

## PROCEEDINGS OF THE NUTRITION SOCIETY

*The Two Hundred and Forty-fourth Scientific Meeting was held jointly with the Society of Endocrinology at the University of Southampton on 21 and 22 April 1972*

### SYMPOSIUM ON 'PROTEIN METABOLISM AND HORMONES'

#### Intestinal absorption of amino acids and peptides

By D. M. MATTHEWS, *Department of Experimental Chemical Pathology, Vincent Square Laboratories of Westminster Hospital, 124 Vauxhall Bridge Road, London SW1*

The aim of this account is to summarize current views on the absorption of the products of protein digestion, amino acids and peptides. Longer reviews of protein absorption (Wiseman, 1968; Matthews, 1969, 1971; Gray & Cooper, 1971) will provide references not given here. Hormonal effects on absorption are reviewed by Levin (1969).

Protein digestion is initiated in the stomach by pepsins and continued in the small intestine by the pancreatic proteases. Though much digestion occurs within the lumen of the gut, the action of proteases adsorbed at the brush border may also play an important part. After a protein meal, the intestinal lumen contains a complex mixture of small peptides and free amino acids, in which peptides predominate. Further breakdown of oligopeptides to dipeptides and free amino acids may occur through the action of brush-border oligopeptidases.

It is now established that though, in general, only free amino acids enter the portal blood during protein absorption, protein digestion products leave the intestinal lumen in two forms (1) as free amino acids and (2) as small peptides (Fig. 1). Since the components of proteins are transported within the body almost entirely as free amino acids, the mucosal epithelium of the small intestine is distinguished from other epithelia by its ability to handle small peptides on a large scale. Comparatively little is known at the present time about the mechanisms of absorption of peptides.

#### *Absorption of amino acids*

Amino acids are absorbed by carrier-mediated transport, and for glycine and most L-amino acids, transport has been shown to be active. In adult small intestine, energy for transport is derived mainly, but perhaps not exclusively (De la Noüe, 1970), from oxidative metabolism. The active transport mechanisms appear to be situated in the plasma membrane of the mucosal poles of the absorptive cells, since amino acids are concentrated in the cells while transport is going on. For some years it was thought that the transport mechanisms were stereochemically specific for L-amino acids, but it has now been shown that several D-amino acids are actively

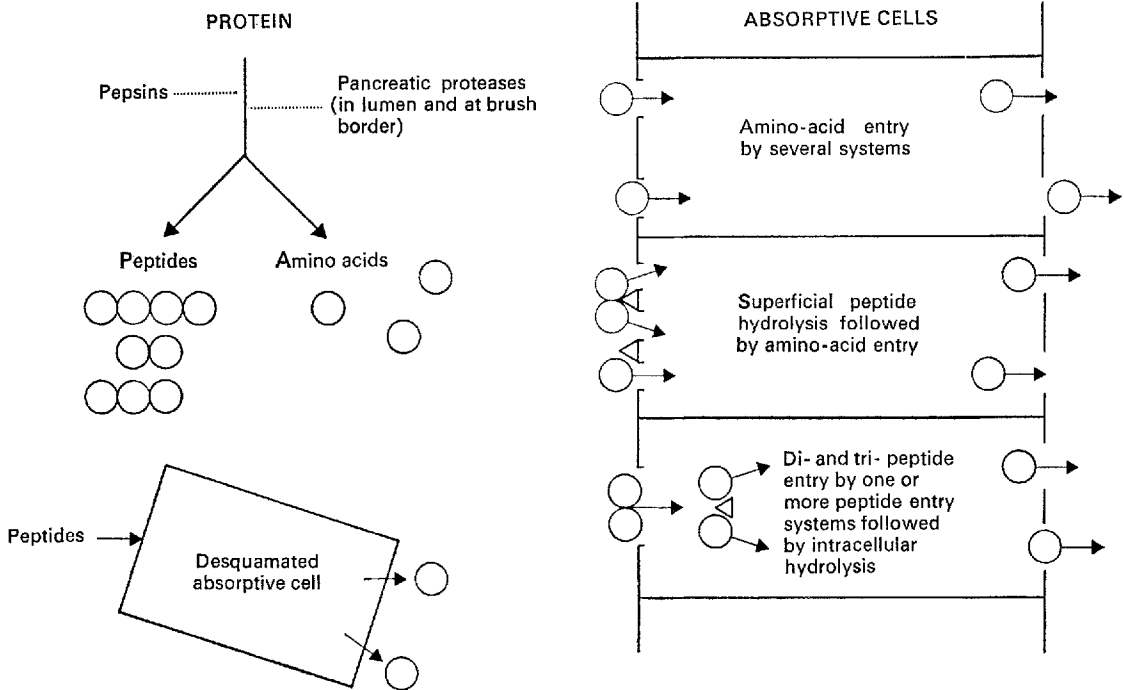


Fig. 1. Tentative scheme of protein absorption. (Reproduced from Matthews (1971), by permission of the Editor of the *Journal of Clinical Pathology*.)

transported (De la Noüe, Newey & Smyth, 1971). Structural requirements for transport are outlined by Wilson (1962), Matthews & Laster (1965) and Wiseman (1968). The active transport of amino acids by the mucosal cells is, like that of many other solutes, sodium-dependent. According to the hypothesis of Schultz & Curran (1970) the transport of sodium and amino acids is coupled, probably by linkage to common carriers. Sodium is pumped out of the cells by the 'ordinary' sodium extrusion system that is found in nearly all animal cells and is linked direct to metabolic energy. This maintains a low concentration of sodium within the cells. Sodium enters the cells down the resulting gradient via the coupled transport systems, bringing amino acids with it and enabling them to be concentrated intracellularly. If this scheme is correct, the transport of amino acids (and several other solutes) is not linked direct to metabolic energy, and thus falls outside any definition of active transport which is restricted to transport coupled direct to energy-yielding chemical reactions. It has been suggested that the term 'secondary active transport' should be used in this situation, and the term 'primary active transport' for transport linked direct to metabolism (Smyth, 1969).

It is believed that carrier-mediated mechanisms are involved in the exit of amino acids from the serosal poles of the mucosal cells, and it has been suggested that exit from the cells is the result of non-sodium-dependent facilitated diffusion (Schultz & Curran, 1970). Comparatively little is known about the exit mechanisms, but they appear to differ in several respects from those for entry.

*Competition for intestinal transport and transport groups*

The results of animal experiments, in particular experiments on competition for intestinal transport (usually in rat or hamster), supported by observations on intestinal transport defects in man, have suggested the existence of several 'transport groups' of amino acids, the members of each group appearing to share a different carrier. The picture is complicated by the existence of species differences (Matthews & Laster, 1965; Nelson & Lerner, 1970), the fact that many amino acids behave as if transported by two or even more carriers, and the demonstration of interactions, involving inhibition or stimulation of transport, between members of different groups. It must be borne in mind that an effect of one amino acid on transport of another does not necessarily mean that they share the same carrier. The interaction may be allosteric – due to attachment of an amino acid to one carrier site inducing configurational changes in another adjacent carrier (Reiser & Christiansen, 1969) – or even due to competition for energy supply.

In all species studied, several transport groups may be distinguished.

1. *Neutral amino acids.* This broad group includes at least two major subdivisions. One subgroup contains many neutral amino acids, in particular those with long side-chains, and the other includes glycine, proline and hydroxyproline, which, in man, behave as if they constitute an almost completely separate transport group. Smyth, Newey and their colleagues (Newey & Smyth, 1964; De la Noüe *et al.* 1971) have described two carriers in the rat: a 'methionine carrier' which handles amino acids including methionine, leucine, glycine and proline, prefers long-chain amino acids, is specific for  $\alpha$ -amino acids and favours L-isomers; and a 'sarcosine carrier' which cannot handle methionine or leucine but transports glycine and proline, prefers short-chain amino acids, is less exacting in its stereochemical requirement and tolerates amino groups which are not in the  $\alpha$  position. The sarcosine carrier probably corresponds to Wilson's carrier for nitrogen-substituted amino acids (Wilson, 1962) and the imino acid carrier described by Munck (1966).

Among the neutral amino acids, there is a relationship between size of side-chain and transport characteristics (Finch & Hird, 1960; Matthews & Laster, 1965). Amino acids with large lipophilic side-chains such as methionine and leucine behave as if they had a high affinity for their transport mechanism. They are rapidly transported from low concentrations of the order likely to be found in the intestinal lumen, but have a low maximal transport velocity and are slowly transported from high concentrations. They are powerful inhibitors of the transport of several other neutral amino acids which use the methionine system. Those with short side-chains, such as alanine and glycine, are slowly transported from low concentrations, but have a high maximal transport velocity. They behave as if they had a low affinity for transport by the methionine system, but are fairly effective inhibitors of the transport of sarcosine (Daniels, Dawson, Newey & Smyth, 1969).

2. *Dibasic amino acids and cystine.* This group includes lysine, arginine and ornithine, and the diaminodicarboxylic amino acid, cystine. The dibasic amino acids are transported relatively slowly. This may be the result of a particularly

slow rate of efflux from the serosal poles of the mucosal cells. Lysine transport is not completely sodium-dependent (Munck & Schultz, 1969).

3. *Acidic amino acids*. The dicarboxylic amino acids, glutamic and aspartic acids, have long been suspected of being actively transported, but the extensive transamination that they undergo during absorption has prevented demonstration of transport against a concentration gradient. They are undoubtedly transported by a carrier-mediated mechanism. It has recently been shown that their transport is saturable and sodium-dependent, and that they compete with each other for transport (Schultz, Yu-Tu, Alvarez & Curran, 1970). The sodium-dependence is strongly suggestive of active transport. The transport of glutamic acid is reported to be inhibited by members of the 'neutral' group: isoleucine, histidine and methionine (Finch & Hird, 1960; Tasaki & Takahashi, 1966). Whether glutamic and aspartic acids have a distinct carrier system of their own has not been definitely established.

There are certain broad similarities in the mechanisms of amino acid transport in small intestine, kidney and other tissues. These are strikingly illustrated by the occurrence of transport defects shared by kidney and intestine. There are, however, many important differences in the details of transport groups and the handling of individual amino acids (Neame, 1967; Christensen, 1968; Binder, 1970; Milne, 1971).

#### *Absorption of amino acid mixtures*

Several investigations have been made of the absorption of mixtures containing many amino acids, either in equimolar proportions or proportions simulating the composition of a protein. The results agree fairly well with what might be expected from the kinetic characteristics of transport of single amino acids *in vitro*, and the results of competition experiments using pairs of amino acids. They suggest that interactions between amino acids, involving inhibition or stimulation of transport, are important in the absorption of mixtures *in vivo* (Gitler & Martinez-Rojas, 1964; Holdsworth, 1972). Adibi, Gray & Menden (1967) studied absorption of equimolar mixtures of eighteen amino acids by intestinal perfusion in man. Individual absorption rates varied widely. The most rapidly absorbed were methionine, leucine, isoleucine and valine, whereas glycine was absorbed comparatively slowly. Lysine, whose transport is inhibited by arginine, and the two dicarboxylic amino acids were absorbed slowest of all. Arginine was absorbed more rapidly than expected from its behaviour when alone, probably because its transport is stimulated by other amino acids. The proportion of each amino acid absorbed in a given time tends to remain constant even when the relative concentrations of the constituent amino acids in a mixture are varied within fairly wide limits. When amino acids are absorbed from a mixture, the absorption of those amino acids whose transport is strongly inhibited by others is retarded until the concentration of the powerful inhibitors has fallen. Gitler & Martinez-Rojas (1964) pointed out that the selective absorption occurring from amino acid mixtures might significantly modify the pattern of amino acids available for utilization in protein synthesis. It has recently

been realized, however, that the characteristics of absorption of amino acid mixtures do not give a true picture of the absorption of protein digestion products, and the importance of selectivity in amino acid absorption now seems more doubtful.

#### *Absorption of peptides*

Newey & Smyth (1959, 1962) showed that several dipeptides were taken up by the intestinal mucosal cells, undergoing cellular hydrolysis and appearing in the blood as free amino acids, and they suggested that this might be an important mode of protein absorption. Much additional evidence has recently accumulated in support of this suggestion (Matthews, 1971, 1972). It has been shown that intestinal transport of amino acids from many dipeptides and tripeptides is more rapid than from the equivalent amino acid mixtures (Craft, Geddes, Hyde, Wise & Matthews, 1968; Adibi, 1971). This occurs with peptides composed of neutral, basic and acidic amino acids in several mammalian species, including man. When a peptide is taken up, competition for transport between the constituent amino acids may be partly or completely avoided (Matthews, Lis, Cheng & Crampton, 1969). The nutritional importance of this mode of absorption is suggested by the finding that tryptic hydrolysates of several proteins, consisting mainly of oligopeptides with a proportion of free amino acids, are absorbed more rapidly than the equivalent amino acid mixtures (Crampton, Gangolli, Simson & Matthews, 1971). In the amino acid transport defects of Hartnup disease and cystinuria, the ability to absorb amino acids from peptides is retained (Milne, 1972), which may explain why such patients are comparatively well-nourished and have no obvious intestinal disturbance after protein meals.

Much work remains to be done on the details of the mechanisms of peptide absorption. There is evidence from Hartnup disease and competition experiments that peptide uptake, or at least the uptake of amino acid residues from peptides, is substantially independent of that of free amino acids (Asatoor, Cheng, Edwards, Lant, Matthews, Milne, Navab & Richards, 1970; Rubino, Field & Shwachman, 1971). The effects of dietary alterations on amino acid and peptide absorption are not identical (Lis, Crampton & Matthews, 1972). The question of whether peptides are hydrolysed superficially in the brush border (Ugolev, 1965), with simultaneous attachment of amino acids to transport sites, or whether they enter the mucosal cells and are hydrolysed within them (Newey & Smyth, 1962; Peters & McMahon, 1970), is controversial. It is possible that both these mechanisms operate. Recent work in the author's laboratory indicates that glycylsarcosine, a peptide which is hydrolysed unusually slowly, is transported into the mucosal cells by an active, sodium-dependent mechanism (Matthews, Burston & Addison, 1972).

The occurrence of mucosal peptide uptake on a large scale may explain several features of protein absorption that were not adequately explained by the hypothesis that protein was completely hydrolysed to free amino acids in the intestinal lumen before absorption took place. It has long been known that protein is absorbed far more rapidly than would be expected if it had to undergo complete intralumen hydrolysis (Fisher, 1954) and it can be absorbed as rapidly as an amino acid mixture

(Gupta, Dakroury & Harper, 1958). Nasset (1965) pointed out that if competition for absorption of free amino acids were important in absorption of a protein meal, the molar ratios between rapidly absorbed and slowly absorbed amino acids should change with time and distance from the pylorus—but that this apparently does not happen. Nixon & Mawer (1970a, b), in an intubation study of protein absorption in man, also concluded that the relative rates of absorption of amino acids from protein meals were different from those from amino acid mixtures, and that experiments on the absorption of amino acid mixtures simulating protein did not give a true picture of the events occurring during protein absorption. Their work suggested that though intralumen hydrolysis might account for the absorption of several neutral amino acids and the dibasic amino acids, it was too slow to account for the absorption of glycine, threonine, serine, proline, hydroxyproline and the dicarboxylic amino acids, which might therefore be taken up as peptides.

#### *Appearance of amino acids in the blood*

The pattern of amino acids appearing in the portal and peripheral blood during protein absorption bears some resemblance to the composition of the ingested protein, but does not reflect it particularly closely (Feigin, Beisel & Wannamacher, 1971). It is modified by many factors, including the rates of release of individual amino acids and small peptides during digestion, the rates at which these compounds are taken up by the intestinal mucosa, the simultaneous digestion and absorption of endogenous protein, metabolic transformation during absorption (which is particularly extensive with the dicarboxylic amino acids) and rates of uptake and release of amino acids by the liver and other tissues. There is no evidence for any general increase in plasma peptides during protein absorption, though one or two individual peptides including peptides of hydroxyproline, which are hydrolysed particularly slowly by the intestinal mucosa, have been shown to enter the blood.

#### REFERENCES

- Adibi, S. A. (1971). *J. clin. Invest.* **50**, 2266.  
 Adibi, S. A., Gray, S. J. & Menden, E. (1967). *Am. J. clin. Nutr.* **20**, 24.  
 Asatoor, A. M., Cheng, B., Edwards, K. D. G., Lant, A. F., Matthews, D. M., Milne, M. D., Navab, F. & Richards, A. J. (1970). *Gut* **11**, 380.  
 Binder, H. J. (1970). *Am. J. clin. Nutr.* **23**, 330.  
 Christensen, H. N. (1968). In *Protein Nutrition and Free Amino Acid Patterns* p. 40 [J. H. Leatham, editor]. New Brunswick: Rutgers University Press.  
 Craft, I. L., Geddes, D., Hyde, C. W., Wise, I. J. & Matthews, D. M. (1968). *Gut* **9**, 425.  
 Crampton, R. F., Gangolli, S. D., Simson, P. & Matthews, D. M. (1971). *Clin. Sci.* **41**, 409.  
 Daniels, V. G., Dawson, A. G., Newey, H. & Smyth, D. H. (1969). *Biochim. biophys. Acta* **173**, 575.  
 De la Noüe, J. (1970). *Biochim. biophys. Acta* **203**, 360.  
 De la Noüe, J., Newey, H. & Smyth, D. H. (1971). *J. Physiol., Lond.* **214**, 105.  
 Feigin, R. D., Beisel, W. R. & Wannamacher, R. W. Jr (1971). *Am. J. clin. Nutr.* **24**, 329.  
 Finch, L. R. & Hird, F. J. R. (1960). *Biochim. biophys. Acta* **43**, 278.  
 Fisher, R. B. (1954). *Protein Metabolism*. London: Methuen.  
 Gitler, C. & Martinez-Rojas, D. (1964). In *The Role of the Gastrointestinal Tract in Protein Metabolism* p. 269 [H. N. Munro, editor]. Oxford: Blackwell Scientific Publications Ltd.  
 Gray, G. M. & Cooper, H. L. (1971). *Gastroenterology* **61**, 535.  
 Gupta, J. D., Dakroury, A. M. & Harper, A. E. (1958). *J. Nutr.* **64**, 447.

- Holdsworth, C. D. (1972). In *Transport Across the Intestine* p. 136 [W. L. Burland and P. D. Samuel, editors]. London and Edinburgh: Churchill Livingstone.
- Levin, R. J. (1969). *J. Endocr.* **45**, 315.
- Lis, M. T., Crampton, R. F. & Matthews, D. M. (1972). *Br. J. Nutr.* **27**, 159.
- Matthews, D. M. (1969). *Klin. Wschr.* **47**, 397.
- Matthews, D. M. (1971). *J. clin. Path.* **24**, Suppl. no. 5, p. 29. (R. Coll. Path.).
- Matthews, D. M. (1972). In *Peptide Transport in Bacteria and Mammalian Gut (Proceedings of CIBA Symposium)*. p. 71 [K. Elliott and M. O'Connor, editors]. London: Churchill Livingstone.
- Matthews, D. M., Burston, D. & Addison, J. M. (1972). *Clin. Sci.* **42**, 29P.
- Matthews, D. M. & Laster, L. (1965). *Gut* **6**, 411.
- Matthews, D. M., Lis, M. T., Cheng, B. & Crampton, R. F. (1969). *Clin. Sci.* **37**, 751.
- Milne, M. D. (1971). In *The Scientific Basis of Medicine Annual Reviews* p. 161 [I. Gilliland and J. Francis, editors]. London: Athlone Press.
- Milne, M. D. (1972). In *Transport Across the Intestine* p. 195 [W. L. Burland and P. D. Samuel, editors]. London and Edinburgh: Churchill Livingstone.
- Munck, B. G. (1966). *Biochim. biophys. Acta* **120**, 97.
- Munck, B. G. & Schultz, S. G. (1969). *Biochim. biophys. Acta* **183**, 182.
- Nasset, E. S. (1965). *Fedn Proc. Fedn Am. Socs exp. Biol.* **24**, 953.
- Neame, K. D. (1967). In *Progress in Brain Research* Vol. 29, p. 185 [A. Lajtha and D. H. Ford, editors]. Amsterdam: Elsevier.
- Nelson, K. M. & Lerner, J. (1970). *Biochim. biophys. Acta* **203**, 434.
- Newey, H. & Smyth, D. H. (1959). *J. Physiol., Lond.* **145**, 48.
- Newey, H. & Smyth, D. H. (1962). *J. Physiol., Lond.* **164**, 527.
- Newey, H. & Smyth, D. H. (1964). *J. Physiol., Lond.* **170**, 328.
- Nixon, S. E. & Mawer, G. E. (1970a). *Br. J. Nutr.* **24**, 227.
- Nixon, S. E. & Mawer, G. E. (1970b). *Br. J. Nutr.* **24**, 241.
- Peters, T. J. & McMahon, M. T. (1970). *Clin. Sci.* **39**, 811.
- Reiser, S. & Christiansen, P. A. (1969). *Biochim. biophys. Acta* **183**, 611.
- Rubino, A., Field, M. & Shwachman, H. (1971). *J. biol. Chem.* **246**, 3542.
- Schultz, S. G. & Curran, P. F. (1970). *Physiol. Rev.* **50**, 637.
- Schultz, S. G., Yu-Tu, L., Alvarez, O. O. & Curran, P. F. (1970). *J. gen. Physiol.* **56**, 621
- Smyth, D. H. (1969). *Gut* **10**, 2.
- Tasaki, I. & Takahashi, N. (1966). *J. Nutr.* **88**, 359.
- Ugolev, A. M. (1965). *Physiol. Rev.* **45**, 555.
- Wilson, J. (1962). *Intestinal Absorption*. Philadelphia and London: W. B. Saunders & Co. Ltd.
- Wiseman, G. (1968). In *Handbook of Physiology* Vol. 3, Sect. 6, p. 1277 [C. F. Code, editor]. Washington, DC: American Physiological Society.