Proteins

Protein absorption

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In the last 20 years, the mechanisms of protein absorption have been studied with increasing intensity, and a large book could be written on this subject alone. In spite of this volume of work, it has recently become clear that our knowledge of protein absorption is still far from complete, since until very recently nearly all investigations have been concerned with the intestinal transport of amino acids. Evidence for the existence of a second important mode of protein absorption—mucosal uptake of small peptides with cellular hydrolysis—is now extremely strong, and the importance of this mode of absorption is maintaining nutrition in cases of intestinal transport defects for amino acids has recently been demonstrated. Yet the details of mucosal oligopeptide transport have hardly begun to be explored. It is therefore difficult to give a balanced account of protein absorption at the present time. This account is selective, most attention being paid to growing points in the field. Several reviews covering the details of amino-acid absorption are available (Wilson, 1962; Wiseman, 1964; Matthews and Laster, 1965; Saunders and Isselbacher, 1966; Fisher, 1967; Wiseman, 1968; Matthews, 1969) and will supply references not given in the present text.

General Considerations

Protein absorption, like the absorption of fat and carbohydrate, is normally a highly effective process, net absorption in man amounting to about 90%. Since much of the faecal nitrogen (totalling 1.2 g per day) is endogenous in origin, the proportion of ingested protein entering the body is probably higher than 90%—possibly 95%. It has been realized in recent years that a good deal of protein enters the gut daily in various forms, and that most of this is digested and re-absorbed along with ingested protein. According to one of the more conservative estimates (Munro, 1966), the protein content of secretions into the human gut (digestive enzymes and mucus) amounts to 20-30 g per day, and another 30 g or more comes from desquamated

epithelial cells. Small amounts of plasma proteins (1-2 g) also enter the gastrointestinal lumen. The sum of protein entering the gut may thus be not much less than the dietary intake. This is one of many reasons why the pattern of amino acids appearing in the portal or peripheral blood after ingestion of a certain protein reflects only rather indefinitely the composition of the protein fed.

There is no reasonable doubt that in man most of the ingested protein is absorbed in the upper two-thirds of the small intestine (Borgström, Dahlqvist, Lundh, and Sjövall, 1957; Booth, 1968; Nixon and Mawer, 1970a and b). Perfusion experiments in man suggest that the absorptive capacity for neutral amino acids is higher in the jejunum than the ileum (Adibi, 1969). However, the absorptive capacity of the small intestine for protein digestion products is good throughout, and resections, unless very extensive, do not seriously impair protein absorption. Mucosal dipeptidase activities are relatively low in the most proximal part of the human duodenum, becoming higher distally (Lindberg, 1966); in the rat, they are maximal in the ileum (Robinson and Shaw, 1960). In the rat, absorption from high concentrations of a dipeptide (L-methionyl-L-methionine) was greater in the jejunum than in the lower ileum (M. T. Lis, R. F. Crampton, and D. M. Matthews, unpublished data). Absorption of protein digestion products is mainly into the portal blood.

Protein Digestion

Protein digestion is initiated by gastric proteinases (pepsins) with pH optima on the acid side of neutrality; two or more such enzymes are distinguishable in man and other animals (Taylor, 1968). While gastric digestion of protein is fairly extensive in the mechanical sense, it is unlikely that it proceeds *in vivo* beyond the stage of producing a mixture of large polypeptides and unaltered protein. The release of free amino acids may well be negligible. Gastric digestion of protein is relatively unimportant, and in pernicious anaemia, with its virtually total

failure of acid and pepsinogen secretion, there is no evidence that protein absorption is impaired. Protein digestion is, in fact, very readily initiated by pancreatic proteolytic enzymes, without any preliminary exposure to pepsin. It is of interest that the source of much popular lore about the digestibility or indigestibility of various foodstuffs is research carried out in the 19th century on the rapidity of peptic digestion (Ewald, 1891).

Once proteins and the products of peptic digestion have left the stomach and are exposed to the proteolytic enzymes of the pancreas, chemical breakdown is extensive and extremely rapid. The pancreatic phase of protein digestion is of great importance. The pancreatic proteases, which have pH optima close to the pH of the duodenal contents (about 6.5), are all secreted in the form of inactive precursors. Activation is initiated by enterokinase, an enzyme produced by the intestinal mucosa, which converts trypsinogen to trypsin. Trypsin then converts the other enzyme precursors to their active forms, and also acts autocatalytically to produce further activation of trypsinogen. Enterokinase is believed to be an enzyme of the brush border of the intestinal mucosal cells (Nordstrom and Dahlqvist, 1970). Recently, cases have been reported in which congenital deficiency of enterokinase was responsible for apparent pancreatic insufficiency in infancy (Tarlow, Hadorn, Arthurton, and Lloyd, 1970a). The pancreatic proteases may be divided into (1) endopeptidases, such as trypsin and chymotrypsin, which attack peptide bonds located within the amino-acid chains of proteins and polypeptides, breaking them into smaller fragments; and (2) exopeptidases, such as carboxypeptidases A and B, which cleave the terminal bonds of proteins or peptides, splitting off amino acids. Study of cleavage sites suggests that the endo- and exopeptidases act in concert, the action of endopeptidases producing peptide substrates suitable for further breakdown by exopeptidases (Keller, 1968). The result, as judged from analyses of luminal contents, is production of a complex mixture of oligopeptides (probably predominantly composed of 2-6 amino-acid units) and free amino acids. Further breakdown of the oligopeptides is brought about by the peptidases of the intestinal mucosa. Reports of the binding of pancreatic proteases to the brush border (Goldberg, Campbell, and Roy, 1969; Woodley and Kenny, 1969) suggest the possibility that much protein digestion occurs in this region and that intralumen and 'membrane digestion' (Ugolev, 1965 and 1968) may not be clearly distinguishable. The concept that adsorbed pancreatic proteases might act in conjunction with superficially placed mucosal peptidases to complete the process of protein digestion (Ugolev,

Jesuitova, Timofeeva, and Chernjahovskaja, 1967) is an attractive one, but here we are entering a controversial area. There are a number of oligopeptidases (peptidases hydrolysing peptides consisting of a few amino-acid units) in the intestinal mucosa, including several dipeptidases. Knowledge of these enzymes and their subcellular location is still very incomplete. While some, including an enzyme which cleaves amino acids sequentially from oligopeptides of some six amino-acid units, may be situated at least partially in the brush border, there is evidence that others are largely located within mucosal cells (Lindberg, 1966; Rhodes, 1968; Peters, 1970). This, in conjunction with transport studies (below), makes it most unlikely that the terminal stages of protein digestion are entirely superficial.

The Forms in which Proteins are Absorbed

Whole proteins are not absorbed in the adult animal except on a very small scale. The occurrence of this in man is suggested by the allergic reactions shown by some individuals to certain foodstuffs. In newborn animals, there is extensive absorption of whole proteins from milk, including maternal antibodies. It occurs by pinocytosis—the invagination of the cell membrane to form minute vesicles (Clark, 1965).

The question to be discussed here is that of the form or forms in which protein is absorbed in the adult mammal. In the latter part of the 19th century, it was widely believed that proteins were absorbed as polypeptides, but following the demonstration by Cohnheim in 1901 that there was peptidase activity ('erepsin') in the 'succus entericus' (fluid collected from the intestinal lumen) it gradually became accepted that proteins were completely hydrolysed to amino acids in the intestinal lumen before absorption, and then absorbed by diffusion. This became the classical view of protein absorption (eg, Verzá and McDougall, 1936). In the years before 1914, several investigators reported that 'peptones' produced by tryptic hydrolysis of protein disappeared from the intestinal lumen more rapidly than amino acids (Messerli, 1913; Verzá and McDougall, 1936), which seemed to suggest that some protein at least might be absorbed as peptides, but as the classical view of protein absorption hardened, these claims were regarded with scepticism or ignored. In fact, by 1935, this view had become so firmly established that Heath and Fullerton (1935), whose results in man suggested to them that 'peptone' was absorbed more rapidly than the same quantity of glycine, stated that their data could not be interpreted in this way, because the peptone 'containing mostly larger molecules than glycine, should theoretically be absorbed more slowly'. The

final vindication of the classical view appeared to be provided by the demonstration, using recently developed chromatographic techniques (Dent and Schilling, 1949; Stein and Moore, 1954), that only free amino acids appeared in the portal and peripheral blood after a protein meal. The concept of protein absorption as involving complete intralumen hydrolysis to amino acids by oligopeptidases in 'succus entericus', though extremely strongly entrenched until quite recently, as reference to textbooks will show, was never in fact fully satisfactory. Thus Cajori (1933) reported that the peptidase activity in the intestinal lumen was apparently inadequate to account for absorption in the form of free amino acids, and Florey, Wright, and Jennings (1941) concluded that peptide hydrolysis was not primarily a function of the 'succus entericus' but of the mucosal cells within it. Fisher (1954 and 1967) made several cogent criticisms of the classical view, and pointed out that complete hydrolysis of protein by known digestive proteases *in vitro* was so slow that it seemed impossible that proteins could be absorbed as free amino acids. This led him to suggest that absorption in some form other than that of amino acids must still be considered. Crane and Neuberger (1960a), using ^{15}N -labelled protein, found that small doses were absorbed in healthy man with remarkable rapidity. In spite of these observations, the majority of investigators continued to concentrate on the absorption of free amino acids or amino acid mixtures simulating the composition of dietary proteins.

In 1959, however, it was shown by Newey and Smyth that the dipeptide glycylglycine was taken up intact from the intestinal lumen and hydrolysed by the mucosal cells. This observation initiated the complete breakdown of the hypothesis of intralumen hydrolysis to amino acids. The present situation is that there appear to be at least two modes of absorption of protein digestion products, (1) absorption of free amino acids and (2) intestinal mucosal uptake of oligopeptides, with hydrolysis to amino acids by the peptidases of the mucosal cells. With a few exceptions, which include some peptides of hydroxyproline (Prockop and Sjoerdsma, 1961; Bronstein, Haffner, and Kowlessar, 1966; Hueckel and Rogers, 1969), and glycylglycine when given in large doses, intact peptides do not appear in the bloodstream. A great deal of work remains to be done on the details of the second mode of protein absorption, and on the question of its nutritional importance, but already recent developments are making it easier to understand some of the previously puzzling features of protein absorption, and breaking new ground in the study of amino-acid transport defects in man.

Mechanisms of Amino Acid Absorption

For many years it was thought that amino acids were absorbed by diffusion. Evidence that simple diffusion could not be responsible was provided by the demonstration that the L-isomers of many amino acids (the forms occurring in foodstuffs) were absorbed much more rapidly than the corresponding 'unnatural' D-isomers (Gibson and Wiseman, 1951). The application of techniques *in vitro* for studying absorption (Wiseman, 1951) made it possible to show that nearly all L-amino acids (Wiseman, 1968) and some D-amino acids (Daniels, Newey, and Smyth, 1969) are absorbed by active transport. This means that their transport across the cell membrane is mediated by a 'carrier' mechanism (the carrier probably being a specific protein) and that transport is driven by metabolic energy. Active transport mechanisms are capable of transporting a substrate against an electrochemical gradient (for example from a region of low concentration to one of high concentration) and probably of accelerating transport in the direction of an electrochemical gradient. It is now established that active transport of amino acids is not driven directly by metabolic energy, but—like that of glucose (Crane, 1968) and some other substrates—indirectly by means of a linkage between amino acid transport and the transport of sodium. This is known as 'secondary active transport'.

When two or more amino acids are present in the intestinal lumen in a mixture, it has been shown that in many cases there is competition for intestinal transport, the presence of one amino acid inhibiting transport of another. Study of this phenomenon has shown that the individual amino acids fall into several different 'transport groups', according to their chemical structure. The members of each group compete among themselves for transport, but on the whole, do not compete for transport with members of other groups. It is believed that the different transport groups may reflect the existence of different carriers, each responsible for the transport of members of one of the groups. This concept is supported by the existence of genetically determined transport defects in man in which the ability to transport members of one group is lost, while transport of other groups is unaffected. Though the subject is complicated by species differences, and the fact that certain amino acids may be transported by more than one mechanism (ie, belong to more than one transport group), a simplified classification of transport groups is as follows:

(1) The monoaminomonocarboxylic (neutral) amino acids: there are strong indications that this group has in fact several subdivisions. In particular,

glycine, proline, and hydroxyproline behave as if transported partly by a separate mechanism. In man, they may be regarded as constituting a virtually separate transport group.

(2) The diamino (basic) amino acids and cystine: this group includes the diamino acids ornithine, arginine, and lysine and the neutral, sulphur-containing amino acid, cystine.

(3) The dicarboxylic (acidic) amino acids: the members of this group, glutamic and aspartic acid, are probably transported by a specific mechanism, but active transport has not yet been demonstrated.

Individual amino acids have widely different affinities for the intestinal transport mechanisms. This leads to preferential absorption of certain amino acids when the small intestine is presented with a mixture (Orten, 1963; Gray, Adibi, and Menden, 1968). Among the neutral amino acids, the length of the side-chain has an important influence on transport characteristics. Long side-chains appear to confer a high affinity for the transport mechanisms, accompanied by a low maximal transport capacity. Such amino acids (eg, methionine and leucine) are particularly effective inhibitors of intestinal transport of amino acids with shorter side-chains, such as alanine.

The intestine is a very active site of protein synthesis and it is probable that a small percentage of amino acids is diverted to this during absorption. In general, there is little metabolic transformation of amino acids during their passage across the intestinal wall, though large amounts of L-glutamic and L-aspartic acids are transaminated during absorption (Matthews and Wiseman, 1953), appearing in the portal blood as alanine. In the dog, transamination is complete except at very high dose levels (Neame and Wiseman, 1957 and 1958). Recent studies on the toxicity of L-glutamate given by mouth, arising out of the 'Chinese restaurant syndrome' due to seasoning with glutamate, appear to have ignored this consideration.

Mucosal Uptake of Oligopeptides and its Mechanisms

Early work with small intestine *in vitro* showed that dipeptides did not cross the intestinal wall except in traces (Agar, Hird, and Sidhu, 1954; Wiggans and Johnston, 1959), though the amino acids resulting from their hydrolysis were transported to the serosal side of the intestine. The first to show uptake of intact oligopeptides by the intestinal mucosa were Newey and Smyth, who found that extracellular hydrolysis could not possibly account for the amounts of amino acid transferred to the serosal side of the gut. In a series of experiments (Newey and Smyth, 1959, 1960, 1962, and 1964; Smyth, 1964)

carried out *in vivo* and *in vitro* they demonstrated that hydrolysis of several oligopeptides was a cellular phenomenon, and, in the case of glycylglycine, probably took place intracellularly following entry of the peptide by a special mechanism. Criticisms of this work on the grounds that the behaviour of glycylglycine, which was used in most of the experiments, might be anomalous (Rhodes, Eichholz, and Crane, 1967) have subsequently been shown to be invalid, and the work has been amply confirmed.

When the study of the absorption of glycine and its peptides was extended to healthy man, a striking phenomenon emerged. Absorption of a given quantity of glycine was most rapid when given as the tripeptide glycylglycylglycine, less rapid from the dipeptide glycylglycine, and least rapid from the free amino acid (Craft and Matthews, 1968; Craft, Geddes, Hyde, Wise, and Matthews, 1968) (Fig. 1). This phenomenon, which at first seemed surprising, was soon found to occur in the rat, and not only with di-, tri-, and tetraglycine, but with the di- and tripeptides of L-methionine (Matthews, Lis, Cheng, and Crampton, 1969). It has now been shown in several species of mammal, including carnivorous,

Fig. 1 Mean changes in plasma glycine nitrogen following oral administration of equivalent doses of glycine, glycylglycine, and glycylglycylglycine to normal human volunteers. The doses (glycine 10 g/70 kg, di-glycine 8.8 g, and triglycine 8.4 g) contained the same number of glycine units. (Reproduced from Craft et al, 1968, by permission of the Editor of Gut.)

herbivorous, and omnivorous species (Lis, Cramp-ton, and Matthews, 1971). The observation that amino acids may be absorbed more rapidly when presented to the intestinal mucosa as oligopeptides than as the equivalent free amino acid entirely confirms the conclusion of Newey and Smyth that peptides must be taken up intact by the intestinal

mucosa. If peptide hydrolysis occurred entirely within the intestinal lumen, the resulting amino acid could not possibly be absorbed more rapidly than the equivalent amino acid presented in the free form (Fig. 2).

Investigations of the intestinal transport of mixed peptides of L-methionine and glycine, carried out

Fig. 2 Scheme representing equivalent doses of glycine and its peptides before and after hydrolysis. After hydrolysis of the peptides doses of all these compounds are identical. If hydrolysis of the peptides occurred within the intestinal lumen, the rates of absorption of glycine from the peptides might approach the rate of absorption of free glycine but could not exceed it.

Fig. 3 Uptake of L-methionine and glycine from the dipeptide L-methionylglycine and the equivalent amino-acid mixture by everted rings of rat small intestine in vitro. When the dipeptide is presented, competitive inhibition of glycine transport is avoided and both amino acids are transported at approximately equal rates. Total transport of amino acid units is very much greater than from the mixture. I = $\pm$ SEM. (Drawn from data of Cheng et al, 1971.)

in vivo and *in vitro*, and including concentrations low enough to be in the likely physiological range, showed an additional phenomenon (Matthews *et al.*, 1969; Cheng and Matthews, 1970; Cheng, Navab, Lis, Miller, and Matthews, 1971). When the amino acids were presented as a mixture, absorption of glycine was inhibited by methionine. When they were presented as the corresponding di- or tripeptide (methionylglycine, glycylmethionine, or methionylglycylmethionine) this inhibition was partly or completely avoided, the resulting absorption of amino-acid units being greater, except at the lowest concentrations, than from the equivalent mixture (Fig. 3). Avoidance of competition between amino acids for transport when presented as oligopeptides is also completely incompatible with the hypothesis that intralumen hydrolysis of peptides precedes uptake of the liberated amino acid, even if this hydrolysis occurs in extremely close proximity to the cell surface. Like the preceding observation, it shows that some stage in peptide transport must precede hydrolysis.

Recent reports (Edwards, 1970; Hellier, Perret, and Holdsworth, 1970) show that more rapid absorption of amino acids from peptides than the corresponding amino-acid mixtures occurs with several mixed peptides composed of various neutral amino acids other than methionine and glycine, and also appears to occur with a dipeptide (glycyl-L-lysine) composed of amino acids of different transport groups. It may not, however, occur with all dipeptides (Asatoor, Bandoh, Lant, Milne, and Navab, 1970a). One interesting feature of dipeptide absorption is that a pair of dipeptides composed of the same two amino acids may show different absorption rates. Thus, in the rat, absorption of the amino acids from glycyl-L-tryptophan is slower than from the equivalent mixture of amino acids, while absorption from L-tryptophylglycine is more rapid (Asatoor, Cheng, Edwards, Lant, Matthews, Milne, Navab, and Richards, 1970b; Edwards, 1970).

Shortly after it was established that mucosal uptake of oligopeptides occurred in healthy man, Milne and his colleagues tested the ability to absorb amino acids from oligopeptides in a disorder of intestinal amino-acid transport—Hartnup disease. The results are discussed elsewhere in this symposium (Milne, 1971). They provide strong support for the importance of oligopeptide uptake in protein absorption in man.

One difficulty in the study of oligopeptide absorption is the great number of oligopeptides. It is undoubtedly significant that almost all the small peptides studied by Newey and Smyth and subsequent workers have provided evidence that they are

taken up intact by the intestinal mucosa (Table I). However, there are about 400 possible dipeptides and 8,000 possible tripeptides, and it is clearly impossible to study the characteristics of absorption of every one. In an attempt to assess the general importance of oligopeptide uptake in absorption of protein digestion products, a study has been made of the rates of absorption of pancreatic digests of several proteins (lactalbumin, lysozyme, bovine albumin, and casein), which consist mainly of oligopeptides of 2-6 amino-acid units, and the equivalent mixtures of free amino acids (Gangolli, Simson, Lis, Cheng, Crampton, and Matthews, 1970; Crampton, Gangolli, Matthews, and Simson, 1971). In all cases, the pancreatic digests disappeared from the intestinal lumen more rapidly than the corresponding amino-acid mixtures. This suggests that relatively rapid absorption from oligopeptides is a general phenomenon, and that oligopeptide uptake is a major mode of protein absorption. It suggests

Peptide	Reference
Glycylglycine ^a	Newey and Smyth (1959, 1960, and 1962); Craft <i>et al.</i> (1968); Matthews <i>et al.</i> (1968); Peters and MacMahon (1970); Asatoor <i>et al.</i> (1970a) Craft <i>et al.</i> (1968); Matthews <i>et al.</i> (1968)
Glycylglycylglycine	Newey and Smyth (1959 and 1960)
Glycylglycylglycylglycine	Newey and Smith (1960); Asatoor <i>et al.</i> (1970b)
Glycyl-L-leucine	Newey and Smith (1959 and 1960); Tarlow <i>et al.</i> (1970b)
Glycyl-L-tryptophan	Newey and Smith (1959 and 1960); Tarlow <i>et al.</i> (1970b)
Glycyl-L-tyrosine	Newey and Smith (1959 and 1960); Tarlow <i>et al.</i> (1970b)
L-leucylglycine	Newey and Smith (1960)
L-methionyl-L-methionine	Craft <i>et al.</i> (1969); Matthews <i>et al.</i> (1970); Cheng and Matthews (1971)
L-methionyl-L-methionyl-L-methionine	Craft <i>et al.</i> (1969); Matthews <i>et al.</i> (1969)
Glycyl-L-methionine	Craft <i>et al.</i> (1969); Matthews <i>et al.</i> (1970); Cheng <i>et al.</i> (1971)
L-methionylglycine	Craft <i>et al.</i> (1969); Matthews <i>et al.</i> (1970); Cheng <i>et al.</i> (1971)
L-methionylglycyl-L-methionine	Matthews <i>et al.</i> (1969)
β -Alanyl-L-histidine (carnosine)	Navab and Asatoor (1970)
L-phenylalanyl-L-phenylalanine	Asatoor <i>et al.</i> (1970b)
Glycyl-L-histidine	Tarlow <i>et al.</i> (1970b)
Prolylhydroxyproline ^a	Hueckel and Rogers (1969)
Glycyl-L-lysine	Hellier <i>et al.</i> (1970)
L-leucyl-L-alanine	Edwards (1970)
L-alanylglycine	Edwards (1970)
Glycyl-L-alanine	Edwards (1970)
L-tryptophylglycine	Edwards (1970)
L-leucyl-L-tyrosine	Edwards (1970)
L-alanyl-L-alanine	M. T. Lis <i>et al.</i> (1971)
γ -Glutamylsteinyglycine (glutathione) ^a	Evered and Wass (1970)

Table I Peptides reported to be taken up intact by the intestinal mucosa

^aThe data come from animal experiments and investigations in man.

^bItalics indicate that absorption of amino acids from the peptide has been found to be more rapid than from the equivalent free amino acid or amino-acid mixture.

^cMucosal hydrolysis is incomplete or does not occur.

also that the observations of nearly 60 years ago (eg, Messerli, 1913) concerning the rapidity of absorption of 'peptones' may have been correct. Further evidence for the occurrence of mucosal oligopeptide uptake in man is provided by an intubation study of the digestion and absorption of test meals containing milk protein or gelatin (Nixon and Mawer, 1970a and b). Certain amino acids, which are absorbed relatively slowly from mixtures (such as proline, glycine, and the dicarboxylic amino acids), were absorbed much more rapidly after ingestion of protein, so that the differences in absorption rates of individual amino acids from such mixtures were partly 'ironed out'. The authors concluded that experiments in which amino acid mixtures were fed did not give a true picture of the absorption rates prevailing after feeding proteins. A preliminary report of experiments in a reptilian species (*C. latirostris*—the cayman) suggests similar results (Coulson and Hernandez, 1970).

Though several lines of evidence now indicate the existence and probable nutritional importance of mucosal oligopeptide uptake, very little is known as yet of the details of the mechanisms involved. The finding that peptide uptake is reduced by anoxia (Newey and Smyth, 1960 and 1962; Cheng *et al*, 1971) suggests that the uptake mechanism is dependent on metabolic energy. An important question is whether peptides are taken up by the same mechanisms that take up amino acids, as indicated by the work of Newey and Smyth (1962) and supported by some subsequent observations (Matthews, Craft, Geddes, Wise, and Hyde, 1968; Matthews *et al*, 1969), or whether there are independent peptide uptake systems as in bacteria (Payne, 1968; Payne and Gilvarg, 1971). An investigation recently carried out in Hartnup disease with the dipeptide L-phenylalanyl-L-phenylalanine (Asatoor *et al*, 1970b) shows that dipeptide uptake must be at least partly independent of amino-acid uptake. The finding that the effects of dietary alterations on dipeptide and amino-acid transport are not identical (Crampton, Lis, and Matthews, 1970) is also compatible with this conclusion. A recent report (Edwards, 1970) suggests that there may be at least two independent dipeptide uptake systems in mammalian gut. Study of the interrelationships between intestinal transport of amino acids and dipeptides is complicated by the fact that amino acids can affect the rates of dipeptide hydrolysis, in most cases causing inhibition, but in some, stimulation (Newey and Smyth, 1962; Cheeseman, Newey, and Smyth, 1971).

The precise site of hydrolysis of oligopeptides during absorption—whether superficial or intracellular—is still uncertain, and, indeed, there may be more than one site. Some experiments have

suggested that hydrolysis is intracellular. The appearance of small quantities of intact glycylglycine in the portal blood during absorption of this peptide (Newey and Smyth, 1959; Peters and MacMahon, 1970) strongly suggests that this peptide can enter the cells intact, and Fern, Hider, and London (1969) found unhydrolysed glycylglycine in the intestinal mucosa in experiments *in vitro*. The hypothesis of intracellular hydrolysis is supported by some of the data on the subcellular distribution of peptidases (Robinson, 1963; Josefsson and Sjöström, 1966; Peters, 1970). However, a feature of many experiments on intestinal dipeptide transport is the appearance of large quantities of free amino acids in the gut lumen or mucosal compartment while peptide absorption is going on. With some dipeptides, this 'back-diffusion' is so extensive as to suggest that much hydrolysis occurs at a superficial site. Fern *et al* (1969) concluded from a kinetic study of the uptake of mixed peptides containing glycine and leucine that hydrolysis of these peptides was entirely superficial, and that there was no initial peptide uptake step. This supports the conclusion of Ugolev and his colleagues (Ugolev, Jesuitova, Timofeeva, and Fediushina, 1964; Ugolev, 1965 and 1968) who maintain that the hydrolysis of oligopeptides is, in general, a superficial process, the site of hydrolysis being external to the transport mechanisms, as is believed to be the case with oligosaccharides (Crane, 1968). Peters and MacMahon (1970) studying absorption of glycine, diglycine, triglycine, and tetraglycine, concluded that the higher peptides were hydrolysed sequentially at the cell surface, only free glycine and the dipeptide entering the mucosal cells. Their conclusions are incompatible with the previous observation that glycine is more rapidly absorbed from triglycine than from diglycine, and the reasons for this discrepancy are not clear.

As a result of work on the kinetics of uptake of oligopeptides of glycine and methionine *in vitro*, including observations (Cheng, Navab, and Matthews, 1969; Cheng and Matthews, 1970; Cheng *et al*, 1971) on the effects of L-amino acid oxidase (an enzyme which destroys methionine but not the peptides) on this, the writer and his colleagues have recently suggested that there may be two modes of uptake: (1) superficial hydrolysis by mechanisms closely linked to the amino-acid entry mechanisms and (2) peptide entry into the cells by a special mechanism or mechanisms followed by intracellular hydrolysis, as originally proposed by Newey and Smyth. This dual hypothesis seems to account for all the experimental and clinical observations at present available.

The complexity of the mechanisms of absorption

of protein digestion products will now be apparent. A tentative summary of protein digestion and absorption (Fig. 4) is as follows. Gastric digestion, followed by pancreatic digestion within the intestinal lumen and at the brush border of the mucosal cells, produces a complex mixture of peptides and amino acids, in which peptides predominate. The peptides may undergo further hydrolysis by mucosal peptidases at the brush border, with uptake of the resultant amino acids; however, there is also substantial uptake of di- and tripeptides by a special mechanism or mechanisms, and this is followed by hydrolysis, probably within the mucosal cells. Some free amino acids may also be liberated from oligopeptides by desquamated cells within the intestinal lumen. The result is that proteins appear in the portal blood almost entirely in the amino acid form, the concerted action of pancreatic enzymes, mucosal peptidases, and systems of mucosal peptide uptake resulting in far more rapid hydrolysis to free amino acids than could be accomplished by pancreatic proteases acting alone. During absorption of protein, the preferential absorption of some amino acids and retardation of absorption of others that occurs

during the absorption of amino-acid mixtures is minimized.

Malabsorption of Proteins

Malabsorption of proteins may be due to gastric, pancreatic, or intestinal defects. It has already been stated that loss of gastric secretions does not seriously affect protein digestion. When defects of protein absorption occur after partial or total gastrectomy, they are apparently the results of other causes such as imperfect mixing of food entering the intestine, derangement of the mechanisms responsible for stimulating pancreatic secretion, and other associated or concurrent disorders such as the presence of blind loops, pancreatic insufficiency, and an adverse effect of protein malnutrition on the absorptive ability of the intestinal mucosa (Crane, 1961; French and Crane, 1963; Neale, Antcliffe, Welbourne, Mollin, and Booth, 1967). After gastrectomy, entry of food into the upper small intestine is often abnormally rapid, with the result that absorption may be faster than normal. This occurs with glucose and ethanol, and has also been shown with

Fig. 4 Tentative scheme of protein absorption. According to this scheme, the only biochemical error likely to produce major disturbance in protein absorption would be a defect in the hypothetical exit mechanism(s) by which amino acids leave the mucosal cells.

glycine and glycyglycine (Craft *et al*, 1968) and whole protein (Richmond and Girdwood, 1962).

Severe impairment of pancreatic secretion causes a serious defect in protein digestion and consequently in absorption, though even after total pancreatectomy the ability to absorb protein is not completely lost. One patient who had both partial gastrectomy and total pancreatectomy apparently absorbed about two-thirds of the protein in a diet containing 100 g a day, and survived for many years (Crane, 1961). In chronic pancreatic disease in adults and children, variable degrees of impairment of protein absorption result. As might be expected, absorption is poor when whole protein is given, but absorption of free amino acids is normal (Christensen and Shwachman, 1949; Anfanger and Heavenrich, 1949; Chinn, Lavik, Babb, Buckaloo, Stitt, and Abbott, 1953; Richmond and Girdwood, 1962; Laster and Matthews, 1963; Bronstein *et al*, 1966; Craft *et al*, 1968).

Intestinal Defects

Resections of the small intestine must be extensive before protein absorption is severely disturbed. In a case with a resection of 2 m of terminal ileum, faecal nitrogen was within normal limits; in another with resection of all the small intestine except the duodenum and about 1.3 m of jejunum, it was raised to about 6 g per day (Booth, 1961). Even when only duodenum remains, survival is possible (Scheiner, Shils, and Vanamee, 1965). Several patients with ileal resections of 1 to 1.5 m had normal glycine absorption, and one with a proximal resection of 1 m absorbed glycine and glycyglycine normally (Craft *et al*, 1968). There is evidence that absorption of free amino acids improves over a period of several months following extensive small-intestinal resections (Althausen, Doig, Uyeyama, and Wieden, 1950).

In coeliac disease and other small-intestinal diseases associated with malabsorption of fat and other substances, protein absorption is probably often severely impaired, but some observations are somewhat puzzling. Many cases show a large increase in faecal nitrogen (Cooke, Thomas, Mangall, and Cross, 1953), which may be contributed to by substantial losses of protein into the gut. Crane and Neuberger (1960b and c) investigated the absorption of ^{15}N -labelled yeast protein and the corresponding pancreatic hydrolysate in idiopathic steatorrhoea. They found that absorption was considerably delayed when the whole protein was given, but much less delayed after the hydrolysate, and suggested that these effects were due to a reduction in total absorbing surface combined with a deficiency of intestinal peptidases. 'Tolerance tests'

using free amino acids have usually shown delayed absorption in generalized disease of the small intestine (Erf and Rhoads, 1940; Butterworth, Santini, and Perez-Santiago, 1958; Craft *et al*, 1968). Brice, Owen, and Tyor (1965) found that uptake of amino acids by intestinal biopsy specimens was reduced. Richmond and Girdwood (1962) gave casein orally to patients with idiopathic steatorrhoea and followed several plasma amino acids; they were unable to find any abnormality by this means. Particularly interesting results have recently been obtained by Douglas and Booth (1969), who measured plasma amino acids after giving test doses of albumin and wheat gluten to cases of adult coeliac disease. After albumin the increase in amino-acid concentration was delayed, but after gluten there was no delay and the increases in concentration were greater than in controls. The results with gluten are not readily explicable at the present time. They do not support the hypothesis that gluten digestion is impaired in coeliac disease.

There have now been a number of investigations of mucosal peptidases in various small-intestinal disorders. Many of these have been stimulated by the hypothesis that coeliac disease is due to deficiency of a peptidase responsible for the final stages of digestion of gluten, allowing accumulation of a toxic peptide that damages the cells (Frazer, 1956). Though Pittman and Pollitt (1966) reported that mucosal digestion of gluten peptides was abnormal in coeliac disease, with failure to liberate proline, they recognized that this might be a secondary phenomenon, and although a toxic peptide of molecular weight 1,000-1,500 can be isolated from gluten, attempts to verify Frazer's hypothesis have not been successful (Messer, Anderson, and Townley, 1961; Douglas and Booth, 1970; Booth, 1970a and b). There is, however, a good deal of evidence that a moderate secondary depression of hydrolase activity against several dipeptides occurs as the result of generalized mucosal disease (Dolly and Fottrell, 1969; Heizer and Laster, 1969; Berg, Dahlqvist, Lindberg, and Nördén, 1970). The depression of activity may be rather more marked in the case of the peptidase splitting dipeptides containing proline, perhaps because it is mainly located in the brush border rather than the interior of the cell (Heizer and Laster, 1969). Whether the reduced peptidase activity in mucosal disease has any significant effect on the absorption of protein digestion products requires further investigation; reduction in mucosal area and impairment of amino acid and oligopeptide transport mechanisms may be equally or more important. The initial investigations of the absorption of glycine and glycyglycine in small-intestinal disease (Craft *et al*, 1968) did not suggest

that deficiency of glycylglycine dipeptidase was usually important. If it had been, it might have been expected that the increases in plasma glycine following the dipeptide would have been depressed relative to those following free glycine. However, extension of this work to a much larger series of cases (Sadikali, 1971), combined with estimation of glycylglycine dipeptidase activity in intestinal biopsies, suggests that in some cases the dipeptidase activity, which is normally relatively low, may be sufficiently depressed to become a rate-limiting factor in absorption of amino acid from this peptide.

It is natural to wonder why it is that though disorders due to intestinal disaccharidase deficiencies are well recognized, no disorders due to deficiencies of mucosal peptidases have yet been described. One reason may be that individual peptides are not ingested in such quantity as individual disaccharides. Though it is possible that disorders due to defects in mucosal peptide hydrolysis or transport will eventually be found, the situation may be that the final stages of digestion and absorption of protein are so complex that with one possible exception (Fig. 4) no single biochemical lesion will seriously impair this process. It may be pointed out that intestinal amino-acid transport defects, even when involving several amino acids, produce no clinical manifestations directly referable to impairment of protein absorption, and very little disturbance in protein nutrition (Milne, 1971). The mucosal peptidases are numerous, and have overlapping specificities, so that a single defective peptidase might produce no clinical disturbance, and its accidental detection by present assay methods would be difficult. Should an 'affected' peptide be absorbed intact, it might be hydrolysed by the liver and fail to appear in the peripheral blood. Even if the peptide entry mechanism(s) were defective, the ability to absorb free amino acids resulting from pancreatic and brush border hydrolysis would still remain. For these reasons the mechanisms of protein absorption may be less vulnerable to biochemical error than the simpler processes of carbohydrate absorption, in which a single transport defect, or a single hydrolase deficiency, produce serious disturbance.

Finally, it may not be out of place to comment on the bearing of recent findings in protein absorption on the use of amino-acid mixtures in nutrition, since diets containing such mixtures are now becoming commercially available for use in metabolic disorders. Some of the older work (see Fisher, 1954) suggested that the growth of animals was not as good on mixtures of free L-amino acids as on protein with the same amino-acid composition. At the time, this was attributed to the possible existence of growth factors such as undiscovered amino acids

or essential peptides. It now seems conceivable that it could have been due to imbalance in the amino acids reaching the tissues, resulting from the differences in absorption rates of individual amino acids from amino-acid mixtures and from the normal digestion products of whole protein. When amino-acid mixtures are absorbed from the intestine, some amino acids are absorbed unduly slowly, and competitive phenomena lead to retardation and 'temporal displacement' of the absorption of others. The time factor is important in amino-acid metabolism, simultaneous presentation leading to optimal utilization. In view of these theoretical considerations, it is reassuring that a 'chemically defined diet', containing only L-amino acids and glycine in place of the protein component, will maintain nutrition in adults (Winitz, Seedman, and Graff, 1970) and support growth in children (McKean, 1970). Nevertheless, unqualified acceptance of the suitability of such diets in health and disease would be premature, reflecting a persistence of the tendency to equate protein absorption with the absorption of free amino acids. It should be pointed out that the use of a 'chemically defined diet' in cases of intestinal amino-acid transport defects would be expected to have serious consequences.

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