Jejunal absorption of an amino acid mixture simulating casein and an enzymic hydrolysate of casein prepared for oral administration to normal adults

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1. An intestinal perfusion technique was used in six normal human subjects to study absorption of sixteen individual amino acids from an amino acid mixture simulating casein and from an enzymic hydrolysate of casein, prepared for oral administration to these subjects, which consisted of a mixture of oligopeptides and free amino acids.

2. Total absorption of α-amino nitrogen was greater from the casein hydrolysate than from the amino acid mixture, and the considerable variation in percentage absorption of individual amino acids from the amino acid mixture was much reduced when the enzymic hydrolysate solution was perfused, as a number of amino acids which were poorly absorbed from the amino acid mixture were absorbed to a greater extent from the casein hydrolysate.

3. These findings indicate that after extensive intestinal resections or in malabsorption there might be significant nutritional advantages in the administration of protein hydrolysates rather than amino acid mixtures.

There is now good evidence that in man as in animals dietary protein is removed from the lumen of the small intestine in the form of small peptides as well as free amino acids (see Matthews, 1972; Milne, 1972; Cook 1973*a*, *b*; Silk, 1974). The results of studies carried out in subjects with two inherited disorders of amino acid transport, Hartnup Disease and cystinuria, have emphasized the nutritional importance of peptide uptake in these conditions (Milne, 1972). The results of recent studies in the rat (Crampton, Gangolli, Simson & Matthews, 1971) and in man (Silk, Marrs, Addison, Burston, Clark & Matthews, 1973) suggest that the mucosal uptake of small peptides is quantitatively important in protein absorption under normal conditions. The aim of the present study was to obtain further information about the role of peptide uptake in protein absorption. An in vivo perfusion technique was used to compare the extent to which individual amino acids are absorbed from solutions containing an enzymic hydrolysate of casein and an equivalent amino acid mixture simulating the composition of casein.

Table 1. *Composition of amino acid solution (mmol/l) used to simulate casein in a perfusion solution administered to six normal adult subjects*

Amino acid		Amino acid	
Histidine	1·87	Glycine	2·53
Lysine	4·23	Alanine	2·97
Arginine	1·87	Valine	4·87
Aspartic acid	2·25	Methionine	1·82
Asparagine	2·44	Isoleucine	3·60
Threonine	6·12	Leucine	5·98
Serine	7·39	Tyrosine	2·74
Glutamic acid	6·12	Phenylalanine	2·92
Glutamine	7·99	Cysteine	0·64
Proline	8·39	Tryptophan	0·62

METHODS

Enzymic hydrolysate and amino acid mixture. The enzymic hydrolysate of casein, which was very readily soluble, was intended for oral administration to normal subjects. It was prepared by hydrolysis with papain (*EC* 3.4.4.10), followed by hydrolysis with hog kidney peptidases by the method described by Clegg & McMillan (1974). It contained about 50% free amino acids and about 50% small peptides of mean chain length 2–3 amino acid residues (Clegg, unpublished results).

The amino acid mixture (Table 1) was made up to simulate the composition of casein as determined by ion-exchange chromatography after complete acid-hydrolysis of the enzymic hydrolysate, except that appropriate amounts of tryptophan and cysteine, as detailed in standard tables giving the composition of casein (Ling, Kon & Porter, 1961), were added because acid-hydrolysis destroys these amino acids. In addition, approximately 10% of the threonine and serine content of casein are destroyed by acid-hydrolysis (Rees, 1946) and appropriate proportions of these amino acids were also added. After acid-hydrolysis, glutamic acid and glutamine, and aspartic acid and asparagine were determined as glutamic acid and aspartic acid respectively. In preparing the amino acid mixture 50% of the 'glutamic acid' obtained by analysis was replaced by glutamine and 50% of the 'aspartic acid' by asparagine, since these amino acids and their respective amides occur in casein in approximately these proportions (Mercier, Grosslande & Dumas, 1972).

Perfusion procedure. Six normal adult volunteers who gave their informed consent, were intubated with a double-lumen perfusion tube incorporating a proximal occlusive balloon as previously described (Silk, Perrett & Clark, 1973). The tube was introduced until the 300 mm perfusion segment was positioned in the upper jejunum; the final position was checked radiologically. The perfusion solutions were pumped at a rate of 20 ml/min through the infusion orifice from bottles maintained at 37° in a water bath. For each subject, after an equilibration period of 30 min for each perfusion solution, a 30 min sample was collected from the distal collecting orifice by a simple syphon procedure.

Each subject was perfused with a solution containing the enzymic hydrolysate of casein and a solution containing the amino acid mixture. The two perfusion solutions,

which were administered in a random order, contained 64 mmol α-amino nitrogen (determined after acid-hydrolysis)/l. Each solution was made iso-osmotic with NaCl; the enzymic-hydrolysate perfusate contained 136 mmol sodium/l and that of the amino acid mixture contained 112 mmol Na/l. The perfusion solutions contained polyethylene glycol (PEG) labelled with 1 μCi [^{14}C]PEG (New England Nuclear Corp., Boston, USA)/l at a concentration of 2·5 g/l. The final pH of the enzymic-hydrolysate perfusate was approximately 7·4, and that of the amino acid mixture was therefore adjusted to pH 7·4 with NaOH before perfusion.

Analytical methods and calculation of results. To measure absorption of individual free plus peptide-bound amino acids from the enzymic hydrolysate of casein and to compare these values with those for absorption of individual amino acids from the equivalent amino acid mixture, portions of the perfusion solutions and their intestinal aspirates were hydrolysed in sealed glass tubes at 110° for 24 h with 6 M-HCl.

Amino acids were estimated by ion-exchange chromatography using a Locarte automatic-loading amino acid analyser (Mark 4 Floor Model; The Locarte Company, 199 North End Road, London W14). ^{14}C radioactivity was measured using a scintillation counter (Corumatic 200 with Diehl Combitron S computor; ICN Pharmaceuticals (UK) Ltd, Tracer Laboratory Instruments Division, Hersham, Surrey) using the procedure described by Wingate, Sandberg & Phillips (1972) and Silk, Perrett, Webb & Clark (1974). The amount of the individual amino acids absorbed was calculated as previously described (Holdsworth & Dawson, 1964; Silk, Marrs *et al.* 1973) and expressed as a percentage of the concentration in the perfusion solution. The significance of differences between the mean values for the six subjects was assessed by the paired *t* test (Snedecor, 1937).

RESULTS

The results (Fig. 1) suggest that there was considerable variation in the extent to which individual amino acids were absorbed from the amino acid solution. For example methionine (75·2 %), leucine (51·9 %) and isoleucine (50·9 %) were well absorbed, whereas threonine (17·0 %) and histidine (16·1 %) were poorly absorbed. The variation in the extent to which the amino acids were absorbed from the enzymic hydrolysate was much less, and there was a tendency for the amino acids which were poorly absorbed from the amino acid mixture to be absorbed more extensively from the enzymic hydrolysate. Seven amino acids (phenylalanine, alanine, tyrosine, serine, aspartic acid (aspartic acid plus asparagine), threonine and histidine) were absorbed to a greater extent from the enzymic hydrolysate than from the amino acid mixture.

Total absorption of α-NH_2 N was greater from the solution containing the enzymic hydrolysate (mean ± SE; 40·3 ± 5·9 %) than from the equivalent amino acid solution (31·2 ± 5·6 %; $P < 0·05$). There was thus a 29 % increase in total α-NH_2 N absorption from the enzymic hydrolysate compared with that from the equivalent amino acid mixture.

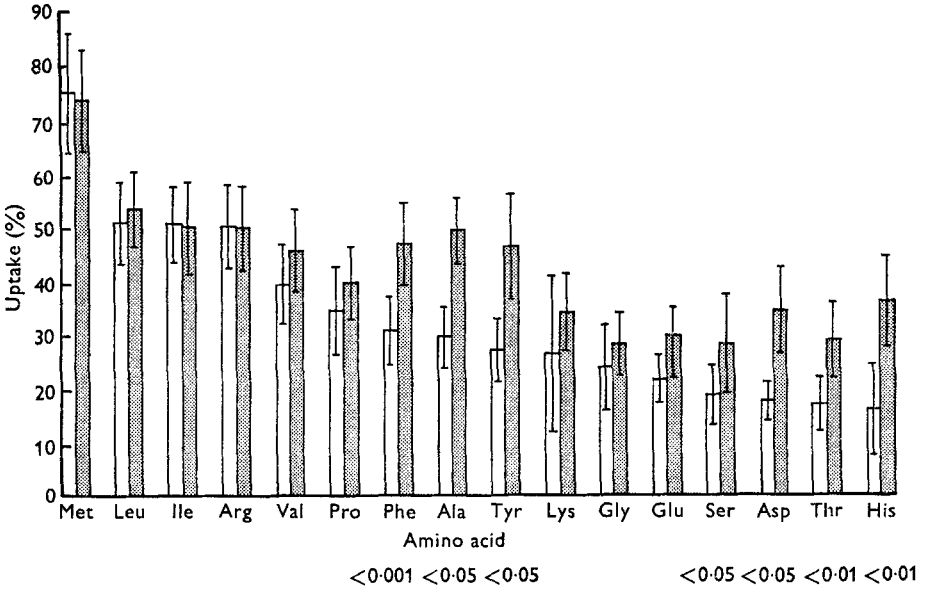

Fig. 1. Amounts of individual amino acids absorbed from a perfusion solution containing either an amino acid mixture simulating casein or an enzymic hydrolysate of casein containing oligopeptides and free amino acids, expressed as a percentage of the amounts in the perfusate. The total height of each column represents the mean value for six adult subjects with the standard error represented by a vertical bar. Where the difference between absorption of individual amino acids from the amino acid mixture and the enzymic hydrolysate was significant, the level of significance (P) is indicated. □, Amino acid mixture; ▨, enzymic hydrolysate.

DISCUSSION

There is evidence to suggest that during protein digestion the intestinal lumen contains a mixture of small peptides and amino acids (Chen, Rogers & Harper, 1962; Nixon & Mawer, 1970; Adibi & Mercer, 1973). An attempt has been made in this study to simulate the composition of intraluminal protein digestion products normally presented to the intestinal mucosa for absorption by perfusing a solution of an enzymic hydrolysate of casein, which contained a mixture of small peptides and free amino acids, through a section of the upper jejunum. The finding that a number of individual amino acids (phenylalanine, alanine, tyrosine, serine, aspartic acid, threonine and histidine) were absorbed to a greater extent from this solution than from an equivalent solution containing only free amino acids provides evidence that they are removed from the lumen of the intestine at least in part as intact peptides. If complete intraluminal hydrolysis of the peptide fraction preceded absorption of liberated amino acids, net uptake of each individual amino acid would have been, at best, the same from the two solutions. The results suggest therefore that under normal conditions uptake of small peptides plays a significant part in protein absorption. With aspartic acid, representing both aspartic acid and asparagine, it was not possible to determine whether the more rapid absorption from the enzymic hydrolysate was due to uptake of aspartic acid or asparagine or both in peptide form. It is important to note that the finding that some amino acids are not absorbed more rapidly from the enzymic hydrolysate than from the amino acid mixture does not necessarily indicate

that they are absorbed from the lumen only in the free form; absorption in peptide form is not precluded.

Many of the present findings are in agreement with those of a recent study comparing the characteristics of amino acid absorption during perfusion of solutions containing a partial hydrolysate of casein prepared by trypsin digestion and an amino acid mixture of similar composition (Silk, Marrs *et al.* 1973). There were, however, slight differences in the rates at which individual amino acids were absorbed from the amino acid mixture; two amino acids (lysine and glutamic acid) which were found to be absorbed to a greater extent from the trypsin hydrolysate than from the amino acid mixture in the earlier experiments were absorbed at similar rates from the two solutions perfused in the present study. In addition, two amino acids (tyrosine and threonine) which were found to be absorbed at comparable rates from the trypsin hydrolysate and its equivalent amino acid mixture (Silk, Marrs *et al.* 1973) were absorbed to a greater extent from the enzymic hydrolysate than from the amino acid mixture in the present study. Although some of these differences might be explained by differences in the composition of the peptide component of the two enzymic hydrolysates of casein, additional factors deserve consideration. First the two studies were done under different experimental conditions, which could have affected absorption values; in the present study the solutions were (*a*) infused at a faster rate than in the previous study and (*b*) contained higher concentrations of α-NH_2 N. Secondly, in the first study the amino acid mixture contained glutamic and aspartic acids but no glutamine or asparagine.

The present results, taken in conjunction with those of previous studies in man (Silk, Marrs *et al.* 1973) and animals (Crampton *et al.* 1971) strongly suggest that total absorption of α-NH_2 N is greater from partial enzymic hydrolysates of protein than from equivalent amino acid mixtures. Thus, if maximally effective absorption is required in a clinical situation, for example, in the treatment of extensive small intestinal resection or malabsorption there would appear to be a significant advantage in administering protein hydrolysates which consist of oligopeptides as well as amino acids rather than amino acid mixtures. Such preparations also have the additional advantage of a lower osmotic pressure than equivalent amino acid mixtures. Finally, the finding of a 'more even' percentage absorption of amino acids during perfusion of protein hydrolysates than during perfusion of amino acid mixtures suggests the possibility that oral administration of protein hydrolysates might result in more effective protein synthesis than oral administration of amino acid mixtures (Cannon, Steffee, Frazier, Rowley & Stepto, 1947; Gitler, 1964; Matthews, 1971).

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