Mechanisms of Peptide and Amino Acid Transport and Their Regulation

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The history of research into mammalian peptide transport has, until recently, been one of frustration as the main features, but not the detail, of a transport process that had first been suggested over 100 years ago were apparent by 1975 [1]. In 1912, it had already been shown that the main products of luminal protein digestion were small peptides [2], but the difficulty of detecting them in the bloodstream [3] led to the view that free L-amino acids were the only significant form in which protein was assimilated in the small intestine. This view was reinforced by two findings: that amino acids were taken up from blood by the tissues [4] and that pancreatic enzymes released only amino acids from dietary proteins during prolonged hydrolysis [5]. Christensen [6] also reported on the failure to detect small peptides in blood and tissues and the implication was clearly that peptides were of little significance in interorgan amino acid trafficking. These views held sway from the 1920s and were reinforced by rapid advances in molecular biology during the period 1950–1960, which showed clearly that tissue proteins could only be synthesized from free amino acids (not peptides) inserted into the nascent polypeptide chain on the polyribosome. As late as 1971, a review of parenteral nutrition [7] could still confidently state that ‘Monopeptides (i.e., amino acids) are the only nitrogenous products the body can use when given intravenously. Therefore, the dipeptides contained in partial hydrolysates are excreted in the urine. If mixtures of essential amino acids are administered, this peptide loss would be avoided’. This review cited the classic series of studies by Christensen et al. [8] as support. Thus when Newey and Smyth [9, 10] showed in 1959 that everted sacs of rat intestine were capable of intact dipeptide uptake as well as surface dipeptide hydrolysis, it was considered to be a physiological curiosity of no great digestive significance. This view can be seen in an excellent summary of intestinal amino acid
transport in the mid 1960s, which went into the specificity of the main groups of transporters in great detail but had only a short section on peptide absorption. The authors commented [11] that ‘these experiments (that is, those of Prockop et al. [12] showing proline peptiduria following gelatin ingestion) conclusively demonstrated that some peptide absorption may occur normally in man’.

At that time, the dominant paradigm was that amino acids were the currency of (i) dietary protein assimilation, (ii) interorgan amino acid flux, and (iii) protein synthesis itself. Several reviews express frustration at the way in which this had led to a low priority being given to peptide transport research [1, 13–15]. The paradigm has, however, changed since human intestinal perfusion studies first demonstrated that there was a kinetic absorptive advantage of dipeptides and tripeptides over mixtures of the equivalent free amino acids [16, 17], and that normal rates of amino acid uptake could be obtained in patients with a genetic defect in the intestinal transporter if they were presented in the form of homologous or mixed dipeptides (for example, in cystinuria [18] and Hartnup disease [19]). This was clear evidence that mucosal brush border hydrolysis was not a prerequisite for dipeptide uptake and is dealt with in greater detail elsewhere in this volume [see the chapter by Silk]. The second aspect of the paradigm was critically reviewed at an international symposium at the Rowett Research Institute [20] because of evidence of persistent peptiduria in animals treated with a dipeptidase inhibitor [21], which was known to increase the intracellular dipeptide and tripeptide pool [22, 23]. Urinary peptide excretion appeared to correlate with rates of whole body protein degradation [24] and as these peptides were intracellular in origin, a transport system was clearly present that could cause them to efflux from the intracellular compartment into urine. In addition, the findings of apparently high blood concentrations of small peptides has suggested that this might be a significant route for interorgan trafficking of amino acid nitrogen [25]. The third part of the paradigm is likely to remain unchallenged.

In this review I will therefore examine the extent of peptide and amino acid transport in the human and the ways in which both processes are affected by disease and malnutrition and could be modified by special diets.

**Methods of Assessing Transport Mechanisms**

The presence of the genetic defect in the dibasic amino acid transport system in renal and intestinal mucosal cell apical membranes in cystinuria (B$^{0,+}$ or y$^+$ in Table 1) has provided a key to unlocking the mechanism of one transporter. It provides a good example of the way in which different techniques can be used to define the structural and mechanistic aspects of the transporter, and in the context of enteral nutrition, the functional characteristics of the system. A simple oral absorption test in cystinuric children given 10 g ornithine showed, in 1961, that much of it appeared in the feces [26], suggesting a specific transport defect. The lesion was further characterized by differential uptake studies
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Table 1. Summary of current knowledge of transporters

<table>
<thead>
<tr>
<th>Transport system</th>
<th>Substrates</th>
<th>Distribution</th>
<th>Transporter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Na⁺-dependent</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Small aliphatics</td>
<td>Widespread, basolateral membrane</td>
<td>SAAT1</td>
</tr>
<tr>
<td>N B⁰⁺</td>
<td>Gln, His, Asn Ala, Lys, Arg, Orn, Gly</td>
<td>Liver, muscle Fibroblasts</td>
<td>GLYT, GLYT-1a, -1b, GLYT-2, BGT-1, PRO</td>
</tr>
<tr>
<td>GLY</td>
<td>Gly</td>
<td>Liver, brain, erythrocytes</td>
<td>GLYT, GLYT-1a, -1b, GLYT-2, BGT-1, PRO</td>
</tr>
<tr>
<td>ASC</td>
<td>Small aliphatics and cysteine</td>
<td>Widespread</td>
<td>ASCT1, ASCT2 SATT</td>
</tr>
<tr>
<td>X⁺/NUL AG</td>
<td>Asp, Glu</td>
<td>Widespread</td>
<td>EAAC1, GLAST, GLT1</td>
</tr>
<tr>
<td>y⁺+L</td>
<td>Leu, Met</td>
<td>Widespread</td>
<td>4F2hc</td>
</tr>
<tr>
<td>y⁺</td>
<td>Gln, homoserine, citrulline</td>
<td>Intestinal, renal</td>
<td>mCAT-1, -2, -2A, CAT-1, -2A, -2B</td>
</tr>
<tr>
<td>IMINO</td>
<td>Pro</td>
<td>Intestinal</td>
<td>SGLT-1, SGLT-2</td>
</tr>
<tr>
<td>Glucose transporter</td>
<td>Glucose</td>
<td>Intestinal</td>
<td>SGLT-1, SGLT-2</td>
</tr>
<tr>
<td><strong>Na⁺-independent</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L b⁰⁺</td>
<td>Leu, Ileu, Val, Phe Lys, Leu, Trp, Met</td>
<td>Widespread</td>
<td>rBAT, D2, NBAT</td>
</tr>
<tr>
<td>y⁺</td>
<td>Basic</td>
<td>Widespread</td>
<td>mCAT-1, -2, -2A, CAT-1, -2A, -2B</td>
</tr>
<tr>
<td>y⁺+L</td>
<td>Leu, Met</td>
<td>Widespread</td>
<td>4F2hc</td>
</tr>
<tr>
<td>x⁻</td>
<td>Glu, Cys</td>
<td>Intestinal, renal</td>
<td>mCAT-1, -2, -2A, CAT-1, -2A, -2B</td>
</tr>
<tr>
<td>Glucose transporter</td>
<td>Glucose and fructose</td>
<td>May have wide distribution</td>
<td>GLUT 1-7</td>
</tr>
<tr>
<td><strong>Proton energized</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptide transporter</td>
<td>Di- and tripeptides, some antibiotics, peptidase inhibitors (e.g. bestatin or captopril)</td>
<td>Epithelial membranes</td>
<td>PEPT-1, PEPT-2</td>
</tr>
<tr>
<td><strong>Organic anion transporter/ATP-binding cassette transporter</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multi-drug resistance protein</td>
<td>Glutathione and glucuronide anionic conjugates (e.g. glutathione aflatoxin B), glutathione itself</td>
<td>Hepatocytes</td>
<td>MRP1, MRP2 (cMRP/cMOAT)</td>
</tr>
</tbody>
</table>
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Table 1. (Continued)

<table>
<thead>
<tr>
<th>Transport system</th>
<th>Substrates</th>
<th>Distribution</th>
<th>Transporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multi-drug resistance protein</td>
<td>xenobiotics</td>
<td>Hepatocytes, enterocytes</td>
<td>MDR1/MDR2/p-glycoprotein</td>
</tr>
<tr>
<td>Organic anion transporting protein</td>
<td>glutathione, conjugated bile salts (e.g., glycococholate) and other conjugates (e.g., dehydroepiandrosterone sulfate)</td>
<td>Hepatocytes</td>
<td>OATP1</td>
</tr>
</tbody>
</table>

Kinetic characteristics defined the transport system (column 1) according to the substrate (column 2). Where a transport protein has been cloned and expressed in *Xenopus laevis* oocytes, this is indicated in column 4. Column 3 gives the tissue distribution of the transporters. Adapted from several sources: amino acid transporters [38, 64, 65, 143, 144]; glucose transporters SGLT1 [65, 145–148] and GLUT [143, 146, 149–151]; peptide transporters [1, 13, 65, 152–157]; organic anion transporters [77, 78, 158–168].

of $^{14}$C-ornithine and $^{14}$C-arginine in human mucosal biopsy specimens from controls and cystinuric patients in 1964 [27]. However, its quantitative significance awaited development of a robust human jejunal perfusion technique by Sladen and colleagues in 1968 [28–30] and in 1973 this technique appeared to show one transport system with simple saturable kinetics [31]. Our perfusion studies have suggested that there may be two ornithine uptake mechanisms in the perfused human jejunum: one with low capacity/high affinity, and an unsaturable component of uptake which may represent passive diffusion [32]. This differs from another perfusion study [33] which showed that $D$-arginine was only slightly absorbed (that is, passive diffusion of $L$-arginine was of little significance), and it is possible that ornithine has access to another transporter not shared with arginine. The presence of multiple transport systems has also been suggested by brush border membrane vesicle uptake studies [34], and a putative transport protein, 4F2hc (Table 1), identified in vesicles [35] and expressed in *Xenopus laevis* oocytes [36].

How can these diverse methods be applied to enteral nutrition in different patient groups? This really depends on the question that is being asked (Table 2). For example, a general question which addresses the effects of enteral diets on global intestinal function could probably be answered adequately by combination sugar absorption tests which approximate to absorptive capacity [37].
### Table 2. Relevance of different methods of studying amino acid/peptide transport to enteral nutrition

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Examples, references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral tolerance test</td>
<td>Easy to administer in health and disease. Antibiotic substrates can be proxy for peptide transport</td>
<td>Extensive intestinal metabolism of amino acid invalidates results (e.g. Arginine and cystinuria)</td>
<td>31</td>
</tr>
<tr>
<td>Perfusion techniques</td>
<td>Functional assessment of human small-bowel absorptive capacity. Appropriate substrate loads can be perfused at multiple sites</td>
<td>Cannot be used in the critically-ill. Animal studies may not relate to human condition</td>
<td>30</td>
</tr>
<tr>
<td>Brush-border membrane vesicles</td>
<td>Simultaneous screening of different transporters in different conditions, regional intestinal adaptation can be measured if biopsy technique is appropriate</td>
<td>Invasive unless intestinal material is available at surgery</td>
<td>98, 172, 773</td>
</tr>
<tr>
<td>Transporter expression</td>
<td>Measurement of mRNA and surface transporter expression in mucosal biopsies gives differential information on nuclear and cytoplasmic control of transporters</td>
<td>Invasive unless intestinal material is available at surgery</td>
<td>174</td>
</tr>
</tbody>
</table>

However, a specific question about the effects of different forms of nitrogen on gut morphology or regional uptake rates could only be answered by collecting baseline data in healthy volunteers, which compared quantitative absorption rates from oral tolerance and perfusion tests with transporter expression in biopsies. This would provide a sound framework for interpreting biopsy data in critically ill patients. Unfortunately, there are many studies that have attempted to extrapolate backwards from biopsy data from humans in order to explain its meaning in terms of malabsorption. This may not be a very fruitful approach because the presence, for example, of reduced absorptive capacity in the proximal small bowel inevitably means that nutrient absorption has moved caudally and may have led to upregulation of transport at distal sites. The net effect may be nil.
Transporters and Transport Systems

Amino Acid Transporters
A review by Christensen et al. [38] in 1994 began with the statement that ‘The study of membrane transport is at a stage so lively that nearly every month a cDNA corresponding to yet another amino acid transporter is reported.’ The earlier studies of amino acid transporters [39] were based on physiological criteria such as ionic specificity of transport and competitive inhibition studies which could group amino acids according to common uptake mechanisms. This classical approach treated the transporter as an enzyme that cannot be isolated (as that would destroy its activity) but which could be identified by its kinetic characteristics [40]. On this basis, several systems were identified (Table 1). Each was very substrate stereospecific (L-amino acids >>> D-amino acids), had low specificity (only a few amino acids transported) but often had overlapping specificity with another system, and finally either cotransported Na\(^+\) or did not. System A (Alanine) is a symporter (Na\(^+\)) for small aliphatic amino acids and is widely distributed. System ASC (Alanine, Serine and Cysteine) is also a Na\(^+\) symporter with similar tissue distribution but unlike system A is not pH sensitive or inducible. System L (Leucine) is not a Na\(^+\) symporter and catalyses uptake of branched chain and aromatic amino acids, while system y\(^+\) is not Na\(^+\) dependent and carries dibasic amino acids, and system X\(^{-}\)AG transports dicarboxylic amino acids with Na\(^+\) (Table 1). In addition, system B or B\(^0\) (Broad specificity), Na\(^+\) symporter, is present in intestinal and renal epithelial cells and should not be confused with system B\(^{0}\)+ (Broad specificity) which is present in fibroblasts and has wide specificity. The intestine also possesses another unique transporter, IMINO, a Na\(^+\) symporter, which catalyses the uptake of proline. There are several reviews of these transporters to which the reader is directed for more information [40–43]. What has revolutionized this field has been the successful cloning of cDNA for transport proteins which have been expressed in X. laevis oocytes as a new transport entity. In some cases, it has been possible to match the protein to the transport system (Table 1) but the final scheme is still unclear. Christensen et al. [38] have attempted to bring some order to this situation by proposing a new naming scheme, and a new consensus is emerging. The beauty of cDNA libraries for these transport proteins is that the transporter can be probed in situ and some interesting questions asked. Thus in the case of the kidney the tubular distribution of transporters, which was defined by Silbernagl [44] with very elegant microperfusion techniques, has been confirmed by immunohistochemistry.

Peptide Transporters
Since the last nutritional review of peptide transport in 1994 [14], there have been major advances in our understanding of peptide metabolism. The peptide transporter has been isolated and characterized in the apical membrane of enterocytes (PepT1) [45] and renal tubular mucosal cells (PepT2) [46]. These two
Transporters have different tissue distributions but a common transport mechanism energized by a proton gradient generated by Na\(^+\)/H\(^+\) exchange at the apical membrane, the Na\(^+\) gradient itself being maintained by the basolateral (Na\(^+\)-K\(^+\))ATPase [47, 48]. PepT1 is considered to be the primary small intestinal transporter in rats and rabbits [49, 50], but is also present in the omasal and ruminal mucosa of sheep and cows [51]. Compared with sucrase, which immunostains over the entire villous surface [52], PepT1 is expressed on the distal two thirds of each villus. The second peptide transporter, PepT2, was thought to be solely of renal origin but in fact occurs in more distal regions of the proximal tubule, the proximal regions immunostaining for PepT1 [53]. PepT2, or rather its mRNA and dipeptide transport characteristics, has been shown to occur in the lung, mammary gland, and brain [54]. In this last case, the blood-brain barrier has usually been considered to be quite impermeable to peptides and some ingenious pharmaceutical strategies have been devised to allow transport of small- to medium-sized peptide drugs into the brain (for example, attachment to a membrane soluble lipid [55, 56]), and Adibi’s group subsequently failed to show intact uptake of glycylglutamine either from organ balance analysis or from uptake by brain capillaries in vitro [57]. However, brain uptake of highly resistant dipeptides such as homocarnosine has been known for some time [58], and PepT2 has recently been demonstrated within astrocyte membranes [59]; thus it probably mediates the uptake of β-lactam antibiotics and glycylsarcosine into brain capillary endothelial cells [59, 60]. Finally, at the subcellular level, PepT1 has been identified in nuclear membranes of pancreatic smooth muscle cells and Schwann cells and on the lysosomal membrane itself [61]. A formal kinetic analysis of lysosomal dipeptide uptake by Adibi’s group has recently shown the presence of a PepT-type of transporter which seems to take the products of proteasome-mediated protein breakdown, that is dipeptides and tripeptides [62], and transports them into lysosomes [63]. PepT1 has a structure in which several relatively hydrophobic sequences form membrane-spanning lipophilic domains (Fig. 1). These domains may form a barrel structure in the membrane which acts as the ‘pore’ [64, 65], as initially suggested by Fei et al. [45], who first reported the cloning and tissue distribution of PepT1. This type of structure is a recurring motif among transporters and may include amino acid-responsive elements within the primary amino acid sequence, or regulatory units which are –S–S– linked to the main catalytic unit forming the transmembrane pore, as may be the case for rBAT and 4F2hc [64]. In addition, transcription of PepT1 may be under the control of amino acid-responsive elements on the gene [66] such that V\(_{\text{max}}\) but not K\(_{\text{m}}\) is altered by de novo synthesis of PepT1.

PepT1 differs from glucose and amino acid transporters in being energized by a proton gradient, and transport is electrogenic (that is, current generating), as originally proposed by Boyd and Ward [67]. This process is aided by submucosal acidification [68] and, as Daniel and Herget [69] have shown, the lowest pH (~6.6) occurs approximately halfway down the villus, increasing to pH 7.4 at the crypt. This pH distribution corresponds to that of pepT1 and of SGLT1.
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Fig. 1. Membrane-spanning model of PepT 1, showing putative amino acid responsive sites Adapted from Fei et al. [45].

(sodium-glucose-linked transporter) [52, 97]. The identity of the main driving force for transport is unclear. While there is a requirement for proton cotransport (compare SGLT1 and Na\(^+\) symporting), transport is not concentrative because hydrolysis of the majority of absorbed intact dipeptides and tripeptides within the enterocyte maintains a large downhill peptide concentration gradient; this has to be balanced against the energy requirements of extruding intracellular free amino acids into the portal circulation through the basolateral membrane. Peptide uptake is indirectly coupled to apical Na uptake, and to basolateral Na\(^+\) extrusion through Na\(^+\)/K\(^+\)-ATPase (Fig. 2). Matthews [70] attempted to define dipeptide transport structure-activity relations in isolated guinea pig preparations and found no clear features among a group of homologous and heterologous dipeptides containing the branched chain amino acids. However, large scale screening of dipeptide uptake by brush border membrane vesicle preparations or Caco-2 cells has identified the structural requirements for PepT1. The original criteria of Daniel et al. [71] are given below with more recent findings in parentheses:

- Di- or tripeptide, not tetrapeptide (unless it is not a peptide [72]);
- Free amino and carboxyl terminus (unless it is a cyclic peptide [73]);
- \(\alpha\) orientation of peptide bond and \(\alpha\)-amino group (unless it is not a peptide at all [74]);
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**PepT1** transporter

Intact transfer

Dipeptides

Tripeptides

Tetrapeptides

Amino acids

Amino acid transporters

Fig. 2. Summary of peptide and amino acid transport in enterocytes. Adapted from Grimble [169].

- **Trans**, not **cis** peptide (but not always [75]);
- Preference for **L**- over **D**-amino acids (but not always [76]);
- If the above are satisfied, hydrophobicity governs rate of uptake [71].

The transporter is therefore remarkably promiscuous with regard to its substrate specificity.

**Organic Anion Transporters**

These have been included because they are intimately linked with entero-hepatic metabolism of the tripeptide glutathione. This group of transporters has similar activity to bacterial multidrug resistance (MDR) proteins, which can catalyze the efflux of antibiotics or toxins and protect the bacterium by preventing the intracellular concentration from reaching therapeutically effective levels. Some tumors which overexpress the transporter are thought to acquire resistance to chemotherapy by this means (Table 1). This is a very active area of research because it is possible that blockade of MDR will sensitize tumors to chemotherapy [77–81]. One of this group of MDRs, OATP1 (or MRP1), catalyses efflux of glutathione from intestinal cells into the gut lumen and from liver cells into bile in the form of glutathione conjugates [82]. This loss of glutathione is balanced by PepT1 (at least in neural tissue), which catalyzes
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uptake of cysteinylglycine, the glutathione precursor [83]. Interestingly, endotoxin exposure will downregulate OATP1/MRP1 through internalization or retrieval of the transporter from the canalicular membrane, whereas expression of the closely related dipeptidase activity, dipeptidyl dipeptidase IV, is unaffected [84–86].

Adaptation of Peptide and Amino Acid Transporters

Figure 2 is an integrated view of amino acid and dipeptide and tripeptide transport in an ‘average’ enterocyte. In reality, at any particular intestinal site, one transporter may be upregulated, another downregulated. The intensity of expression of brush border digestive enzymes and transporters reflects the site at which there is highest exposure to the substrate. Under normal circumstances, glucose assimilation occurs in the proximal jejunum, as suggested by the rapidity of glycemia following ingestion of glucose or maltodextrins [87], a kinetic observation which is reflected in the higher concentration of brush border disaccharidases at human jejunal (rather than ileal) sites [88, 89]. SGLT1 has a similar distribution [90, 91]. However, glucose uptake at other sites can be modulated. We have demonstrated this for SGLT1 by feeding animals with oral dextran, an \( \alpha \)-1,6-linked glucose polymer which is resistant to \( \alpha \)-amylase and to most brush border \( \alpha \)-glucosidases except for isomaltase [92–94]. At distal small intestinal sites, there was considerable luminal overspill of significant amounts of dextran digestion products and glucose in contrast to maltodextrin-fed animals. SGLT1 expression in distal brush border membrane vesicles was markedly increased in dextran-fed animals [95]. Similar results have been observed in diabetic animals because of the marked increase in ileal glucose concentration as a result of hyperphagia [96]. In the ileum, although SGLT1 is expressed mainly at the villus tip, diabetes recruits more proximal enterocytes in the mid-villus region into glucose transport [97]. The expression of SGLT1 is therefore a clear reflection of sites of glucose assimilation [98]. The same analysis applied to human brush border peptidases has revealed that aminopeptidase and dipeptidylpeptidase IV expression increases towards the ileum [88, 89]. More recently, Bai [99, 100] has shown that the gradient for rabbit and rat endopeptidase-24,11 and dipeptidylpeptidase was negative, while for endopeptidase-2 it increased towards the ileum. As the amount of PepT1 tends to decrease towards the ileum in the rat [50], it is likely that humans will differ, and expression of PepT1 should be higher in the ileum than in the jejunum.

Earlier studies in rats showed increased intestinal neutral amino acid uptake in response to protein restriction or semistarvation [101–103]. The effect was site-specific, because although the jejunum and ileum were shown to be the main sites of leucine and glycylsarcosine uptake, respectively, there was a relative suppression of rates of uptake at both sites by starvation, such that the ileum became the main site of uptake for dipeptides and amino acids [104, 105]. An
intriguing examination of life-long nutrient restriction in mice has indicated that transporters for fructose, glucose, and proline respond positively, with an expression about 40% above that of age-matched *ad libitum*-fed controls. The effect is clearly long-term because a month of dietary restriction for aged *ad libitum*-fed mice had no effect on transport capacity [106]. Presumably upregulation represents a conservative mechanism which, despite the energy burden of maintaining the extra transport proteins, may be offset by a compensatory increase in mucosal cell longevity. Protein restriction also alters the villus distribution of PepT1. This has been elegantly shown by Cheeseman [107], using microautoradiography to identify sites of transporter expression, and by Ogihara *et al.* [108], who used immunofluorescence labeling of PepT1. Protein restriction transiently stimulated peptide uptake (but depressed amino acid uptake), whereas starvation markedly increased the amount of PepT1, an effect which was reversed by amino acid feeding. Most PepT1 expression was at the villus tip and although protein restriction appeared to move it down towards the crypt, in reality the enterocytes which started to express PepT1 were 32 h old, regardless of dietary treatment [107]. It is therefore clear that studies on relative maintenance of intestinal amino acid transporters or PepT1 should be treated with caution. For example, it has recently been shown that starvation increased PepT1 expression [108] but earlier studies had not shown that the functional peptide absorptive capacity, that is $V_{\text{max}}$, was increased [105]. However, when quantitative transport data were recalculated in relation to body weight (because the starved animals were smaller), it could be shown that peptide and amino acid transport had been stimulated by 223 and 217%, respectively [105].

If luminal glucose stimulates SGLT1 expression, do dietary peptides upregulate PepT1? Two animal studies have addressed this but found that, while an increase in dietary ‘protein’ content from 18 to 54% of the diet upregulated carnosine transport (a model peptide), there was no differential effect of casein, casein hydrolysate, or amino acids. Interestingly, the high amino acid diet down-regulated brush border peptidase activity slightly [109, 110]. The most recent findings from the Tokushima group [66] have suggested that the PepT1 gene has sequence elements that are responsive to dipeptides and single amino acids such as phenylalanine. It is thus unlikely that the type of dietary nitrogen will alter PepT1 expression in man, but there may be differences in expression of brush border peptidases in response to the dietary peptide load.

The second question should be whether amino acids or peptides or proteins are most beneficial for the recovering patient with evidence of gastrointestinal dysfunction. The earliest report of comparative effects of these diets came from Jorpes *et al.* [111] in 1946, who used a partial casein hydrolysate, Aminosol, and glucose as a supplement to breast milk for preterm infants. This peptide amino acid supplement increased weight gain, and was more effective than whole casein/glucose supplements. Their summary of these data [111] has a curiously modern ring about it: ‘The results achieved up to now indicate that by administering this supplementary food we can shorten the stay in hospital
of these infants’. The possibility of achieving similar results with specialized peptide or predigested diets in other groups of patients has exercised researchers and manufacturers ever since.

In rats, both Itoh et al. [112] and Baro et al. [113] have shown that for preparations with comparable amino acid composition, proteins and amino acids or proteins and protein hydrolysates produced similar weight gain and body composition. Moriarty et al. [114] found that the nitrogen balance elicited by each form of nitrogen was the same in man. This is not surprising as the basis of the question relates to the relative rapidity of uptake of peptides and amino acids, and in neither situation was this an issue because feeding patterns were essentially unconstrained. Under conditions of rapid intestinal infusion, amino acids from peptides (that is, protein hydrolysates) certainly appear in the portal circulation faster [115] and are emptied from the stomach faster than milk protein [116], thus potentiating a greater insulin response. Under the highly artificial situation of continuous duodenal infusion of a protein-free diet, with daily stomach gavage of the nitrogen source as two boluses, Monchi and Rérat [115] showed that milk protein hydrolysate led to a doubling of net protein utilization compared with whole protein. In addition, plasma amino acid and plasma insulin concentrations were markedly increased [115]. This is an interesting approach which may be justified on the grounds that hospital patients in France often receive their entire daily ration as a 9-hour enteral infusion (~10 g protein/h). In healthy subjects, these high rates of infusion have also been shown to accentuate the absorptive difference between whole protein and partially hydrolyzed protein. A peptide diet was shown to increase oxidative $^{13}$C-leucine disposal even though whole body protein synthesis was increased [117]. The same approach in patients undergoing abdominal surgery led to augmented insulin release and improved nitrogen balance [118, 119]. Rats that were allowed to eat ad libitum received a diet that contained either whey protein, a partially hydrolyzed whey, or an amino acid control. Growth rate and nitrogen balance were increased in the peptide-fed group, but in starved-refed animals a marked increase in intestinal morphometry in the group receiving a whole protein diet was surprising [120, 121].

Boza et al. [122–124] have repeated these studies with casein and whey hydrolysates in order to define the starve-refeed cycle better. There was some evidence of increased nitrogen balance in those animals fed the casein hydrolysate, and reduced intestinal permeability to ovalbumin [122–124]. Brinson et al. [125, 126] have also produced interesting evidence that intestinal function in hypoproteinemic rats can be best preserved with peptide-based diets.

Finally, we have observed in rats that in a direct comparison of enteral diets based on protein hydrolysate or its amino acid control, there were no differences in body composition except for a doubling in size of the cecum. There is no framework for discussing this surprising observation, but it clearly indicates that the peptide diet was associated with a component which either induced malabsorption or provided a specific adaptive signal to the cecum [127].
As can be gathered from this discussion, the published reports are confusing. This may reflect the nature of the protein hydrolysate itself. The concept of peptide diets has been built around the existence of a peptide transporter, and attempts to exploit uptake by this route. It is therefore logical that the peptides in such a diet should be presented as true substrates for PepT1, and should not require prior hydrolysis by brush border peptidases. Because of this we have explored the way in which subtle differences in peptide chain length distribution affect nitrogen uptake in the perfused human jejunum [128, 129]. The first question we attempted to answer was what is meant by the terms ‘degree of hydrolysis’ (DH) and ‘peptide chain length’. This is not a new question, and in fact it was exhaustively discussed by Nehring et al. [130] nearly 30 years ago. Each hydrolysate is a complex mixture of peptides of different chain length together with free amino acids, which can be defined by a global value known as its DH. This is the fraction of peptide bonds that have been cleaved by a global value known as its DH. This is the fraction of peptide bonds that have been cleaved in the starter protein [131]. For any well-defined hydrolysis scheme, this is invaluable for quality control purposes, but it cannot be used to compare different hydrolysis schemes. For example, two protein hydrolysates made by different methods (for example, larger oligopeptides/significant free amino acids vs. mainly di- and tripeptides/little free amino acid) may have a similar DH even though their absorption kinetics are likely to be quite different. For this reason, it is better to use the term ‘peptide chain length profile’, even if this is analytically difficult to obtain [132].

We have found that the most robust methods to profile the mixtures are Cu(II)-sephadex chromatography [133] together with stochastic sequencing [132]. Fine detail can be obtained by gas chromatography mass spectrometry [134], high performance liquid chromatography mass spectrometry [135], or tandem mass spectrometry [136], which will sequence many of the dipeptides or tripeptides present in the mixture. Size exclusion chromatography is of little use for mixtures of peptides with a molecular weight <2000 daltons unless hydrophobic interactions between peptides and column packing are suppressed [132]. We have therefore investigated absorption of short- and medium-chain hydrolysates of ovalbumin, whey, and casein, and found that the dipeptide- and tripeptide-based mixtures are more rapidly absorbed than those based on tetra- and higher peptides (Fig. 3) [14]. Although originally made by a triple enzyme process, we are currently investigating the use of single thermostable enzymes to achieve the same end but with better quality control [137]. These short-chain preparations could be used as low-osmolarity intravenous substrates, because of the rapidity of hydrolysis of dipeptides and tripeptides infused into the plasma compartment [138, 139]. As proposed before, these would make ideal substrates for total peripheral parenteral nutrition [14].

Elsewhere in this book, Silk argues that most comparative clinical studies of amino acids, peptides, and proteins have failed to show clear-cut differences. In two senses, this is not surprising given the artificial nature of enteral nutrition. We were surprised to observe how poor the metabolic response to continuous
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Fig. 3. Summary of perfusion studies comparing uptake of short- and medium-chain protein hydrolysates. Data from references 128, 170, and 171.

Nasointestinal nutrition was in healthy volunteers [140] and the way in which it stimulated colonic water secretion as a harbinger of diarrhea [141]. Both criteria have been used to determine the effectiveness of the different forms of nitrogen in several clinical trials, when the real issue is that of manipulating the rapidity of amino acid uptake, which would not be apparent during continuous tube feeding. Perhaps it is time to re-examine these questions using bolus feeding as the correct frame of reference [142]. It is possible that dipeptide- and tripeptide-based hydrolysates may prove to be the means of optimizing uptake through PepT1, an intestinal transporter which seems to be preserved in the face of malnutrition and trauma.

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Discussion

**Dr. Bentsen:** I've been interested in PepTI and was involved in mapping the gut for its presence some years back. I have always been puzzled by a Finnish paper that was published a few years ago [1] in which malnourished children were fed an extensively hydrolyzed diet. This was changed to an amino acid-based diet in the course of the treatment because of poor weight gain. All the children had significantly better weight gain and longitudinal growth after the change. This does not fit with my view of the role of the PepTI transporter. Do you have any comments on that?

**Dr. Grimble:** You always have to compare apples with apples rather than with oranges. You would probably find that the amino acid composition in that study differed quite markedly in the two feeding regimens. One piece of work that led people to cease using intravenous hydrolysates in the early days was the discovery that a fibrin hydrolysate originating from America was limiting in valine and phenylalanine [2]. These amino acids had been partially removed by an activated charcoal purification step during manufacture. In addition, Patel *et al.* [3] did a nice study showing that oral cottage cheese (i.e. casein) was better than intravenous casein hydrolysate, again for the same reason. If you were to go back to that Finnish study, you would need to look at the amino acid composition and compare it quite carefully before laying the blame at the door of the peptide diet. The work of Itoh *et al.* [4] has put the whole thing into perspective because they controlled everything in relation to peptide, amino acid and whole protein diets, even down to the feeding pattern and taste aversion from the taste of hydrolysates, by using space pair feeding for the animals and getting them to eat the diet in a very short period of time. They could find no difference in growth rates between any of them. So the form of nitrogen, I think, is irrelevant. It's the amino acid composition which seems to be important in normal feeding and growing conditions.

**Dr. Young:** What is the recent thinking with respect to the quantitative significance of di- and tripeptide absorption during the normal course of digestion of intact protein versus the absorption of free amino acids during that same period?

**Dr. Grimble:** I don’t think anyone knows. You can get some clues from our perfusion studies looking at the sodium dependence of uptake, where it appears that the majority of the protein was being taken up as peptides. Also, the fact that in the perfused intestine you will always get faster rates of amino acid uptake in the form of dipeptides does suggest that quantitatively the transporter is more active than single amino acid transporters. Thirdly, if you look at the pattern of amino acids taken up from partially hydrolyzed protein, you tend to find a normalization of amino acids that have rather low rates of uptake when given in the free form.

**Dr. Haschke:** One of your slides indicated that dietary tripeptides inhibit the activity of some brush-border membrane enzymes, such as maltase. Is this inhibitory effect also present when you give tetra- and pentapeptides together with di- and tripeptides or whole protein?
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Dr. Grimble: We’ve no data. It’s possible that the preparation was at fault. There may have been some inhibitory factor that arose during manufacture. I’m not sure how that could have happened because the tetra- and pentapeptide preparation went through the same procedures, but certain stages of the hydrolysis were longer. The other part of the mystery is why you don’t get bigger interactions, say between sodium and glucose uptake, or between glucose and peptide transport, or between peptide and amino acid transport, because ultimately they’re all dependent on the basolateral sodium potassium ATPase for extrusion into portal blood. Thus if peptide transport is very rapid, you would expect alterations in the intracellular environment but with impact, for example, on glucose uptake, and that’s not been found in general.

Dr. Reeds: In relation to the actual quantities of peptides that are transported under real conditions it puzzles me why the brush border is full of aminopeptidases. Why is there such a high expression of peptidases in the brush border if peptide transport is the predominant means of getting the peptides into the enterocyte?

Dr. Grimble: That’s a very interesting question. Steinhardt et al. [5] did a lovely experiment in patients with pancreatectomy, feeding whole protein or hydrolysates, and found that about two thirds of the whole protein had been assimilated in the small intestine, and a third of it had gone into the colon and been fermented, whereas with hydrolysate all of it was assimilated. So the implication is that brush-border hydrolysis is really part of oligopeptide hydrolysis rather than di- and tripeptide hydrolysis. It’s there to look after quite large protein fragments and clearly was doing that in Steinhardt’s experiment. But the more interesting question is, why is there a caudal gradient of peptidases? It’s obviously responding to substrate in some way, and presumably it’s responding to endogenous protein loads.

Dr. Barbul: Could you speculate on the parenteral use of peptides? As a clinician, it’s a very attractive notion and I think anything that could reduce the osmotic load would be of benefit. Technically we’re not in the 1970s any more, when there were great problems with the purity of the preparation. Do you think it’s feasible now, and what do you think is necessary to bring this back into the clinical arena as a parenteral source of nitrogen?

Dr. Grimble: I think about this most weeks, and wonder why we haven’t got further than we have. You are right that on paper, it’s a beautiful concept because to have hypo-osmolar TPN regimes that you can give peripherally removes one of the biggest risks related to parenteral nutrition. There is much evidence that we are well adapted to intravenous peptides, with a half life of 3 min or so. We would like to make a protein hydrolysate that’s exactly the same as the equivalent mixture of synthetic di- and tripeptides, and then persuade the regulatory authorities that it really is synthetic di- and tripeptides; then they wouldn’t feel quite so bad about its past history!

Dr. Barbul: We’re coming at this from another direction because we are using peptides to provide various amino acids that are not stable in solution, for a variety of reasons, so we’re making boutique-type peptides. Unfortunately as a whole protein concept it’s not well advanced even though it’s so attractive on theoretical grounds.

Dr. Grimble: That’s right. Casein is about 8–10% glutamine, so an appropriate di- and tripeptide-based casein hydrolysate could be a stable carrier. In addition, this hypothetical peptide preparation would also contain sufficient tyrosine and cysteine in soluble peptide form. We did find that our preparations lost some glutamine in processing. In the original Swedish casein hydrolysate, Aminosol, all the glutamine was lost in processing because it had been liberated as a free amino acid and converted to oxoproline. But I agree that a modernized protein hydrolysate would solve a lot of formulation problems in one hit, and at the same time could allow modest supplementation with synthetic dipeptides, if necessary to bring the essential amino acids, or conditionally essential amino acids, up to reasonable levels. But it’s quite a steep hill to climb, I think, in persuading people.
Dr. Lundholm: As a clinician I believe that albumen is an excellent protein substrate and amino acid source. We have recalculated the human data in various metabolic studies to take account of albumen and red blood cell transfusions, and we find that most patients are in nitrogen balance, or they are in negative nitrogen balance if we calculate only on the basis of the provision of free amino acids. I think large proteins are of at least conceptual interest, even for nutrition, especially when combined with their physiological use in preserving colloid osmotic pressure, which is one of the main reasons for giving albumen in critical situations. So I think we could achieve two different goals in one: a nutritional one, and a physiological one. What do you think about that? Would there be any limitation to providing large proteins intravenously or intra-intestinally?

Dr. Grimble: Well, with large proteins there’s the obvious issue of antigenicity, and you have regulatory issues relating to the source of the protein and problems of transmission of viral vectors. Some data also suggest that although you can partially replete critically ill patients, you cannot do so very quickly, and the bioavailability of the infused albumen is rather poor—about 0.18 g/kg/day [6].

Dr. Rowe: I was interested in the data you presented on the persistence of the peptide transport system during malnutrition and chemotherapy. Are you aware of any evidence that peptide transport persists in other clinical conditions, such as with prolonged TPN or following injury or stress?

Dr. Grimble: The difficulty with this field is how you determine whether transport function has increased or decreased. From some of the early work on the effect of starvation on amino acid and peptide transport in young rats, it was quite clear that there was a net suppression of the peptide transporter and the amino acid transporter [7]. But when you express those results per kilogram body weight, there is actually an upregulation—it went up by about 200%. So in some ways the intestine has preserved that function at the expense of other things. Transport functions seem to be the last things to go.

Dr. Roessle: A Danish group has fed stressed rats with casein hydrolysate preparations that were obtained using different enzymes, and the nitrogen balance results were very different on these different preparations [8]. Thus with a given amino acid profile and a comparable peptide size the clinical results were quite different. How could you explain these findings?

Dr. Grimble: I saw those data last week at ESPEN and tried to figure them out myself. They were using the same triple enzyme mixture that we were using to produce the intravenous peptide preparations, but it is quite clear that there was an absorptive difference between the two preparations. Why they got the results they did, I still don’t know. I think it is probably related to the load of amino acids entering the portal blood. Because of the rapidity of di- and tripeptide uptake, or the very rapid load reaching the liver, what it’s partially doing is to relieve the intestinal efflux of amino acids for early acute phase protein synthesis, and by giving a very rapidly absorbed protein source you would actually achieve a much better acute phase protein-modulating effect.

Dr. Lundholm: You showed that long proteins may improve protein synthesis in the splanchic area. There may be a very simple explanation for that, that they induce better insulin secretion. I think we should consider providing patients with longer proteins rather than breaking them down into small peptides or isolated amino acids, because that is the way we normally function. All the classical studies on refeeding show that the more complex the diet the better the response on a whole organ basis. The best way to stimulate liver protein synthesis is to give the patient a steak. That’s far superior to giving free amino acids or hydrolysates. I think we miss out when we apply too much technical processing to the diet.

Dr. Déchelotte: I wonder whether the issue of the absorption of nitrogen as protein or peptide is clinically relevant. It could be relevant if enhanced absorption of peptides provided metabolic advantages. It could also be relevant if proteins are not well absorbed
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and peptides are better absorbed in certain categories of patients, but I am not sure that this problem of absorption is a real problem in many patients. We used to assume that diarrhea in intensive care patients receiving enteral nutrition was caused by malabsorption of substrate. But now when we use closed systems with sterile preparations we have far less diarrhea, and when we measure the stool output in these patients, even when they have diarrhea, it is mostly water and salts with very little nitrogen. So I think the issue of nitrogen absorption as protein or peptide may not be clinically relevant in most patients.

Dr. Grimble: Dr. Lundholm was advocating gastronomy over technology, I think, and suggesting that one should try to mimic the normal meal feeding pattern. One of the issues in enteral nutrition is that we give continuous nasoenteral feeds which rarely switch the patient out of the fasting motility pattern. We have shown previously that continuous nasoenteral feeding will cause colonic secretion of water, which you only eventually inhibit by increasing the feed rate or by feeding post-pylorically [9]. So there is something very artificial about continuous nasoenteral feeding. I believe we should try to mimic the normal meal feeding pattern as far as possible, with bolus feeding to provoke aminoacidemia and an insulin response. Under those circumstances I would have thought that a substrate that is very rapidly absorbed, such as a peptide preparation, would be advantageous.

Dr. Hunter: In your clinical indications for the oral use of peptide feeds you didn’t mention Crohn’s disease and its nutritional management. We find such feeds are very valuable in this situation, because of their osmotic advantages which result in much less diarrhea. Why didn’t you mention it? Are you against it?

Dr. Grimble: Because I only had 20 min! Various multicenter trials have suggested there may be an advantage in using amino acid or peptide-based diets in Crohn’s disease, but it’s a very confused picture. It may have nothing to do with the nitrogen source, but rather be related to the fat source and to the anti-inflammatory effects of a low fat diet.

Dr. Young: My understanding is that there is a large excess capacity in the gastrointestinal tract for peptide and amino acid absorption. I wonder to what extent this has to be reduced before dietary protein utilization is physiologically compromised.

Dr. Grimble: Most of the evidence that we have comes from ileostomy patients who have disrupted motility. Normal motility patterns are one of the major factors controlling the efficiency of absorption, so it’s quite difficult to work back from human studies. You need to go to animal studies which indicate that the safety margin is about 100% in relation to peptides, glucose and amino acids [10]. The limit of adaptation in cold-adapted mice goes up a bit, but you end up with a safety margin of about 40% when they’re hyperphagic. By and large I think the same situation holds in man – a safety margin of probably around 100%. For example, pancreatic lipase is present in about nine times greater amounts than we need for fat digestion.

Dr. Haschke: There are data in pediatric patients with short bowel syndrome. Infants with only one third or one quarter of the small intestine remaining need to be on peptide or amino acid diets for months before they can tolerate intact proteins [11, 12]. So there are human data available.

Dr. Grimble: I think that’s quite an artificial situation.

Dr. Roessle: Would you comment on the use of peptides with postpyloric feeding techniques which are becoming more frequent? Do you think that this type of feeding requires different sources of protein or peptides from intragastric feeding.

Dr. Grimble: If you feed casein-based diets, you get quite slow gastric emptying because of coagulation of casein in the stomach, while gastric emptying with the peptide diets is almost instantaneous. It has been shown that in healthy volunteers peptide-based diets will empty as if they were water and they give an insulin response that’s greater than the response with an isocaloric amount of glucose [13]. I believe there are no particular precautions that need to be taken so far as diet is concerned if you’re feeding
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postpylorically, because the issue of casein coagulation and gastric emptying no longer arises. Virtually anything will do.

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