic destaining device.[3] The intensities of protein bands on polyacrylamide gel were measured by a densitometer[4] at 570-nm wavelength and at a scanning rate of 2.5 cm/min.

Amino acid analysis. Each heated intestinal sample was cooled to room temperature and then centrifuged at 2000 rpm for 10 min. The supernate was filtered through Whatman No. 1 filter paper. The protein content of the filtrate was removed by the addition of 6% sulfosalicylic acid and centrifugation at 2000 rpm for 10 min. The protein-free filtrate was divided into two fractions: one fraction was used for determination of free amino acid content by an ion-exchange chromatography technique (3), using an automated amino acid analyzer;[5] the other fraction was used for determination of amino acid concentrations in the peptide form. The peptides present in each sample were hydrolyzed to their free amino acid constituents for measurement by the ion-exchange chromatography technique. The concentration of amino acid in peptide form was the difference between the concentration of the same amino acid in the protein-free filtrate before and after hydrolysis. Peptide hydrolysis was achieved by adding 1 vol of the protein-free filtrate and 2 vol of 6 N HCl to a vacuumized test tube. The test tube was then heated for 24 h at 100°C. Preliminary studies indicated that except for cystine, methionine, and tryptophan, other free amino acids (aspartic acid, threonine, serine, proline, glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine) were 98%–100% recoverable after the acid hydrolysis treatment. Methionine, cystine, and tryptophan recoveries were 90%, 86%, and 0%, respectively. Asparagine and glutamine were recovered only as aspartic and glutamic acids, respectively, after the acid hydrolysis treatment.

The intracellular concentrations of free amino acids were calculated by determining the concentrations of free amino acids in the mucosal tissue and in plasma obtained at the same time from each individual. After being weighed, the mucosal tissue was transferred to a grinding tube and an appropriate volume of 6% sulfosalicylic acid (1 ml/100 mg of tissue) was added. The tissue was homogenized in a Potter-Elvehjem homogenizer, while the sample was kept chilled. The tissue supernate was obtained by centrifugation of the tissue homogenate for 10 min at 2,000 rpm in a refrigerated (4°C) centrifuge. The supernate was then stored at −20°C until the time for free amino acid analysis. The concentration of each free amino acid inside

[1] Sigma Chemical Company, St. Louis, Mo.

[2] Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N. J.

[3] Canalco, Inc., Rockville, Md.

[4] Gilford Instrument Laboratories, Inc., Oberlin, Ohio.

[5] Model 120 C amino acid analyzer, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.

the mucosal cells was calculated by the following formula:

$$\text{intracellular concn. of free amino acid } (\mu\text{mol/ml}) = \frac{\text{concn. in tissue } (\mu\text{mol/g}) - \text{concn. in plasma } (\mu\text{mol/ml}) \times \text{inulin space (ml/g)}}{\text{total tissue water (ml/g)} - \text{inulin space (ml/g)}}$$

Using a similar peroral biopsy procedure in normal human volunteers, Thier, Segal, Fox, Blair, and Rosenberg (7) previously determined the inulin space (15.5% of the wet tissue weight), as well as the total tissue water (84.4% of the wet tissue weight) of jejunal mucosa. We have used the above values for the calculations of intracellular concentrations. In previous publications from this laboratory, the necessary assumptions for determinations of free amino acid concentrations per whole tissue (8) or per cell (9) have been discussed. The concentrations of free amino acids in plasma were determined by the method previously described (10).

The paired *t* test was utilized for the statistical analysis of data (11). The statistical significance of a correlation between the amounts of individual amino acids in the fed protein and the changes in the amino acid concentrations of intraluminal contents and plasma was determined by the method of the Spearman rank correlation coefficient (12). Since glutamine and glutamic acid, and asparagine and aspartic acid could not be separated by the acid hydrolysis of bovine serum albumin, these amino acids were not included in the comparison of changes in free amino acid pools with the amino acid content of the fed protein.

RESULTS

Protein profile of intraluminal contents. Data regarding purity and electrophoretic pattern of the fed albumin in the test meal are presented in Fig. 1. This pattern was always compared with the pattern obtained in each intestinal aspirate (Fig. 1). Without exception, protein analysis of jejunal and ileal aspirates obtained before meals or during the 4 h after the protein-free meal failed to show any protein in each individual. In contrast, the

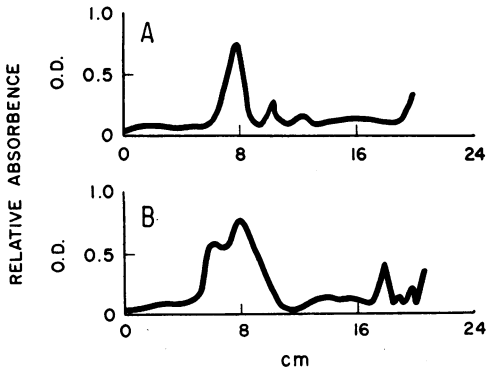

FIGURE 1 Densitometric scans of protein-stained polyacrylamide gels performed on 20 μg of bovine serum albumin (A) and on 20 μl of ileal aspirate (B) obtained 4 h after the protein-rich meal. The two minor peaks in (A) represent extraneous proteins. Migration is toward the anode from right to left.

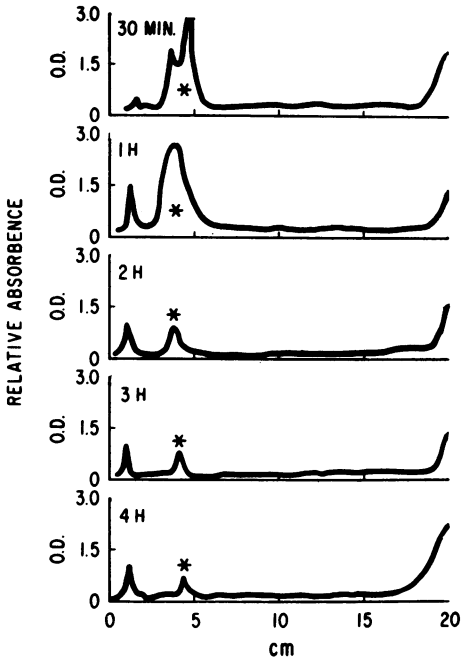

FIGURE 2 Densitometric scans of protein-stained polyacrylamide gels performed on jejunal aspirates obtained at regular intervals after the protein-rich meal in one subject. The patterns given in this Figure were also seen in the other subjects. The amount of aspirate used for each electrophoresis was 20 μl. The protein peaks, identified by an asterisk, correspond to the peak of the fed protein (bovine serum albumin). Migration is toward the anode from right to left.

intestinal aspirates after the protein-rich meal almost always contained protein. Typical examples of these studies are shown in Figs. 2 and 3. Within 30 min after the protein-rich meal, electrophoretic scans of the jejunal aspirates showed the presence of protein corresponding to the peak of the fed albumin. The amount of this protein in the jejunal content progressively decreased during the 4 h after the meal. The ileal aspirates showed no protein during the 1st h after the protein-rich meal; however, electrophoretic scans of the ileal aspirates obtained at the 2nd h revealed several protein bands, one of which could be identified as the fed protein (Fig. 3). The amount of this protein, which increased at the 3rd and 4th h, was usually considerably less than the amount detected in jejunal aspirates. The unidentified protein bands in the electrophoretic scans of jejunal and ileal aspirates after the protein-rich meal (Figs 2 and 3) were never found in any intestinal aspirate obtained before ingestion of meals or during the 4 h after the protein-free meal. The protein bands, therefore, probably represent partially digested forms of the fed albumin.

Intraluminal concentrations of free and peptide amino acids. Within 30 min after the ingestion of the protein-

rich meal, the jejunal concentrations of most amino acids both in free and peptide forms were increased, and they remained increased during the 4 h of the study period. Because of wide variations, there were no statistically significant differences in amino acid concentrations in intestinal aspirates obtained between 30 min and 4 h after the meal. Therefore, for economy of presentation, the concentration values are detailed only for one interval in Table I. At each examined interval, the increase was more marked for the amino acids in peptide than in free form; for example, at the 3rd h the average total concentration of free amino acid in the jejunum was increased by 26.08 μmol/ml while that of peptide amino acid was increased by 102.03 μmol/ml ($P < 0.02$).

The phenomena described in the jejunum were also observed in the ileum. However, the ileal concentrations of amino acids were not remarkably altered within the 1st h after the protein-rich meal but increased later. Furthermore, the differences between increases of amino acids in peptide and free forms were not as marked in the ileum as in the jejunum (Tables I and II). For example, in the ileum at the 3rd h, the average total concentration of free amino acid was increased by 14.29 μmol/ml while that of peptide amino acid was increased by 51.80 μmol/ml ($P < 0.05$).

Free amino acids such as lysine and leucine showed the greatest increases, and glycine, methionine, proline, and isoleucine exhibited the smallest increases in the free amino acid pool composition of the jejunum (Table I). With few exceptions, the same general relationship was also found for the increases in concentrations of peptide amino acids after the protein-rich meal (Table I). The notable exceptions were aspartic acid and glycine, which increased disproportionately to the amounts in the fed protein. The disproportionate increase in the concentration of glycine can be accounted for by considering that in addition to hydrolysis of peptides, the acid hydrolysis results in the release of glycine from the glycine-conjugated bile salts which enter the gut lumen in response to the meal. This explanation is supported by the finding of a high concentration of glycine relative to the other amino acids in the peptide fraction even when the test meal lacked protein (Table I).

Ileal aspirates after the protein-rich meal were characterized by markedly increased concentrations of free aspartic and free glutamic acids (Table II). Glycine and aspartic and glutamic acids contributed the most, and methionine and histidine contributed the least to the rise in amino acid concentrations of the peptide fraction of ileal aspirates.

Comparisons of the amino acid composition of bovine serum albumin with the increases in jejunal and ileal concentrations of peptide amino acids yielded significant correlations for most of the individual aspirates. Among

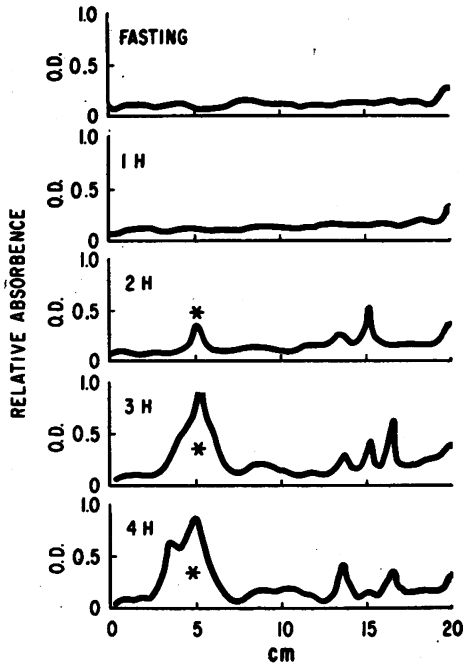

FIGURE 3 Densitometric scans of protein-stained polyacrylamide gels performed on ileal aspirates obtained at regular intervals after the protein-rich meal in one subject. The patterns given in this figure were also seen in the other subjects. The amount of aspirate used for each electrophoresis was 20 μl. The protein peaks, identified by an asterisk, correspond to the peak of the fed protein (bovine serum albumin). Migration is toward the anode from right to left.

16 jejunal and 14 ileal aspirates, the correlations were statistically significant for all except 3 and 6 aspirates respectively. The correlations were statistically more significant for jejunal (r values ranged between 0.79 and 0.62 with all P values < 0.01) than for ileal (r values ranged between 0.58 and 0.49 with all P values < 0.05) studies. Similar statistical evaluations for increases in the jejunal and ileal concentrations of free amino acids showed significant correlations for most of the jejunal aspirates (14 out of 18) but for few ileal aspirates (5 out of 15).

In contrast to the protein-rich meal, there were no statistically significant changes in concentrations of free and peptide amino acids, but there was a tendency for these amounts to decrease after the ingestion of the protein-free meal[6] at all the examined intervals. This finding was true in the jejunum as well as in the ileum (Tables I and II).

Free amino acids in plasma. The total concentrations of free amino acids before and at regular intervals after

[6] Amino acids analysis of the protein-free meals, after acid hydrolysis, revealed traces of amino acids. The total concentration of amino acid was 0.04 μmol/ml of the test meal.

TABLE I

Concentrations (μmol/ml) of Amino Acids in Jejunal Contents before and 3 h after Test Meals (Mean±SEM in Four Subjects)

Amino acids	Bovine serum albumin*	Free amino acids			Peptide amino acids		
		Before	After protein-rich meal	After protein-free meal	Before	After protein-rich meal	After protein-free meal
	μmol/g						
Aspartic acid	663.7	0.05±0.02	1.37±0.70	0.05±0.01	1.63±0.54	15.87±2.51‖	1.45±0.60
Threonine	430.0	0.12±0.03	1.14±0.23‖	0.12±0.04	1.00±0.31	8.10±2.32‡	0.63±0.30
Serine	358.0	0.10±0.02	1.05±0.21‖	0.12±0.04	1.17±0.45	5.74±1.44‡	0.96±0.34
Asparagine and glutamine		0.15±0.03	1.21±0.34§	0.17±0.06			
Proline	373.4	0.17±0.03	0.85±0.59	0.09±0.02	0.17±0.02	9.10±3.28‡	0.94±0.38
Glutamic acid	939.3	0.11±0.04	2.62±0.94‡	0.07±0.02	1.74±0.81	16.15±5.00‡	1.24±0.48
Glycine	232.6	0.21±0.05	0.74±0.17‖	0.20±0.04	3.96±1.77	13.96±1.52‖	3.86±1.80
Alanine	573.5	0.17±0.04	1.88±0.29‖	0.18±0.07	0.92±0.33	10.91±2.35‖	1.06±0.42
Valine	359.9	0.23±0.08	1.69±0.37‖	0.23±0.10	0.92±0.40	7.43±1.89§	0.71±0.28
Methionine	50.1	0.05±0.005	0.60±0.01‖	0.06±0.02	0.12±0.09	0.17±0.10	0.04
Isoleucine	130.5	0.08±0.03	0.89±0.09‖	0.17±0.08	0.63±0.28	2.40±0.58‡	0.44±0.18
Leucine	695.4	0.32±0.10	3.02±0.43‖	0.28±0.12	0.85±0.38	7.99±1.24‖	0.79±0.26
Tyrosine	251.0	0.27±0.09	1.69±0.07‖	0.21±0.09	0.28±0.18	0.94±0.50	0.19±0.09
Phenylalanine	349.8	0.23±0.07	1.76±0.20‖	0.18±0.08	0.54±0.18	2.84±1.16	0.38±0.14
Lysine	1072.6	0.65±0.17	5.63±0.86‖	0.29±0.22	1.18±0.42	12.09±4.02‡	0.40±0.23
Histidine	242.2	0.07±0.004	1.90±0.09‖	0.04±0.04	0.49±0.16	3.90±1.23‡	0.18±0.11
Arginine	367.0	0.23±0.07	2.06±0.11‖	0.20±0.16	0.34±0.17	0.38±0.12	0.22±0.13
Total amino acid		3.21±0.59	29.29±3.97‖	2.33±1.31	15.94±5.97	117.97±26.91§	13.51±5.30

* The amino acid composition of the protein in the test meal as determined by an acid hydrolysis method.
‡, §, ‖ *P* values <0.05, <0.02, and <0.01, respectively.

the protein-rich and the protein-free meals are compared in Fig. 4. The list of individual free amino acids included in this study is presented in Table III. The total concentrations of free amino acids significantly increased after the protein-rich meal (*P* values at 1st and 2nd h were < 0.01). In contrast to the protein-rich meal, the protein-free meal failed to elevate the levels of free amino acids in plasma. The mean ±SEM of the concentrations of individual free amino acids before and 1 h after meals are given in Table III. Lysine, alanine, leucine, and valine showed the greatest increases, and methionine, tryptophan, and glycine showed the smallest increases in the concentrations after the protein-rich meal. The plasma concentrations of individual free amino acids were either decreased or remained unaltered after the protein-free meal; however, statistical significance could be shown only for the decreases in leucine and isoleucine levels. Comparisons of the amino acid composition of the bovine serum albumin with the increases in the concentrations of free amino acids in all plasma specimens showed significant correlations in 12 of the 13 subjects studied (*r* values ranged from 0.70 to 0.96 with *P* values of < 0.01 in 10 subjects).

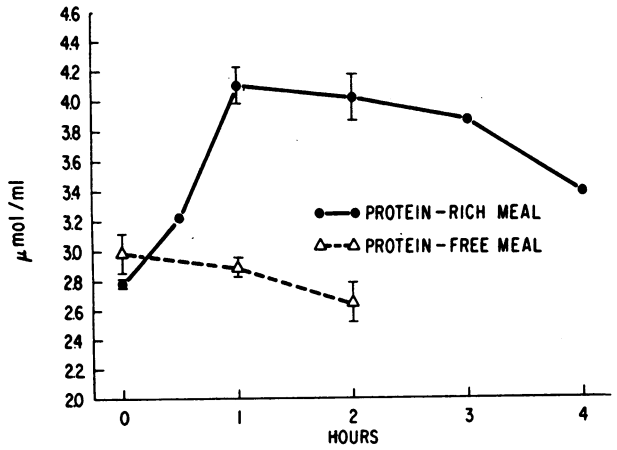

FIGURE 4 Total concentrations of free amino acids in plasma before and at regular intervals after the protein-rich and the protein-free meals. The list of free amino acids included in total concentration, as well as the number of subjects used for each study, is given in Table III.

Free amino acid levels in intestinal mucosa. The intracellular concentrations of free amino acids in jejunal mucosa before and after the protein-rich meal are summarized in Table IV. In both basal and postcibal states the intracellular concentration of each amino acid was

TABLE II

Concentrations (μmol/ml) of Amino Acids in Ileal Contents before and 3 h after Test Meals (Mean±SEM in Four Subjects)

Amino acids	Free amino acids			Peptide amino acids		
	Before	After protein-rich meal	After protein-free meal	Before	After protein-rich meal	After protein-free meal
Asparatic acid	0.11±0.03	3.34±3.18	0.33±0.25	1.26±0.25	10.23±3.65*	0.96±0.32
Threonine	0.16±0.06	1.09±0.69	0.16±0.03	1.22±0.19	3.72±0.41§	0.82±0.22
Serine	0.14±0.08	0.40±0.13	0.10±0.03	1.10±0.21	3.51±0.84*	1.14±0.44
Asparagine and glutamine	0.19±0.06	0.46±0.16	0.10±0.02			
Proline	0.36±0.06	0.54±0.22	0.15±0.03‡	0.95±0.22	3.06±0.65*	0.76±0.20
Glutamic acid	0.44±0.14	7.30±2.17‡	0.53±0.39	1.15±0.34	7.88±3.88	0.82±0.35
Glycine	0.86±0.26	0.99±0.31	0.38±0.08	2.68±0.33	15.39±5.40	1.19±0.32
Alanine	0.51±0.15	0.86±0.24	0.40±0.18	0.90±0.21	4.23±0.59§	0.88±0.35
Valine	0.58±0.16	0.58±0.13	0.25±0.07	0.70±0.08	3.34±0.46§	0.56±0.14
Methionine	0.06±0.01	0.29±0.11	0.04±0.01	0.12±0.02	0.57±0.37	0.06±0.04
Isoleucine	0.32±0.11	0.38±0.10	0.14±0.03	0.70±0.32	1.73±0.24*	0.37±0.13
Leucine	0.57±0.18	0.98±0.38	0.31±0.08	0.78±0.29	4.06±0.89‡	0.76±0.23
Tyrosine	0.26±0.07	0.34±0.07	0.14±0.04	0.27±0.02	1.03±0.28*	0.17±0.08
Phenylalanine	0.30±0.08	0.36±0.08	0.16±0.05	0.35±0.06	1.65±0.33§	0.29±0.13
Lysine	0.21±0.11	1.15±0.25‡	0.26±0.09	0.64±0.03	2.70±0.26§	0.38±0.08*
Histidine	0.14±0.03	0.28±0.09	0.06±0.02	0.45±0.22	1.03±0.13	0.14±0.03
Arginine	0.24±0.11	0.40±0.04	0.10±0.03	0.32±0.04	1.26±0.12§	0.15±0.03‡
Total amino acid	5.45±1.13	19.74±6.60*	3.63±1.28	13.59±2.10	65.39±15.81‡	9.45±2.91

*, ‡, § P values <0.05, <0.02, and <0.01, respectively.

considerably greater than the concentration of the same amino acid in plasma (Table III). Glutamate, aspartate, alanine, and glycine exhibited the highest concentrations among the examined free amino acids. Although the mean values of individual free amino acid concentrations tended to be slightly increased after the protein-rich meal, the differences were not statistically significant. Comparison of the concentration of each individual free amino acid (Table IV) with the concentration of the same free amino acid in the lumen (Table I) revealed that, except for a few amino acids, the intraluminal concentrations were greater than the intracellular concentrations. The serine concentrations were similar, but the intracellular concentrations of glutamate, aspartate, alanine, and glycine were greater than the intraluminal concentrations of these free amino acids. Therefore, except for a few of the examined amino acids, the in vivo transport of free amino acids across the microvillous membrane does not appear to be achieved against any noticeable concentration gradient. It is pertinent to note that the rises in luminal concentrations of free amino acids after the meal were modest. In situations when the intestinal mucosa is presented with higher concentrations of free amino acids (3, 5, 13–15), there may be changes in intracellular concentrations.

DISCUSSION

Previous studies have suggested that there is mixing of exogenous and endogenous proteins in the gut lumen. Nasset maintained that there is a several-fold dilution of dietary proteins with endogenous proteins (16). Nixon and Mawer estimated that after protein meals as high as 53% of the intraluminal proteins may have their origin from endogenous sources (2). In contrast, our protein electrophoretic patterns of intestinal fluids failed to show any protein when the meal lacked protein. It is possible that endogenous proteins are either water insoluble or present in such limited amounts (less than 0.002 mg) that they are not detected by the method used in the present investigation. Nevertheless, the proteins should express their presence in the gut lumen by contributing to the products of protein catabolism, such as free amino acids and peptides. When protein was excluded from the meal, the free and peptide amino acid content of jejunal fluids did not increase. Since the ileum has been considered the intestinal site where the catabolism of endogenous protein may occur (17), ileal aspirates were also examined for the products of protein digestion. Again, there was no increase in free and peptide amino acid content of ileal fluids when the meal lacked protein.

TABLE III

Concentrations (μmol/ml) of Free Amino Acids in Plasma before and after Either the Protein-Rich or the Protein-Free Meal (Mean±SEM)

Amino acids	Protein-rich (*n*-13)*			Protein-free (*n*-4)*		
	Before	1 h after	Changes‡	Before	1 h after	Changes‡
Threonine	0.136±0.006	0.190±0.009¶	+0.054	0.121±0.008	0.120±0.008	−0.001
Serine	0.121±0.005	0.168±0.005¶	+0.047	0.115±0.005	0.121±0.007	+0.006
Asparagine and glutamine	0.658±0.026	0.753±0.028§	+0.095	0.746±0.035	0.762±0.036	+0.016
Proline	0.200±0.016	0.254±0.012‖	+0.054	0.290±0.037	0.297±0.042	+0.007
Glutamic acid	0.041±0.006	0.058±0.005¶	+0.017	0.038±0.001	0.042±0.006	+0.004
Glycine	0.227±0.007	0.245±0.005§	+0.018	0.206±0.006	0.203±0.003	−0.003
Alanine	0.364±0.020	0.508±0.021¶	+0.144	0.370±0.045	0.360±0.034	−0.010
Valine	0.238±0.007	0.364±0.016¶	+0.126	0.235±0.016	0.220±0.022	−0.015
Methionine	0.027±0.001	0.030±0.001§	+0.003	0.024±0.001	0.021±0.001	−0.003
Isoleucine	0.076±0.003	0.101±0.006¶	+0.025	0.071±0.004	0.060±0.006§	−0.011
Leucine	0.138±0.004	0.275±0.012¶	+0.137	0.141±0.001	0.123±0.006§	−0.018
Tyrosine	0.071±0.003	0.112±0.005¶	+0.041	0.066±0.004	0.064±0.002	−0.002
Phenylalanine	0.064±0.002	0.097±0.004¶	+0.033	0.059±0.002	0.056±0.002	−0.003
Lysine	0.231±0.011	0.566±0.108§	+0.335	0.231±0.020	0.224±0.018	−0.007
Histidine	0.100±0.010	0.154±0.016§	+0.054	0.103±0.006	0.087±0.004	−0.016
Tryptophan	0.050±0.010	0.052	+0.002	0.052±0.014	0.046±0.006	−0.006
Arginine	0.126±0.016	0.248±0.016¶	+0.122	0.122±0.018	0.112±0.013	−0.010
Total amino acid	2.79±0.03	4.11±0.12¶		2.99±0.13	2.90±0.07	

* Number of subjects used; ‡ positive values indicate increases and negative values indicate decreases in plasma levels.
§, ‖, ¶ *P* values <0.05, <0.02 and <0.01, respectively.

In the present studies, changes in the intraluminal amino acid contents were determined only in the upper jejunum and upper ileum. Therefore, the possibility existed that there may be other sites in the intestine where the intraluminal fluids may show a noticeable rise in their amino acid content after the intake of the protein-free meal. The studies of changes in plasma amino acid levels after meals were, therefore, helpful in eliminating this possibility. Our data clearly show that while there is a rise in plasma levels of all measured individual free amino acids after the protein-rich meal, plasma levels of the same free amino acids are either lowered or unaltered after the protein-free meal (Table III). The plasma studies fail to reveal any evidence of the influx of free amino acids from the gut into the periphery when protein is excluded from the meal.

TABLE IV

Intracellular Concentrations (μmol/ml) of Free Amino Acids in Jejunal Mucosal Cells before and 1 h after the Protein-Rich Meal (Mean ± SEM in Four Subjects)

Amino acids	Before	After
Aspartic Acid	4.00±0.89	3.35±1.57
Threonine	0.72±0.06	0.76±0.15
Serine	0.96±0.11	1.07±0.32
Asparagine and glutamine	1.10±0.19	1.31±0.31
Glutamic acid	5.28±0.34	6.90±0.89
Glycine	1.85±0.25	2.17±0.44
Alanine	1.68±0.13	3.17±0.84
Valine	1.02±0.18	0.92±0.25
Methionine	0.20±0.04	0.12±0.04
Isoleucine	0.38±0.06	0.43±0.07
Leucine	1.09±0.30	1.39±0.29
Tyrosine	0.32±0.06	0.55±0.12
Phenylalanine	0.34±0.06	0.56±0.13

Our studies support the contention that dietary and not endogenous proteins serve as a principal source of added amino acids in the gut contents or plasma during the course of digestion. Statistically significant correlations were obtained when the increases in intraluminal concentrations of either free or peptide amino acids were compared with the amounts of the corresponding amino acids in the ingested protein. The magnitude of increases in concentrations of amino acids, however, did not exactly mirror the relative amounts of the same individual amino acids in the fed protein. The absence of an exact correlation is not surprising, because of the differences in absorption rates of individual amino acids and peptides (3, 5, 13–15), the interaction between the amino acids when they are presented to intestinal mucosa (3, 13), and the differences in rates of release of amino acids

from the protein undergoing enzymatic hydrolysis (18). The small amounts of proteins used by other investigators may have been insufficient to significantly alter the intraluminal concentrations of free amino acids (18, 19).

A relationship between the magnitude of increases in plasma levels of amino acids and the amounts of amino acids in the fed protein was also observed. In view of the complexity of transport and metabolic steps interposed in the process of amino acid movement from the gut into the periphery, the absence of a precise relationship is understandable. Nevertheless, the amino acids that were most or least abundant in the fed protein exhibited the greatest and smallest rises in their plasma levels, respectively.

One unexpected finding was that, within the range of usual protein intake, the digestion of ingested protein extends over a long period (longer than 4 h) and distance (more distal than the upper ileum). The previous studies have concluded that protein digestion is quite rapid (20) and is completed in the upper jejunum (1). The differences in the design and methodology of the experiments may account for the varied conclusions; for example, the intraluminal fate of ingested protein was not directly examined in the previous studies. Furthermore, in addition to differences in the protein content, the test meal used in the present study contained considerably more fat and carbohydrate than the ones previously used. In fact, in one study no fat was included in the test meal (20). It is well known that dietary lipids influence a wide variety of gastrointestinal functions, including inhibition of gastric emptying (21). Therefore, a slow rate of gastric release may have allowed the longer survival of the fed protein in the intestine of our subjects.

Previous studies have emphasized the changes in intraluminal concentrations of free amino acids (16, 18, 19). The present data show that there are more amino acids in peptide than in free form in the luminal contents after a protein meal. Our investigations of the sizes of peptides which are fragments of the intraluminal proteolysis are not yet complete. Our preliminary evidence indicates that they are mostly small peptides. This information is based on the visual inspection of the chromatograms obtained after the ion-exchange chromatographic analyses of unhydrolyzed intestinal aspirates. Many unidentified peaks resembling di-, tri-, and tetrapeptides are seen on the chromatograms. In addition, larger oligopeptides are generally poorly water-soluble and would not accumulate in the peptide fractions of the present experiments.

Of the oligopeptides, only the intestinal fate of dipeptides has been examined in man (5, 22). Matthews and others, however, have investigated the intestinal transport of several species of oligopeptides in the intestine of experimental animals (23). Studies in our own laboratory have established that the human intestine could absorb dipeptides before their hydrolysis by intraluminal or membrane-bound enzymes (5). Furthermore, the rate of absorption of an amino acid is more rapid when it is linked to another amino acid than when it is free. Therefore, rapid peptide absorption may be responsible for the lack of a dramatic rise in the intraluminal concentration of free amino acids after a protein meal.

In conclusion, the present studies provide a qualitative description of the phenomena of protein digestion which differs in several aspects from prevailing concepts. Based on the information gained in the present studies, additional investigations are currently in progress to quantitate the in vivo digestion of dietary protein in the human intestine.

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