Digestion and absorption of dietary protein in man

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Although it has become clear that brush border hydrolysis and consequent utilization of the monosaccharide transport system is the predominant method of absorption of the luminal products of carbohydrate digestion in man, it now appears certain that two major mechanisms are involved in the absorption of the luminal products of protein digestion; on the one hand transport of liberated free amino acids by group specific active amino acid transport systems, and on the other hand uptake of unhydrolysed peptides by mechanisms independent of the specific amino acid entry mechanisms.

Protein sources

Exogenous protein. Dietary protein is derived from animal and vegetable sources and makes up 11–14% of the average energy intake. In Western diets this amounts to about 70–100 g of protein/d.

Endogenous protein. Significant amounts of protein derived from endogenous sources such as gastric biliary, pancreatic and intestinal secretions also require assimilation by the human gastrointestinal tract. Although the initial animal studies of Nasset and co-workers suggested that the endogenous pool was considerably larger than the exogenous pool (Nasset *et al.* 1955; Nasset & Ju, 1961), more recent studies performed in man indicate that the endogenous pool is approximately one-third the size of the exogenous protein pool (Nixon & Mawer, 1970*a*,*b*; Johansson, 1975).

Site of protein assimilation

The bulk of ingested protein appears to be absorbed in the proximal jejunum (Borgstrom *et al.* 1957; Nixon & Mawer, 1970*a*,*b*; Johansson, 1975; Silk *et al.* 1979). Small amounts reach the ileum and are absorbed at this site (Adibi & Mercer, 1973; Silk *et al.* 1979; Chung, Kim *et al.* 1979*b*), and as judged by the protein content of ileostomy effluent, absorption of protein is not completed in the small intestine (Gibson *et al.* 1976). The site of endogenous protein assimilation in man has not been characterized, but recent animal studies implicate the colon as the major site (Curtis *et al.* 1978).

Luminal protein digestion

The initial step in protein digestion is its denaturation at acid pH in the stomach by the action of several pepsins with varying substrate specificities (Taylor, 1968; Turner, 1968; Whitecross *et al.* 1973). Negligible amounts of free amino acids are released, and large polypeptides enter the duodenum to be further hydrolysed by pancreatic proteolytic enzymes.

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The intraluminal digestion of proteins by pancreatic proteolytic enzymes has been reviewed in some detail by Gray & Cooper (1971) and the zymogenenzyme exopeptidases, by Keller (1968). Each of the proteolytic pancreatic enzymes is secreted as an inactive precursor. Trypsinogen is then activated by contact with enterokinase, an enzyme that has been isolated in a highly purified form from the brush border membrane fraction of human intestinal mucosa (Lobley *et al.* 1973; Schmitz *et al.* 1973; Hermon-Taylor *et al.* 1977). Trypsin then hydrolyses bonds in the other zymogens to form the active enzymes.

In addition to pancreatic proteolytic enzymes, recent studies indicate the presence of solubilized intestinal brush border and cytoplasmic intestinal mucosal amino oligopeptidases in intestinal contents (Josefsson & Lindberg, 1967; Silk *et al.* 1976*a*). In the jejunum, these enzymes are unlikely to have a functionally significant role in the terminal stages of protein digestion. In the ileum the activity of luminal peptidases is higher so that here significant quantities of luminal peptides may be hydrolysed in the gut lumen rather than at the surface of ileal mucosal cells.

The products of luminal proteolysis are free amino acids and small peptides having a chain length of 2–6 amino acid residues (Chen *et al.* 1962; Nixon & Mawer, 1970*a*,*b*; Adibi & Mercer, 1973). Analysis of post prandial intestinal contents aspirated from human jejunum reveals that approximately only a third of the total amino acid content exists in the free form (Adibi & Mercer, 1973). The nature of the oligopeptides in terms of distribution of chain length frequency and amino acid composition has not yet been characterized.

Free amino acid transport

As with glucose transport, results of in vitro experiments have shown that active amino acid transport is dependent on a gradient of sodium ions across the brush border membrane of intestinal epithelial cells (Schultz & Curran, 1970). The absorptive patterns of free amino acids have been studied in some detail in man using various in vivo steady state perfusion techniques. In these experiments saturation of transport was reached with increasing solute concentration compatible with the existence in man of carrier mediated mechanisms for amino acid transport (Adibi & Gray, 1967; Adibi *et al.* 1967; Adibi, 1969, 1970; Hellier *et al.* 1973). Also, different affinities of different free amino acids for these mechanisms were indicated by variations in the absorption rate of individual free amino acids when perfusion studies were performed with the use of equimolar mixtures of different acids (Adibi & Gray, 1967; Adibi *et al.* 1967; Adibi, 1969). To date, the absolute Na dependency of free amino acid transport has not been demonstrated in vivo in man (Silk & Dawson, 1979).

The results of competition studies in animals have indicated the likely existence of three major group-specific active transport systems (Matthews, 1971). (1) monoamino monocarboxylic (neutral amino acids), (2) dibasic amino acids and cystine, (3) dicarboxylic (acidic) amino acids. It should be borne in mind that the subject is complicated by species differences and by the fact that certain amino

acids may be transported by more than one mechanism, e.g., glycine, proline and hydroxyproline. In man the in vitro and in vivo studies of amino acid transport in patients with Hartnup disease and cystinuria have firmly established the existence of mechanisms (1) and (2) (see Reviews, Matthews, 1975; Matthews & Adibi, 1976; Silk & Dawson, 1979).

Intestinal handling of peptides—historical aspects

Nineteenth-century physiologists believed that dietary protein was absorbed in the form of polypeptides, a view that seemed to be confirmed when Nolf (1907) and Messerli (1913) showed that 'peptones' produced by tryptic hydrolysis of protein disappeared from the lumen of the small intestine more rapidly than equivalent amounts of free amino acids. When Cohnheim (1901) demonstrated that intestinal juice was capable of hydrolysing peptones to amino acids, some early workers suggested that protein must be hydrolysed to amino acids before being absorbed. This hypothesis gained ground when all known free amino acids were detected in intestinal contents during protein absorption in vivo (Abderhalden & Lampe, 1912; Cohnheim, 1912, 1913) and when studies in vivo showed that complete hydrolysates of protein (consisting of free amino acids) disappeared rapidly from the lumen of the small intestine (Cathcart & Leathes, 1905; Abderhalden & London, 1910). When it was found that only amino acids could be isolated from the portal circulation during protein absorption (Abel *et al.* 1913), the idea that protein was completely hydrolysed to amino acids within the intestinal lumen became the classical view of protein absorption (Verzar & MacDougall, 1936). This view was held despite later observations that intraluminal peptidase activity was insufficient to account for the absorption of peptone in the form of free amino acids (Cajori, 1933). The final vindication of the classical view of protein absorption appeared to be provided by the demonstration, with the use of ion exchange chromatography, that only free amino acids appeared in peripheral plasma after protein was administered to human subjects (Stein & Moore, 1954).

Fisher (1954) strongly criticized the classical view of protein absorption. He pointed out that it had previously been shown that upward of 200 h were required for the liberation of 90% of the amino acids from different proteins subjected to successive action of pepsin, trypsin, and erepsin and made the following statement: '. . . even on the most generous assumption the time course of liberation of amino nitrogen is too slow to fit with the view that protein must be digested to amino acids before they are absorbed,' and he suggested that the idea of absorption of protein in the form of peptides deserved serious consideration.

Mucosal transport of peptides

Initial experiments in vitro with dipeptides showed that small amounts of unhydrolysed glycyl-glycine and glycyl-L-leucine crossed the intestinal wall (Agar *et al.* 1954). Similar observations were made when glycyl-glycine was studied in vitro (Wiggans & Johnston, 1958, 1959) and in vivo (Newey & Smyth, 1959) by other workers. Newey & Smyth (1960) demonstrated that dipeptides could be

taken up intact by intestinal mucosa and concluded that the products of protein digestion could be transported into the mucosal cell in the form of oligopeptides as well as amino acids (Newey & Smyth, 1962).

The concept of intact peptide uptake as a second mode of protein absorption, although not disputed, was not thought to be quantitatively significant, as it seemed much more likely that absorption of peptides, analogous to disaccharides, would involve brush border hydrolysis with subsequent absorption of the released amino acids by amino acid transport systems.

The modern era of our knowledge of peptide absorption stems from the results of oral load experiments carried out by Matthews and his colleagues in man (Craft *et al.* 1968). They found that a given quantity of glycine was absorbed faster when administered orally as the dipeptide or tripeptide than in the free form. It was concluded that the glycine peptides were transported unhydrolysed into the mucosal cell because, if hydrolysis had preceded uptake, then, at the best, the net rates of glycine transport from the free and peptide forms would have been the same.

Evidence for intact transport of dipeptides in man

Of all the experimental results available in favour of dipeptide transport in human intestine, the most persuasive is still derived from experiments performed in patients with Hartnup disease and cystinuria. In Hartnup disease there is an intestinal transport defect for free neutral amino acids and in cystinuria for dibasic amino acids and cystine (see Milne, 1971). Despite these transport defects the 'affected' amino acids have been shown to be absorbed normally or near normally when presented to the mucosa in the form of homologous or mixed dipeptides (Nawab & Asatoor, 1970; Asatoor *et al.* 1970*a*,*b*, 1972; Hellier *et al.* 1972*b*; Silk *et al.* 1975*a*).

If any of the dipeptides administered to these patients had been hydrolysed to a substantial degree in the bulk phase of the gut lumen, or by brush border peptidases before transport of released amino acid by specific active transport processes, then absorption of the affected amino acids would not have occurred. This was clearly not the case in these experiments.

Additional evidence supporting the existence of intact dipeptide transport in human small intestine has included the following. First, the competition between free amino acids for mucosal uptake is avoided or much reduced when solutions of dipeptides instead of corresponding free amino acid mixtures are instilled into the gut lumen (Adibi, 1971; Hellier *et al.* 1972a; Silk *et al.* 1973a; Silk, 1974). Similar observations, but on a wider scale, have been made in animal studies (Matthews, 1975). Secondly, in all the human dipeptide perfusions in vivo faster rates of uptake of at least one of the constituent residues has been observed from dipeptide than corresponding free amino acid solutions (Adibi, 1971; Cook, 1972; Hellier *et al.* 1972*a*; Silk *et al.* 1973*a*). This line of evidence is open to criticism because the same phenomenon has been observed in three perfusion studies with disaccharides (Cook, 1973; Fairclough *et al.* 1977*a*; Sandle *et al.* 1977) which are

thought to be hydrolysed at the brush border and not to be transported intact. Nonetheless, unlike the sugar experiments, the kinetic advantage conferred by dipeptides on amino acid transport has been a consistent finding and of much greater magnitude.

Finally, unhydrolysed glycyl-glycine has been detected in peripheral plasma samples during intestinal perfusion in vivo (Adibi, 1971), and in other studies carnosine, anserine and hydroxyproline peptides have also been detected in the peripheral circulation during oral feeding experiments in man (Prockop & Sjoerdsma, 1961; Prockop *et al.* 1962; Perry *et al.* 1967; Hueckel & Rogers, 1970).

Characteristics of dipeptide transport in man

Matthews and co-workers have shown in vitro that intestinal transport of glycyl sarcosine and carnosine occurs via a carrier mediated Na dependent process (Addison *et al.* 1972; Matthews *et al.* 1974). Doubt has subsequently been thrown upon the Na dependency of peptide transport, because Rubino *et al.* (1971) have shown appreciable influx into rabbit ileal mucosa in vitro after Na replacement. Cheeseman & Parsons (1974) have reported that transport of glycyl-leucine by the small intestine of *Rana Pipiens* in vivo was unaffected by replacement of intralumen Na by potassium whereas transport of glycine and leucine from the equivalent mixture of free amino acids was inhibited.

In man saturation of transport of three dipeptides has been reached during perfusion in vivo of increasing solute concentrations (Adibi, 1971; Silk, 1977). Competitive inhibition between two dipeptides has been observed (Adibi & Soleimanpour, 1974) which confirms the existence of a carrier mediated dipeptide transport mechanism in human small intestine. The Na dependency of this transport process has not yet been investigated.

Free amino acids have not been shown to substantially alter the absorption rates of either glycyl-glycine or glycyl-leucine in vivo, which suggests that the carrier mediated transport system for dipeptides in human small intestine is not shared by free amino acids (Adibi & Soleimanpour, 1974).

The question as to whether a single or more than one carrier mediated transport system exists for dipeptides in the small intestine of animals or man has not yet been fully resolved. In an elegant series of in vitro animal experiments, Matthews *et al.* (1979) have provided evidence in favour of a single peptide transport system; my associates, however, have provided evidence suggesting the existence of more than one transport system (Lane *et al.* 1975; Fairclough *et al.* 1977*b*; Chung *et al.* 1979*a*). Further work in this area is clearly needed before firm conclusions can be drawn.

Appearance of free amino acids in gut lumen during dipeptide perfusion

A consistent finding during in vivo dipeptide perfusion experiments in human small intestine is the detection of free amino acids in intestinal contents aspirated from the distal end of the perfusion segment (Adibi, 1971; Cook, 1972; Hellier *et al.* 1972*a*,*b*; Silk *et al.* 1973*a*, 1974*a*, 1975*a*). The rate of appearance of free

amino acids during perfusion of different peptides varies (Adibi, 1971; Silk *et al.* 1973*a*) and substantially faster rates of appearance have been observed during ileal compared to jejunal dipeptide perfusion (Adibi, 1971; Silk *et al.* 1974*c*).

Intraluminal peptidase activity is insufficient to account for the appearance of more than a small proportion of the released free amino acids (Adibi, 1971; Silk *et al.* 1973*a*), which implies that, analogous to disaccharide transport, a close relationship exists between the mucosal transport and hydrolysis of dipeptides. Numerous studies have now shown that there are two distinct groups of mucosal peptidases, one located within the cytoplasmic compartment and the other at the brush border of the cell (for review see Kim, 1977 and Norén *et al.* 1977). It seems very likely therefore that the appearance of free amino acids during dipeptide perfusion is related to hydrolysis of components of luminal peptide by brush border peptidases, and that a proportion of the liberated free amino acids diffuse back into the bulk phase of the gut lumen (Silk, 1974; Matthews, 1975; Matthews & Adibi, 1976). Animal studies support this view as the rate of appearance of free amino acids during absorption of two dipeptides was related to the differential activity of the brush border rather than cytoplasmic peptidases obtained from intestinal mucosa at the end of the experiments (Silk *et al.* 1976*b*).

Models of dipeptide transport and hydrolysis

At first sight a scheme proposing that dipeptides are transported intact and hydrolysed by cytoplasmic peptidases would appear to explain most of the experimental results, especially the findings of normal transport of dipeptides in the face of transport defects for free amino acids in Hartnup disease and cystinuria.

Nevertheless, the appearance of free amino acids during dipeptide transport has been a consistent finding. As mentioned above, this is likely to be related to brush border hydrolysis. We believe therefore (Silk, 1974; Silk & Dawson, 1979) that a dual hypothesis for peptide transport is applicable. Thus on the one hand, those peptides with a high affinity for brush border peptidases are predominantly hydrolysed by these enzymes and absorbed as free amino acids whereas those with low affinities for the surface enzymes are absorbed predominantly intact.

Tripeptide absorption in man

Absorption of three tripeptides has been investigated in man (Craft *et al.* 1968; Silk *et al.* 1974*a*; Adibi *et al.* 1975). During the in vivo intestinal perfusion studies (Silk *et al.* 1974*a*; Adibi *et al.* 1975), the rate of amino acid uptake has been shown to be significantly greater from tripeptide than from corresponding free amino acid solutions. Whether this is indicative of intact transport of tripeptide or of constituent dipeptide released following brush border hydrolysis is not entirely clear. In all these experiments, constituent free and dipeptide bound amino acids were released into the lumen during tripeptide perfusion, as with dipeptides, the relative rates of appearance of hydrolysis products varied according to the chemical structure of tripeptide perfused. Subfractionation studies using human intestine

reveal approximately equal distributions of tripeptidase activity between brush border and soluble fractions (Nicholson & Peters, 1977*a*,*b*), so it seems reasonable to conclude that substantial proportions of at least two of the infused tripeptides alanyl-glycyl-glycine and tri-leucine were hydrolysed at the brush border prior to uptake of consituent dipeptide and amino acid by a peptide and a free amino acid transport mechanism respectively. As with dipeptides, a dual mechanism for the intestinal handling of tripeptides may be proposed as the available results indicate that substantially greater proportions of triglycine were absorbed intact. Again the quantitative importance of the brush border hydrolysis versus intact transport mechanisms is likely to be dictated by the affinity of the tripeptide substrate for the brush border peptidases.

Tetrapeptide transport in man

The absorption of only one tetrapeptide, tetraglycine has been studied in human intestine in vivo (Adibi & Morse, 1977). No evidence of intact absorption was found, and hydrolysis of the peptide at the brush border appears to be the rate limiting step in absorption.

The results of animals are more conflicting, for evidence has been presented in one in vivo study to suggest that significant components of L-leucyl-triglycine may be absorbed intact and subsequently hydrolysed by cytoplasmic peptide hydrolases (Chung *et al.* 1979*a*). However, in vitro work recently performed by Matthews and co-workers indicated that tetrapeptides are not absorbed intact but hydrolysed to tri- and dipeptides by brush border peptide hydrolases prior to uptake (Burston *et al.* 1979).

Nutritional significance of oligopeptide transport in man

The studies carried out in Hartnup disease and cystinuria have emphasized the nutritional importance of oligopeptide transport in these two conditions. As there are 400 possible dipeptides and 8000 possible tripeptides, it would clearly be impossible to assess the over-all nutritional importance of peptide absorption by studying the characteristics of absorption of each in turn.

A number of recent perfusion studies, however, do support a concept that mucosal uptake of peptides has an important, or possibly a major role to play in protein absorption.

Total absorption of amino nitrogen has been shown to be consistently greater during perfusion of solutions containing partial enzymic hydrolysates of protein (consisting mainly of peptides of chain length 2–6 amino acid residues) than during intestinal perfusion of the corresponding free amino acid mixtures of identical amino acid compostion (Silk *et al.* 1973*b*, 1975*b*; Fairclough, 1978). In addition, there was less variation in the extent to which individual amino acids were absorbed from the peptide solutions compared to that from the amino acid solutions.

The possible importance of this evening out of amino acid absorption rates conferred by the peptide component of the hydrolysate on the pattern of amino

acid transport in relationship to protein synthesis has been discussed in detail (Payne & Matthews, 1975).

Clinical studies have shown that the kinetic advantage conferred by peptides on rates of amino acid transport is maintained even when the absorptic function of intestinal mucosa is reduced, as for example in untreated adult coeliac disease (Adibi *et al.* 1974; Silk *et al.* 1974*b*). Moreover, there is experimental evidence from animal studies suggesting that long-term protein restriction causes a decrease in absorption of free amino acids but not peptides (Lis *et al.* 1972).

There seems, therefore, to be some reason to believe that there could be advantages in administering oligopeptide mixtures (i.e. partial enzymic hydrolysates of protein) rather than free amino acid mixtures orally to patients with severe long-standing protein–energy malnutrition caused by disorders of intestinal mucosal function.

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