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The digestion and absorption of protein in man

2. The form in which digested protein is absorbed

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1. Intestinal contents, collected from the human jejunum after a test meal (milk-protein, gelatin or low-protein) were fractionated by centrifugation and gel filtration on G-75 Sephadex. The fractions were hydrolysed and the proportion of the total amino acid in each fraction was determined. The amino acids were measured with an EEL Amino Acid Analyser.

2. The free amino acid concentrations were determined in samples of the contents of the small intestine collected from various levels after the three types of test meal.

3. Intestinal contents collected from two levels of the jejunum after a milk-protein meal, were incubated in vitro at 37° for periods up to 80 min and the rates of release of the individual free amino acids were determined.

4. There was a rapid breakdown of the proteins of the test meals to fragments of molecular weight under 5000. The further breakdown (during incubation in vitro) to free amino acids was sufficiently rapid to account for the absorption in the free form of arginine, lysine, tyrosine, valine, phenylalanine, methionine and leucine. It was not rapid enough to account for the absorption of glycine, threonine, serine, the imino acids or the dicarboxylic amino acids in the free form.

5. The free amino acid concentrations in the intestinal lumen bore very little relationship to the concentrations in hydrolysates of the test meals or to those in hydrolysates of the intestinal contents. Many of the free amino acids in the intestinal samples were present in approximately equimolar concentrations.

6. It is suggested that experiments in which amino acid mixtures, simulating a dietary protein, are fed to experimental animals to determine the rates of amino acid absorption do not present a true picture of the events in the small intestine following the ingestion of a protein meal.

Digestion is not a rate-limiting step in the overall process of digestion and absorption of protein (Dent & Schilling, 1949; Crane & Neuberger, 1960). Because of this, Adibi, Gray & Menden (1966), when studying absorption in man, used amino acid mixtures instead of whole protein. They found that, when amino acid mixtures simulating protein hydrolysates were perfused through the human jejunum, the rates of amino acid absorption depended on the concentrations and characteristic absorption rates of the individual amino acids. Glutamic acid and aspartic acid were absorbed slowly; leucine and methionine were absorbed rapidly. The amount of glutamate and aspartate which was absorbed was less than that of other amino acids although glutamate and aspartate constituted a larger proportion of the mixture of amino acids perfused.

In a previous paper (Nixon & Mawer, 1970) we have reported the results of a series of experiments in which human volunteers were fed protein-containing test meals (milk protein and gelatin) and net absorption of the amino acid constituents (the differences between the amino acid concentrations in the gut and in the test meal,

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related to polyethylene glycol) was determined at various levels of the small intestine. It was found that by a level of 230 cm from the nose about 75% net absorption of each of the amino acids of the milk-protein meal had occurred; absorption appeared to occur at rates proportional only to the concentrations in the meal. Glutamic acid was the principal component (about 18%) of this meal. In the course of this work, intestinal samples which were collected after the ingestion of the test meals were fractionated by precipitation and gel filtration techniques and the individual fractions were analysed for their amino acid content, with an EEL Automatic Amino Acid Analyser, in an attempt to answer the following questions: (1) what is the rate of release of free amino acids in intestinal contents after a protein-containing test meal? and (2) do the amino acids of a protein meal leave the intestinal lumen in the free form?

EXPERIMENTAL

Details of the six human experimental subjects, the intubation technique and the composition of the test meals (milk-protein, gelatin and a low-protein meal) have been given previously (Nixon & Mawer, 1970).

Intestinal samples

The samples of intestinal contents to be fractionated by centrifugation and gel filtration were frozen immediately after collection and were stored at $-25°$ until the analysis was to be done. 5 ml samples were taken from the region 100–190 cm from the nose for fractionation.

After the milk-protein meal five samples were taken during the period 15 min to 1·5 h after the ingestion of the meal, from a level of 110 cm. Although there were variations in the total concentrations of amino acids, the distribution of the various components showed no significant differences with time of collection, and the results of each fractionation experiment were therefore combined.

Three samples taken after the gelatin meal were fractionated; two were from a level of 110 cm and one was from a level of 190 cm. They were collected between 1 and 1·5 h after the ingestion of the meal. No differences were found between the samples from the two levels and the results of each experiment were combined.

Two samples taken from a level of 100 cm 1 h after the ingestion of the low-protein meal were fractionated.

Samples (5 ml) for free amino acid determination were collected as soon as intestinal contents began to siphon after the ingestion of the meal (5–10 min after the ingestion of the meal, from the upper intestinal levels, and 10–20 min, from the lower levels) and again, 1 and 2 h after the ingestion of the meal. The preliminary experiments showed that the free amino acid concentrations in each subject varied little with time of collection and therefore the three samples collected in each experiment were pooled. Immediately after collection, the 5 ml samples were put into 25·0 ml of 1% (w/v) picric acid.

Free amino acids were determined in twelve experiments after the milk-protein meal, at intestinal levels ranging from the proximal part of the duodenum to the

lower jejunum (80–200 cm from the nose), in four experiments after the gelatin meal at levels ranging from 110 to 190 cm and in four experiments after the low-protein meal at levels ranging from 110 to 240 cm.

Fractionation of intestinal contents and protein-containing test meals by centrifugation and gel filtration

G-75 Sephadex was soaked in 0·1% (w/v) NaCl for 24 h. After removal of fines it was made up into a column, 85 cm by 1·5 cm and equilibrated at 4° with ammonium acetate buffer, pH 5·6, ionic strength 0·08. The column was developed with the same buffer; a pressure head of 10–20 cm gave a flow rate of 15 ml/h. Ammonium acetate buffer was chosen so that the electrolyte could be removed later by freeze-drying. The mean pH of the intestinal samples used in these experiments was 5·6. The column was calibrated with Blue Dextran (molecular weight, 2000000), pepsin (molecular weight, 36000; Dixon & Webb, 1964), trypsin (molecular weight, 23800; Dixon & Webb, 1964) and a mixture of free amino acids. The positions of the protein and aromatic amino acid peaks were determined by their extinction at 280 nm. Except where stated the whole of the fractionation procedure was carried out at 4° using a cold room and a refrigerated centrifuge.

Fractionation of the dried milk powder

Humanized Trufood (Trufood Ltd) (700 mg) was suspended in ammonium acetate buffer to give a final volume of 10·0 ml; 5·0 ml were centrifuged for 30 min at 23600 *g*. The supernatant fraction was filtered through Whatman no. 5 filter-paper which was then washed twice with 1 ml portions of buffer. The filtrate with washings was concentrated by rotary evaporation to about 3 ml which was then loaded on to the Sephadex column; 3·5 ml fractions were collected and their extinction at 280 nm was determined.

Fractionation of gelatin

A solution of gelatin (10·0 ml/mg) was made up in ammonium acetate buffer and 4·0 ml was applied to the column at room temperature (at 4° the solution set rapidly to a stiff jelly on the surface of the gel). The fractions were collected and the E_{280} was determined as before.

Fractionation of the intestinal samples

Portions (5·0 ml) of intestinal contents collected after a protein meal were frozen immediately in polyethylene tubes and kept at −25° until the fractionation was to be done. It was then allowed to thaw at 4° and centrifuged at 23600 *g* for 30 min. The supernatant fraction was decanted and the residue washed with 1 ml of the acetate buffer. It was then recentrifuged and the washing added to the supernatant fraction. The washing was repeated once.

After centrifugation the supernatant fraction and washings were combined and dialysed against 100 ml of the ammonium acetate buffer for 24 h. The contents of the dialysis sac were filtered through Whatman no. 5 filter-paper which was then washed twice with 1 ml of buffer. The filtrate and dialysate were recombined and freeze-

dried. The residue was dissolved in the buffer and then applied to the column. Intestinal samples taken after the low-protein meal were treated in a similar fashion but, because of the lower protein concentrations in these samples, 20·0 ml portions were fractionated. The samples were applied in volumes of approximately 4 ml as a layer between the surface of the gel and the buffer above the column, by means of a fine catheter attached to a 10 ml syringe.

Gel filtration on G-25 Sephadex

In some experiments, using intestinal contents after the milk-protein meal, Fraction IV (Fig. 3) was subjected to further filtration on G-25 Sephadex. A column, 45 cm by 1·9 cm, was made up and equilibrated with the ammonium acetate buffer. The totally excluded volume was measured with Blue Dextran and the elution positions of tyrosine, phenylalanine and tryptophan were noted.

Fraction IV was freeze-dried and redissolved in ammonium acetate buffer. After application of the sample to the column, fractions were eluted and read at 280 nm as before.

A 5 ml sample of intestinal contents, after precipitation of protein by picric acid, was treated in a similar fashion.

Hydrolysis of the fractions

The eluate from the column of G-75 Sephadex after filtration of the soluble component of the intestinal contents, could be conveniently divided into three fractions on the basis of the extinction at 280 nm (Fig. 3).

The 3·5 ml portions of each fraction were combined, with washings, into a 500 ml flask. They were freeze-dried and the residues were dissolved in 6 N-HCl containing $SnCl_2$, 1·5 mg/ml. Suitable concentrations were obtained when Fractions II and III were made up to 10·0 and 4·0 ml portions were hydrolysed. Fraction IV was made up to 20·0 ml and 5·0 ml portions were hydrolysed.

The residue after centrifugation of the original sample (Fraction I) was suspended in 6 N-HCl, with $SnCl_2$, to give a final volume of 10·0 ml, and 4·0 ml portions were hydrolysed.

The filter-paper, used for filtering the intestinal samples after dialysis, was washed with 6 N-HCl, with $SnCl_2$, to give a final volume of washings of 10·0 and 4·0 ml portions were hydrolysed. The amino acid content of this fraction was found to be very small and the concentration values were added to those of Fraction I.

Hydrolysis was carried out in sealed ampoules under an atmosphere of nitrogen for a period of 18 h at a temperature of 115°.

A separate portion of intestinal contents, prepared for the determination of the free amino acid concentrations by picric acid precipitation of protein, was subjected to acid hydrolysis in the same way as Fraction IV.

Determination of the free amino acid concentrations

The free amino acid concentrations in intestinal contents were determined by the method of Moore & Stein (1954).

After removal of the samples for hydrolysis from Fraction IV, 4·0 ml were freeze-dried directly and the residue was dissolved in 10·0 ml of 0·1 N-HCl for the determination of the free amino acid concentrations in this fraction.

Incubation of fresh intestinal contents

In two experiments, intestinal contents, (*a*) from a level of 110 cm from the nose and (*b*) from a level of 170 cm from the nose, were collected for 2 consecutive hours into polyethylene bottles embedded in crushed ice. The sample which was collected during the 2nd hour was mixed by swirling gently and 5·0 ml portions were transferred to 35 ml stoppered centrifuge tubes. These were fitted into a mechanical shaker and placed in a water bath at 37°. At intervals of 0, 5, 10, 15, 20, 40 and 80 min, 25·0 ml of 1% (w/v) picric acid was added to successive tubes in duplicate and the samples were subsequently treated as for the free amino acids.

RESULTS

Fractions of the intestinal contents and the test meals

Fraction I. Fraction I was the sediment at 23 600 *g*. The microscopic examination of haematoxylin and eosin stained sections showed no evidence of intact mucosal cells in this insoluble fraction of intestinal contents; 50% of the total amino acid of the milk-protein test meal was in this fraction but none of the gelatin was insoluble (Fig. 1).

Constituents containing amino acids were found in this fraction of all the intestinal samples. The mean proportions of the total amino acid content of the intestinal samples varied from 8% after the gelatin meal to 16% after the other two meals. (The total amino acid content in the samples was derived from acid hydrolysis of the unfractionated material.) The total concentrations of amino acids varied from 2·5 μmoles/ml after the low-protein meal to 8·5 μmoles/ml after the milk-protein meal (Fig. 2).

Fraction II. Fraction II was that fraction of the soluble material which was totally excluded from G-75 Sephadex and which was eluted in the volume from 45 to 65 ml (Fig. 3).

Although only 7% of the amino acids of the milk-protein meal and as much as 94% of the amino acids of the gelatin meal were contained in this fraction, in jejunal contents after the three meals the proportion of the total amino acid varied only from 7 to 17% (Fig. 1). The total concentrations of the amino acids varied from 2·5 μmoles/ml after the low-protein meal to 5·5 μmoles/ml after the gelatin meal (Fig. 2).

Fraction III. Fraction III was the fraction which was eluted from the column in the volume from 65 to 110 ml. The reference proteins, pepsin and trypsin, were eluted in this region of the chromatogram (Fig. 3); 20% of the amino acid-containing components of the milk-protein meal and 2% of the components of gelatin were in this fraction.

In jejunal samples after the three meals the proportion of the total amino acid varied from 7% after the milk-protein meal to 18% after the gelatin meal. The total

concentrations of amino acids varied from 2 μmoles/ml after the low-protein meal to 13 μmoles/ml after the gelatin meal.

Fraction IV. Fraction IV was that fraction which was eluted from the column in the volume from 110 to 180 ml. It included that region of the chromatogram in which a standard solution of an amino acid mixture was eluted (Fig. 3).

Only 2% of the total amino acid of the protein test meals was eluted in this region whereas 35–57% of the total amino acid content of jejunal samples after all three meals was contained in this fraction (Fig. 1). The total concentrations of amino acids varied from 6·5 to 41·5 μmoles/ml of intestinal contents (Fig. 2).

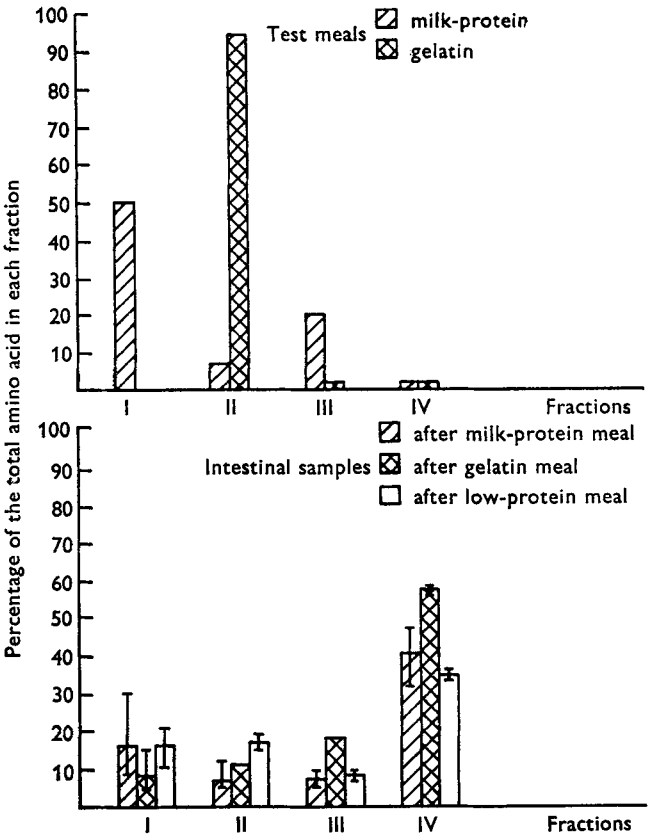

Fig. 1. Proportions of the total amino acid contained in each of the four fractions of the protein-containing test meals and of the intestinal samples from young adults after the test meals. The results are the means of five experiments after the milk-protein meal, of three experiments after the gelatin meal and of two experiments after the low-protein meal. The vertical lines represent the ranges.

Comparison between the test meals and the fractions of the intestinal contents

A rapid breakdown of the large molecules of the protein of the test meals had occurred in the lumen of the small intestine. By 15 min after the ingestion of the test meal, the distribution of the various components in the intestinal samples was similar to that found in samples collected up to 2 h later.

The breakdown was particularly noticeable with gelatin where the bizarre amino acid pattern allowed the processes of digestion to be studied more easily. The relative molar concentrations in the fractions indicate the qualitative changes which had occurred. The molar concentrations of the amino acids relative to leucine in the four fractions show that the whole of the gelatin components in the intestinal contents was contained in Fractions III and IV (Table 1); 75% of the hydroxyproline content of the intestinal samples after the gelatin meal was contained in Fraction IV and the remaining 25% in Fraction III.

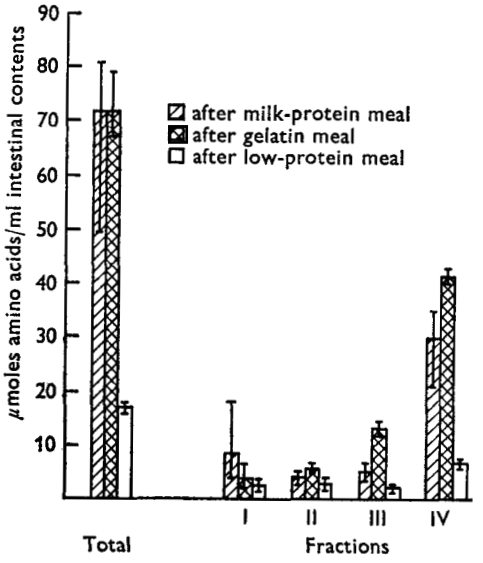

Fig. 2

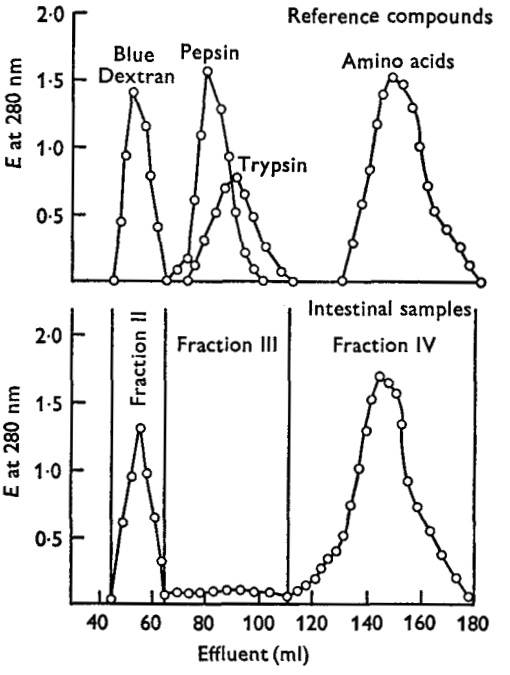

Fig. 3

Fig. 2. Concentrations (μmoles/ml) of the amino acids in hydrolysates of the unfractionated intestinal samples from young adults and of the four fractions of the samples after the three test meals. The results are the means of five experiments after the milk-protein meal, of three experiments after the gelatin meal and of two experiments after the low-protein meal. The vertical lines represent the ranges.

Fig. 3. The three arbitrary fractions of intestinal contents based on the extinction at 280 nm when the soluble component from 5 ml of intestinal contents from young adults was fractionated by gel filtration on a column 85 cm by 1·5 cm of G-75 Sephadex. (Fraction I was the insoluble component of the intestinal contents.) The upper part of the figure shows the elution positions of the reference compounds from G-75 Sephadex.

Even after the low-protein meal approximately one-third of the total amino acid content of the intestinal samples was in the form of amino acids or relatively small peptides.

Further investigation of Fraction IV

To exclude the possibility that Fraction IV was a result of continued enzyme activity during the gel filtration procedure, the amino acid content of this fraction, before and after hydrolysis, was compared with that of intestinal samples after pre-

cipitation of protein with picric acid (the 'picric acid supernatant') immediately on collection (as in the procedure for determination of free amino acids) .The percentages of the total of each amino acid in the unfractionated intestinal samples, contained in each of these two fractions are shown in Table 2. With the exception of glycine the percentages are very similar for both fractions. The lower glycine figure in the 'picric acid supernatant' may have been due to loss by adsorption of bile salts on to Dowex 2 during the removal of excess picric acid, taurine, a prominent component of hydrolysates of Fraction IV, was absent from hydrolysates of the 'picric acid supernatant'.

Table 1. *Molar concentrations relative to leucine (leu = 1) of the amino acids in hydrolysates of the fractions of the intestinal contents compared with those in the test meals*

Amino acid	Fraction I			Fraction II			Fraction III			Fraction IV			Test Meals		
	MP	LP	G	MP	LP	G	MP	LP	G	MP	LP	G	MP	LP	G
Asp	1·1	1·7	1·6	1·3	1·4	1·3	1·3	1·5	2·0	1·3	1·5	2·0	0·93	0·67	1·8
Thr	0·75	0·94	0·90	0·88	1·0	1·2	1·3	0·70	1·0	0·78	0·71	0·81	0·68	0·61	0·72
Ser	1·0	1·1	1·3	1·0	1·1	1·0	1·1	1·3	1·7	0·88	0·88	1·5	0·72	0·78	1·4
Pro	0·89	0·89	1·6	0·76	0·93	1·7	1·2	0·90	6·7	0·93	0·71	5·0	0·85	0·81	4·4
Glu	1·5	1·2	1·6	1·2	1·1	1·3	2·3	1·1	3·6	2·3	1·7	3·5	1·8	0·97	2·8
Gly	1·0	2·8	5·0	1·0	1·3	1·9	0·94	4·3	14·1	2·0	7·9	12·8	0·39	0·55	12·8
Ala	0·78	1·0	1·5	1·0	1·0	1·2	0·89	0·90	4·3	0·76	1·1	4·5	0·64	0·48	4·4
Val	0·76	1·1	1·0	0·94	1·1	1·0	1·1	1·0	1·1	0·78	0·88	0·85	0·70	0·84	0·94
Met	0·11	0·12	0·12	0·06	0·05	0·05	0·11	0·10	0·10	0·16	0·18	0·21	0·19	0·19	0·04
Ileu	0·52	0·65	0·64	0·50	0·57	0·57	0·63	0·65	0·55	0·64	0·56	0·50	0·58	0·61	0·56
Tyr	0·29	0·47	0·30	0·44	0·36	0·33	0·26	0·45	0·15	0·25	0·35	0·17	0·28	0·26	0·08
Phe	0·39	0·59	0·42	0·44	0·43	0·46	0·29	0·40	0·45	0·35	0·47	0·42	0·35	0·45	0·50
Lys	0·67	0·65	0·68	0·59	0·50	0·61	0·42	0·55	0·84	0·84	0·79	1·3	0·68	0·65	1·1
His	0·22	0·29	0·27	0·26	0·28	0·30	0·24	0·25	0·21	0·23	0·29	0·26	0·20	0·42	0·22
Arg	0·27	0·47	0·47	0·32	0·36	0·39	0·18	0·30	0·34	0·40	0·44	1·1	0·21	0·36	1·4
Hyp	—	—	—	—	—	—	—	—	5·0	—	—	4·5	—	—	3·3

MP, milk-protein; LP, low-protein; G, gelatin.

In unhydrolysed samples the amino acids in Fraction IV constituted a slightly greater percentage of the total amino acids in the unfractionated intestinal sample compared with those in the 'picric acid supernatant'. This was most noticeable for methionine but was also true of tyrosine, lysine and arginine. Some release of free amino acids within the fraction may, therefore, have occurred without altering the total amino acid concentrations in the fraction.

The proportions of the total amino acids in Fraction IV and in the 'picric acid supernatant' which were present in the free form are shown in the right-hand column of Table 2. For about half the amino acids measured the proportion was less than 15%, but free tyrosine and arginine constituted 50–60% of the total tyrosine and arginine content of this fraction.

Gel filtration of Fraction IV and the 'picric acid supernatant' on Sephadex G-25 gave the elution patterns shown in Fig. 4. For comparison the positions of Blue Dextran (which was totally excluded from the gel), tyrosine, phenylalanine and tryptophan are shown. Neither of the samples contained any components which were excluded from the gel.

The agreement between the results of the analyses of Fraction IV and the 'picric acid supernatant' suggests that these are similar fractions although they were derived by different methods.

Table 2. *Comparison between Fraction IV and the intestinal contents of young adults after removal of protein with picric acid; the percentage in each of these fractions of the total amino acid present in the intestinal sample*

Amino acid	Hydrolysed (A)		Unhydrolysed (B)		The free amino acids as % of the total in each fraction (B/A × 100)	
	Fraction IV	PSN	Fraction IV	PSN	Fraction IV	PSN
Asp	47	42	5	5	11	8
Thr	43	56	5	4	11	7
Ser	45	48	4	4	8	8
Pro	47	56	—	—	—	—
Glu	51	52	6	8	12	16
Gly	54	21	4	4	7	17
Ala	42	44	6	5	13	12
Val	45	44	6	4	14	9
Met	50	46	14	6	27	13
Ileu	47	48	3	2	7	5
Leu	44	38	5	3	11	8
Tyr	37	35	21	15	56	46
Phe	40	46	13	10	32	24
Lys	52	49	25	17	48	35
His	46	36	13	11	28	32
Arg	43	36	27	21	63	59

Fraction IV was that fraction of the intestinal contents of young adults which was not excluded from Sephadex G–25; the PSN was the fraction of intestinal contents which was soluble in picric acid.

Recovery of the amino acids containing components from the fractionation procedure

The sum of the total amino acids in hydrolysates of each fraction of test meal or of intestinal contents compared with the total amino acid content in the unfractionated material is a measure of the recovery. This varied from 68% from intestinal samples after the milk-protein meal to 98% from gelatin (Table 3). The losses probably occurred before gel filtration; when the amino acid content of the three fractions eluted from the column was compared with that of the material loaded on to the column, 95–100% recovery was observed.

The percentage recovery of amino acids was greater for gelatin and for intestinal samples collected after gelatin than it was for the milk-protein meal or for intestinal samples collected after this meal. This may have been because a greater percentage of the total amino acid was in a soluble form in intestinal contents following gelatin compared with that after the milk protein. Certainly the gelatin itself was more soluble than the casein of the milk protein.

Loss may therefore have occurred (*a*) of insoluble residue, due to non-uniform sampling for hydrolysis, or (*b*) of proteins which may have come out of solution during dialysis and adhered to the dialysis sac.

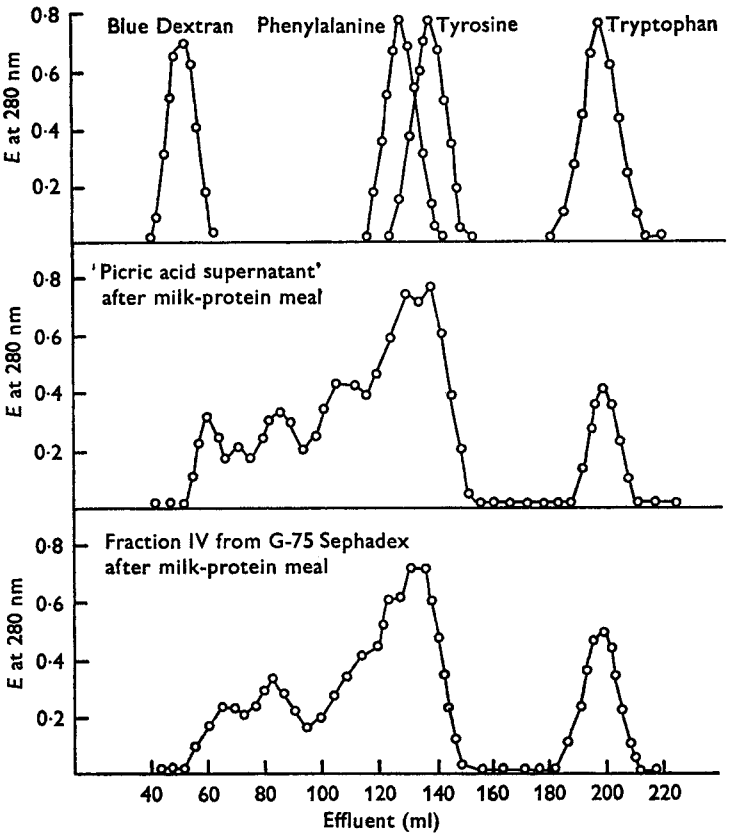

Fig. 4. Elution pattern from G-25 Sephadex of intestinal contents from young adults after removal of protein by precipitation with picric acid ('picric acid supernatant') compared with that of Fraction IV of the intestinal contents. The elution positions of the reference compounds are shown in the upper part of the figure.

Table 3. *Percentage recovery (mean and range) of total amino acid when the test meals and intestinal samples from young adults were fractionated by centrifugation and gel filtration on G-75 Sephadex*

(The single numbers in parentheses show the number of experiments which were done)

Meals		Intestinal samples after		
MP	G	MP	LP	G
80 (2)	98 (2)	62 (56–72) (5)	75 (74–76) (2)	93 (90–98) (3)

Percentage recovery of total amino acid when the soluble component of intestinal contents of young adults after a milk-protein meal was fractionated on G-75 Sephadex:

98 (95–100) (3)

MP, milk-protein; G, gelatin; LP, low-protein meal.

Concentrations of the free amino acids

The determination of the free amino acids in intestinal contents was complicated by the presence of ninhydrin-positive material, probably small peptides, which emerged from the ion exchange column immediately after the buffer peak, corresponding with the change from pH 3·25 to 4·25 buffer. This was particularly noticeable in samples collected after the gelatin meal where the peaks corresponding to cystine, valine, methionine, isoleucine and leucine, which were present in small concentrations, were

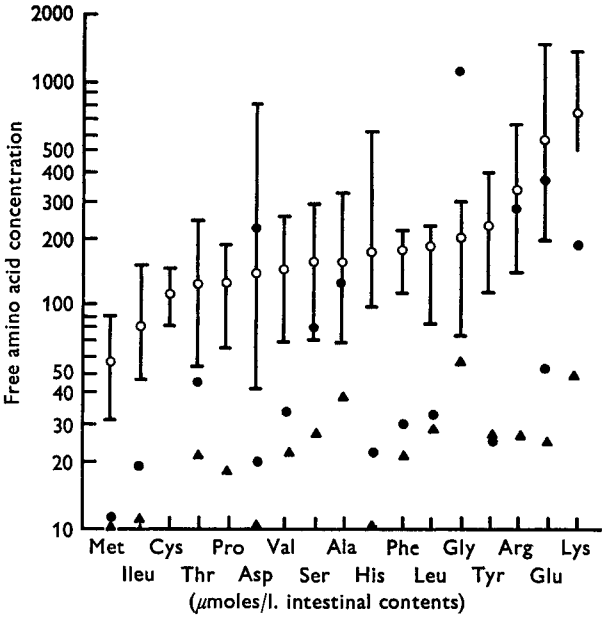

Fig. 5. Free amino acid concentrations (μmoles/l.) in 3 h collections of small intestinal contents from young adults after the three test meals. The values for samples after the milk-protein meal are the means and ranges from experiments at intestinal levels ranging from the proximal part of the duodenum to the lower jejunum (80–200 cm from the nose). The values for samples after the gelatin test meal are the means of four experiments at levels ranging from 110 to 190 cm from the nose. The values for glutamate and aspartate are given separately for the two subjects who took this meal. The values for samples after the low-protein meal are the means from four experiments at levels ranging from 110 to 240 cm from the nose. A log scale has been used. ○, after milk-protein meal; ●, after gelatin meal; ▲, after low-protein meal.

partially obscured by much larger peaks due to unidentified compounds. In many instances it was not possible to determine the actual concentrations of these amino acids.

The free amino acid concentrations showed little variation with level of sampling. The concentrations of aspartate, threonine, serine, alanine and histidine, after the milk-protein meal were lower in the duodenal samples than in the jejunal samples. Lindberg (1966) found that dipeptidase activity was low in the proximal duodenum but rose sharply in the distal part of the duodenum. The concentrations of tyrosine and arginine

were lower in the lower jejunum than in the upper intestinal samples. However, more experiments would be necessary to show significant differences with level of sampling.

Fig. 5 shows the free amino acid concentrations (μmoles/l., mean and range) in samples from all levels in the six subjects after the milk-protein meal. The ranges were greatest for the dicarboxylic amino acids; high concentrations of free glutamate and aspartate in jejunal contents appeared to be a characteristic feature of the subjects B.N. (Fig. 6).

The concentrations in the intestinal contents after ingestion of the other two meals are also shown in Fig. 5. Except for glutamate and aspartate, the values are the means of four values from two subjects. The values for glutamate and aspartate in the intestinal contents after the gelatin meal are given separately for the two subjects, one of whom was the subject B.N.

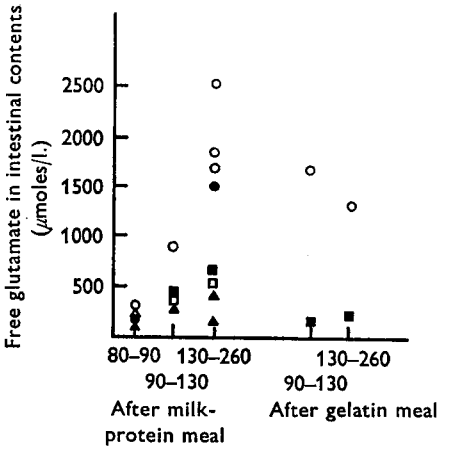

Fig. 6. Concentrations (μmoles/l.) of free glutamate in 3 h collections of intestinal contents from the six young adults. Each value is the result from one experiment. ○, B. N.; ●, B. W.; ▲, G. M.; △, B. H.; □, A. T.; ■, G. H.

The concentrations in the samples after the gelatin and the low-protein meals were, in general, lower than those after the milk-protein meal. The relative concentrations bore little relationship to the total amino acid concentrations in the test meals or to those in the intestinal contents. Proline and hydroxyproline, which together represented 21% of the amino acid content of the gelatin meal, were not detectable in the free state in the intestinal samples taken after this meal. (The limits of detection were approximately 50 μmoles/l. in each instance). Many of the amino acids in each sample were present in approximately equimolar concentrations.

The free amino acid concentrations expressed as percentages of the total amino acids in the intestinal contents were very similar in samples collected after the milk-protein meal and after the low-protein meal. Fig. 7 shows the mean values (and ranges) of the concentrations expressed in this way in samples after the milk-protein meal. Also shown on the figure are the mean values for the samples obtained after the other two meals (the values for glutamate and aspartate are again given separately for the two subjects taking the gelatin meal).

The proportion of the total amino acid which was present in the free form ranged from 2% for proline to 20% for arginine in samples taken after the milk-protein meal.

Release of free amino acids during in vitro incubation of fresh intestinal contents

Samples from two intestinal levels, 110 and 170 cm from the nose, were incubated. The total amino acid concentrations (μmoles/l.) were very similar in both types of sample; therefore the results from each level will be directly compared.

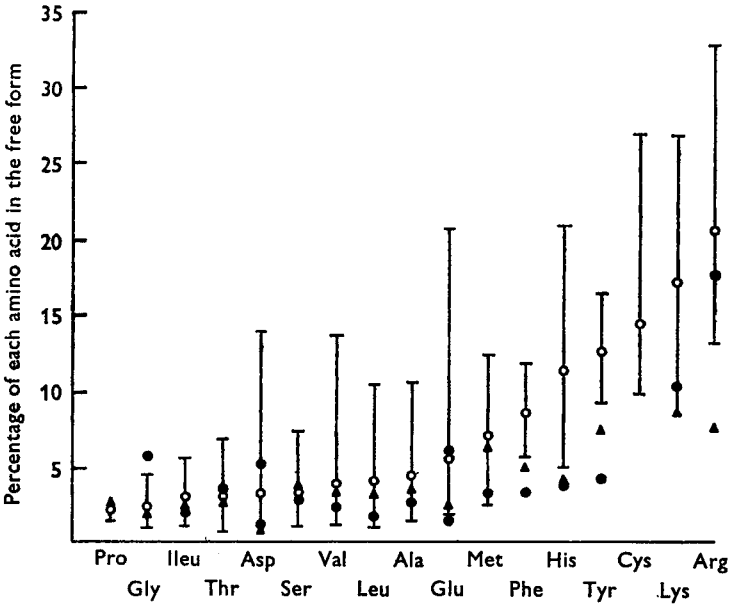

Fig. 7. Free amino acid concentrations in 3 h collections of intestinal contents from young adults expressed as percentages of the corresponding amino acid concentrations in hydrolysates of each intestinal sample (e.g. 2% of the total proline was present in the free form.) The values were obtained as indicated in Fig. 5. ○, after the milk-protein meal; ●, after the gelatin meal; ▲, after the low-protein meal.

The amino acids fell roughly into three groups with regard to the rate of increase in free amino acid concentrations during incubation. In Fig. 8*a*, *b*, the concentrations expressed as percentages of the totals, are plotted against the time of incubation for various representatives of the three groups.

Upper level (*Fig.* 8*a*). Arginine in samples from a level of 110 cm from the nose increased from 380 to 625 moles/l. during the first 10 min of incubation. Thereafter the rate of release declined, the concentration rising only to 775 μmoles/l. over the next 70 min. This concentration amounted to over 60% of the total arginine present. The proportions of lysine, methionine and tyrosine in the free state increased in a similar fashion. In contrast a group of amino acids, including proline, glycine and the acidic amino acids, showed little or no increase in concentrations during the period of incubation. Where an increase was observed it appeared to occur during the time when the rate of release of the rapidly released amino acids had declined. Between these

extremes there was a group, including histidine, alanine, phenylalanine and the branched-chain amino acids, which showed a gradual but continued rise in concentration over the incubation period.

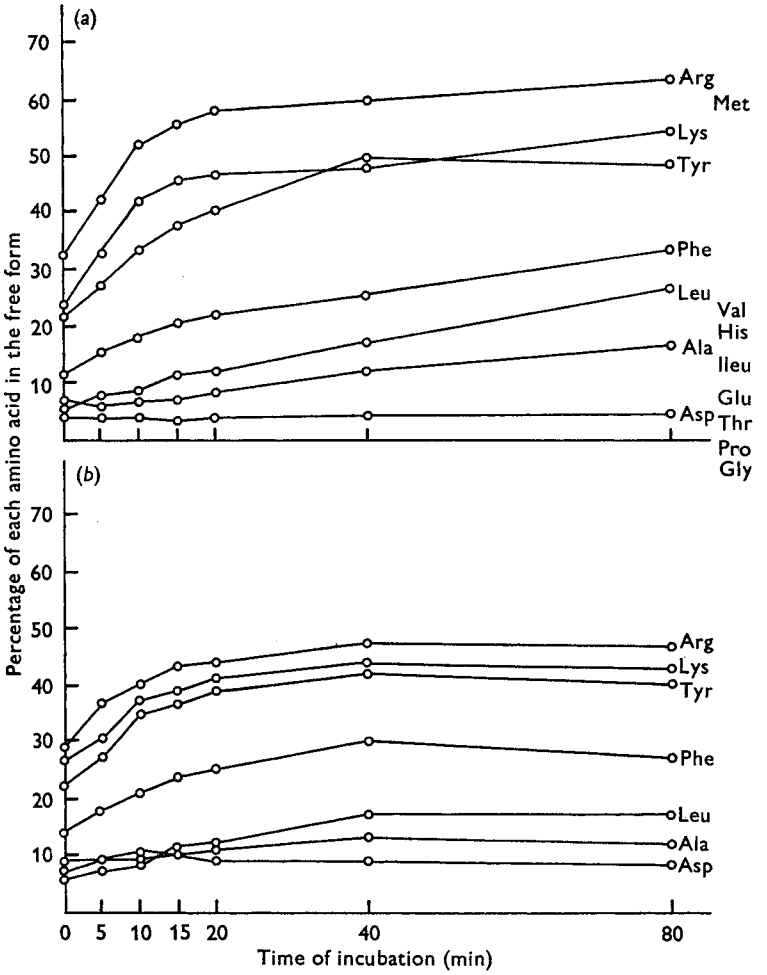

Fig. 8. Increase in the free amino acid concentrations, expressed as percentages of the corresponding amino acid concentrations in hydrolysates of each sample, when small intestinal contents, (*a*) from a level of 110 cm from the nose and (*b*) from a level of 170 cm from the nose, were incubated at 37°. Representative amino acids only are shown in each instance. The approximate final concentrations of the others are shown in (*a*).

Lower level (*Fig.* 8*b*). The same grouping was observed after incubation of the samples from the lower level. The initial free amino acid concentrations were sometimes slightly greater than those in the upper level sample but often were almost identical. The concentrations of free aspartate and glutamate in the sample from the lower level were approximately double those in the sample from the upper level.

The initial rate of release of the rapidly released amino acids was less in the samples from the lower level than in those from the upper level. After 40 min of incubation

there was no further release of any of the amino acids. The results for the group comprising phenylalanine, alanine, histidine and the branched-chain amino acids were almost identical at both levels over the first 40 min.

DISCUSSION

Digestion

There was a rapid breakdown of the food proteins in the small intestinal lumen. It was not possible to make definitive statements about the fate of the milk protein for the following reasons: (1) there was incomplete recovery from the fractionation procedure; (2) there was no way of differentiating between dietary and endogenous protein; (3) by the time the test meal had reached the level of sampling, 110 cm from the nose, a large part of the dietary protein had probably already been absorbed (Nixon & Mawer, 1970). These criticisms did not apply to the same extent to the intestinal samples after the gelatin meal. In these samples, where hydroxyproline could be used as a marker, the dietary protein of molecular weight over 50000 (Table 1; Gelotte, 1964) had been digested to the extent where 75% of the hydroxyproline was present in fragments of under 5000 molecular weight. The close relationship between Fraction IV and intestinal samples in which protein had been precipitated immediately on collection (Table 2) confirmed that the breakdown had occurred in the intestinal lumen.

Table 4. *Estimated rates (μmoles/min) of release of the free amino acids in the jejunal lumen of young adults after a milk-protein meal*

Amino acid	Upper level	Lower level	Amino acid	Upper level	Lower level
Lys	71	40	Ser	6·6	7·1
Val	30	13	Ileu	5·6	5·8
Arg	24	12	Glu	5·1	7·1
Leu	21	22	Thr	3·6	3·7
Tyr	20	21	His	2·1	2·5
Phe	15	12	Asp	1·7	3·0
Met	14	13	Pro	1·5	1·0
Ala	7·0	8·2	Gly	0·5	2·1

Although the samples which were fractionated were collected 1 h after the ingestion of the meal, most of the breakdown probably occurred during the passage of the test meal from the stomach to the sampling level. As test meal (containing polyethylene glycol, the intestinal marker) could be collected from the upper intestinal levels 5–10 min after the ingestion of the meal, the time for transit from the stomach to a level of 110 cm from the nose need be no more than 10 min.

The results of this work are therefore in agreement with the findings of Börgstrom and his colleagues (Borgström, Dahlqvist, Lundh & Sjövall, 1957) who found that the degree of hydrolysis of iodinated albumin rapidly reached 50–60% in the duodenum. The extent of hydrolysis in their experiments was determined by the decrease of ^{131}I

which was precipitable in phosphotungstic acid with a concomitant increase in the ^{131}I associated with the phosphotungstic acid-soluble material.

The incubation experiments showed that, in samples after a milk-protein meal, the rapid breakdown could continue to the free form for some amino acids, e.g. arginine, lysine, methionine, tyrosine and phenylalanine (Fig. 8). The initial slope of the concentration–time curve gives an estimate of the rate of release of the free amino acids in the intestinal contents at the time of removal. Enzyme autolysis, substrate exhaustion and product accumulation each tends to reduce the rate of release of the free amino acids in vitro. The slopes during the first 10 min appeared to be linear and theoretical rates (μmole/min) of amino acid release in vivo were calculated from the rise in free concentrations during this interval. In view of the delay between the contents leaving the lumen and the start of the incubation each value underestimates the actual rate of release in vivo. The estimated rates of amino acid release within the lumen at 110 and 170 cm are shown in Table 4. The amino acids released most slowly showed only minor increases in concentration after 10 min; in these the slope was calculated from the increase over the initial 40 min period.

For many of the amino acids, the rates of release were almost identical at both levels. However, the release of lysine, valine and arginine was approximately 50% slower at the lower level than at the upper level, while glutamatic acid, aspartic acid and glycine were released more rapidly at the lower level.

The mode of release of these rapidly released amino acids by digestive enzymes is well known; lysine and arginine are rapidly released from proteins by the combined actions of trypsin and carboxypeptidase B and tyrosine and phenylalanine by the actions of pepsin, chymotrypsin and carboxypeptidase A (Dixon & Webb, 1964). These were also the amino acids which were present in the free form as greater percentages of the totals in intestinal samples (Fig. 5).

The mode of release of serine, threonine and the dicarboxylic amino acids is not so well documented. These amino acids were released very slowly on incubation and the free forms constituted very small proportions of the totals in the intestinal samples. It is possible that the enzymes normally releasing these amino acids are intimately associated with the jejunal mucosa. Intestinal mucosa contains a number of dipeptidases (Gailey & Johnson, 1941), the most important one being leucine aminopeptidase, isolated in a highly purified form by Spackman, Smith & Brown (1955). A wide variety of synthetic amides and peptides are split by this enzyme although it is most active against dipeptides with an N-terminal leucine. Other dipeptidases have been described, one splitting ala-gly, one splitting gly-gly and one splitting pro-gly (Gailey & Johnson, 1941). Peptides containing these amino acids may therefore be split on the mucosal surface of the intestinal wall (Ugolev, Iesuitova, Timofeeva & Fediushina, 1964) or in the mucosal cells (Newey & Smyth, 1959; Wiggans & Johnston, 1959).

Form in which amino acids are absorbed

Table 5 gives an estimate, based on the theoretical rates of release in vivo (μmoles/min) at the upper jejunal level, of the time required for the release of all the amino

acids of the milk-protein meal. The entire content of seven of the amino acids could probably be released during the 3 h of the experiments. It is therefore not necessary to postulate anything other than complete digestion within the lumen with the subsequent absorption of the free amino acids for lysine, valine, arginine, tyrosine, phenylalanine and methionine; this might also be true of leucine. These are the amino acids which are absorbed most rapidly from mixtures of free amino acids (Orten, 1963; Adibi *et al.* 1966). However, for the amino acids such as proline, glycine and the dicarboxylic amino acids some other mechanism must be postulated. After 80 min incubation, 90% or more of the total acidic amino acids were still peptide-linked.

Table 5. *Estimated time to release all the amino acids of the milk-protein test meal in the upper jejunum of young adults*

Amino acid	Time (min)	Amino acid	Time (h)
Lys	72	Ala	11·6
Val	175	Ser	13·6
Arg	66	Ileu	13·0
Leu	358	Glu	45·5
Tyr	108	Thr	23·6
Phe	175	His	11·9
Met	104	Asp	71·0
		Pro	74·5
		Gly	98·5

In a previous paper (Nixon & Mawer, 1970) we have shown that the rates of net absorption of the amino acids after a milk-protein test meal were proportional to the concentrations of the amino acids in the meal. Thus by the time the meal had reached an intestinal level of about 200 cm from the nose, 70% of the glutamate (amounting to 12 m-moles) and 70% of the arginine (amounting to 1·5 m-moles) had been absorbed. It has been suggested that because of the higher concentrations of glutamate in the meal it can compete effectively for transport mechanisms (Gitler & Martinez-Rojas, 1964). In these experiments however, the free amino acid concentrations bore little relationship to the concentrations in acid hydrolysates of test meals or to those in acid hydrolysates of the intestinal contents. Although the concentrations of the acidic amino acids showed great subject variation they were in many cases similar to, or even smaller than, the concentrations of the basic or neutral amino acids.

Alternative suggestions are (*a*) that these amino acids are absorbed as peptides, or (*b*) that the peptides are hydrolysed at the mucosal surface of the intestinal wall. There is evidence for the absorption of small peptides. Hydroxyproline-containing peptides have been shown to be absorbed as such after a gelatin meal in man (Prockop, Keiser & Sjoerdsma, 1962). In the work being described, almost 50% of the hydroxyproline of the gelatin meal had disappeared by a level of 190 cm from the nose (Nixon & Mawer, 1970) yet free hydroxyproline was never demonstrated in the intestinal contents. Glycyl peptides, which have also been demonstrated to be relatively resistant to enzymic hydrolysis, can be transported across the gut wall as such (Wiggans & Johnson, 1959; Newey & Smyth, 1959).

Either of the suggested mechanisms could result in a more rapid absorption of

certain amino acids after a protein meal than that which occurs from a mixture of amino acids. If absorption of glutamate occurs in the form of peptides then those peptides may compete more effectively than free glutamate for transport mechanisms. If hydrolysis of the peptides occurs on the mucosal surface of the cell then, possibly by binding of the released amino acids at the surface, the subsequent transport of glutamate may be accelerated.

The evidence is therefore consistent with the view that absorption mainly in the free state occurs for lysine, arginine, methionine, tyrosine, phenylalanine and leucine. It is not consistent with the view that glycine, threonine, serine, the imino acids or the dicarboxylic amino acids are released in the lumen before absorption. Experiments where amino acid mixtures, simulating a dietary protein, are fed with the subsequent determination of the rates of amino acid absorption are not presenting a true picture of the events in the small intestine following the ingestion of a protein meal.

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