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THE SIGNIFICANCE OF PEPTIDES IN CLINICAL NUTRITION

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INTRODUCTION

Classical models of protein metabolism view nitrogen intake in terms of the flux of free amino acids from dietary protein and their exchange between plasma and intracellular compartments and between free and protein-bound amino acids (138). However, this view is misleading because it neglects any flux of amino acids through intermediate pools of small peptides, the least obvious of which is that produced by intracellular protein degradation (26). Other peptide pools comprise intermediates of dietary protein digestion, filtered peptides in the renal tubular lumen, and circulating peptides from dietary sources or from intracellular protein degradation.

These peptides enter the general free amino acid pool after peptidase hydrolysis, the total capacity of which must be as great as the whole-body flux of amino acids from protein turnover, currently estimated to be 1.5–4.5 g kg^{-1} d^{-1} (100–300 g d^{-1} for a 70 kg person) in healthy humans. Surgical stress, cancer cachexia, and liver disease can increase this capacity (35). Thus, whole-body peptidase activity is capable of upregulation.

Earlier studies defined the major organs involved in peptide metabolism (and in analogous glucose polymer metabolism) (Table 1). If all activity were expressed, the total capacity to hydrolyze dipeptides and maltose would be of the order of 4.4 kg d^{-1} and 230 g d^{-1}, respectively. This hypothetical calculation explains why metabolism of i.v. synthetic dipeptides is so rapid compared with that of maltose.

This review describes aspects of peptide metabolism from the basic premise that widespread peptide transport or peptidase activity can be exploited to improve organ (or whole-body) nutrition in specific disease states.

SOURCES OF PEPTIDES IN BLOOD AND TISSUES

Dietary Protein

Dietary peptides, which are resistant to luminal digestion and to enterocyte brush-border hydrolysis, can be detected in the circulation, and significant urinary excretion of specific peptides has been observed after oral ingestion (70, 98). Measurable amounts of peptide-bound amino acids are present in peripheral blood or urine after a protein-rich meal (39). As a proportion of portal amino acids, the amount is surprisingly high: 15% in the guinea pig (71) and 52%, 65%, and 78% in the rat, sheep, and cow, respectively (172). The late David Matthews concluded that in humans, peptides only comprise a small proportion (10–15%) of human plasma amino acids (129) but wryly noted that if analytical methods reveal only amino acids, then "If we continue to look only for free amino acids, we shall find only free amino acids: peptides cannot

Table 1 Hydrolase activities in various rat tissues[a]

Tissue	Depeptidase		Maltase	
	μmol min $^{-1}$ organ $^{-1}$	% Total	μmol min $^{-1}$ organ $^{-1}$	% Total
Liver	4650	33.27	13.25	2.93
Kidney	2800	20.04	40.27	8.90
Muscle	2750	19.68	2.92	0.64
Ileum	2200	15.74	—	—
Jejunum	1500	10.73	388.49	85.87
Blood	75	0.54	7.50	1.66
Total	13975	100	452.42	100

[a] Data recalculated from Refs. 6 and 215. Data for intestinal brush-border maltase activity not given.

be expected to declare their presence." (129). The most recent animal studies (172) suggest that circulating peptides may be of great significance.

Intracellular Protein Degradation

Although early attempts to detect significant tissue quantities of small peptides met with failure (41), Scornik demonstrated the presence of a cytoplasmic pool of di- and tripeptides generated by intracellular protein degradation (26). This pool is of small size but has rapid turnover and may efflux from liver or other tissues in pathological states, such as uremia or pre-eclampsia (105, 220). Urinary excretion of small peptides may reflect rates of whole-body protein breakdown (153) and may include a small component of D-peptides from colonic bacterial or L-peptides from endogenous protein degradation.

PEPTIDE METABOLISM IN THE HUMAN INTESTINE

Kinetic Evidence for Peptide Uptake

Until the early 1950s, the absorption products of protein digestion were widely thought to be free amino acids for which there were several discernable transport mechanisms. The most significant paper, by Newey & Smyth (148), demonstrated absorption of the constituent amino acids of poorly hydrolyzed dipeptides by intact intestinal preparations in vivo or in vitro, but the mechanism of the process was unclear. Matthews and colleagues performed the seminal study that suggested that intact peptide transport occurs in the small intestine. Oral doses of glycine in the form of glycine, glycyl-glycine, or glycyl-glycyl-glycine yielded more rapid absorption from the di- and tripeptides, thereby effectively ruling out a mechanism by which brush-border hydrolysis had preceded glycine uptake from the peptides (44). Human jejunal perfusion studies have yielded similar results (9) in that competition between

free amino acids for uptake was avoided or reduced when they were in dipeptide form (175). Furthermore, uptake of amino acid residues was faster from partial enzymic hydrolysates of protein than from the equivalent free amino acid mixture (54, 177), and the relative uptake of amino acids was more "even" (54, 77). The physiological significance of peptide transport was confirmed in patients with inborn errors of cysteine and dibasic amino acid transport, lysinuric protein intolerance, Hartnup disease, or cystinuria. Despite the intestinal/renal transporter defect in cystinuria, uptake of dibasic amino acids is never entirely abolished because of a passive component (137), which is also responsible for D-arginine absorption (91). However, free amino acid presented to the intestinal mucosa in dipeptide form was absorbed to a significant extent (179).

Enzymic Evidence for Peptide Uptake

Release of solubilized brush-border peptidases into medium bathing mucosal preparations cannot account for the release of amino acids from dipeptides (175). Instead, the specificity of brush-border and cytosolic peptidases is consistent with brush-border hydrolysis and intracellular hydrolysis of tetra/higher peptides and di- and tripeptides, respectively (151, 152).

Dual Modes of Dietary Nitrogen Assimilation

Most evidence for intestinal peptide uptake was obtained between 1962 and 1974 from kinetic evidence without isolation of a transporter protein. This work provided the basis for dual mechanisms of dietary amino acid assimilation (Figure 1). Only di- and tripeptides, which remain after luminal and brush-border peptidase digestion, are absorbed intact; free amino acids liberated by luminal or brush-border digestion are absorbed via the free amino acid transport systems (Figure 1). Tetra- and higher peptides require prior brush-border hydrolysis before their hydrolysis products can be absorbed (9).

Electrophysiological and Biochemical Characteristics of Peptide Transport

Amino acid transport shows strong Na^+ dependency (32). The elegant microelectrode studies of individual giant enterocytes of the worm, *Necturus maculosus,* by Boyd & Ward (29) suggested that uptake of dipeptides was electrogenic and related to H^+, not Na^+, cotransport. Subsequent studies with brush-border membrane vesicles (BBMV) from small intestine, renal tubule, or placenta (all of which lack the basolateral membrane Na^+/K^+/ATPase transporter) confirmed the need for an inwardly directed H^+ gradient during peptide uptake (66, 67, 69). As in the buccal cavity (53), intact peptide uptake probably occurs in the large intestine. The acid microenvironment adjacent to the mucosal brush border provides the motive force for di- and tripeptide uptake (68).

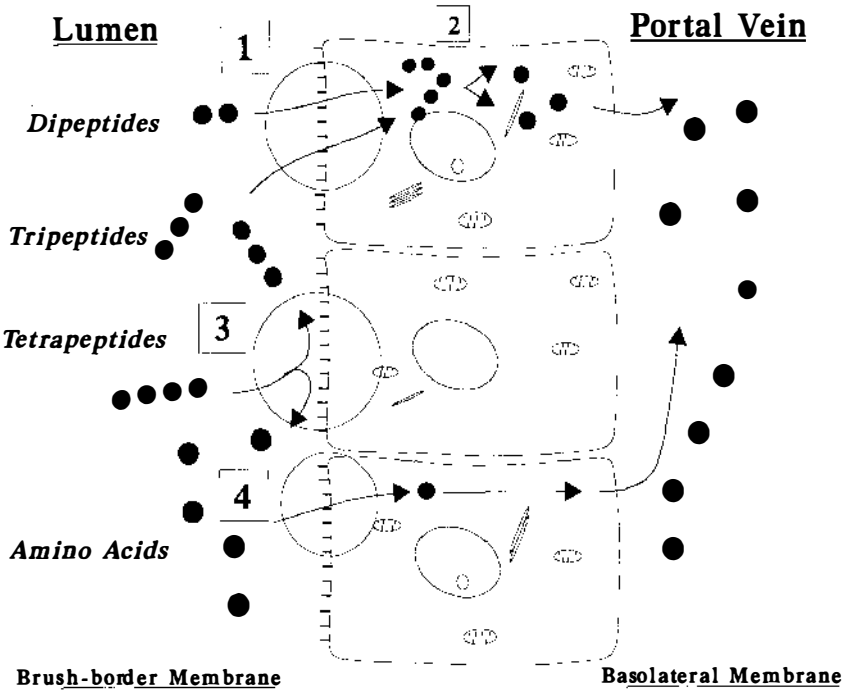

Figure 1 Peptide and amino acid absorption in the small intestine. An idealized view of the intestinal wall is shown. Luminal di- and tripeptides are absorbed by a specific transporter (step 1) and hydrolyzed by intracellular peptidases (step 2). Tetra- and higher peptides are hydrolyzed by brush-border peptidases (step 3). Free amino acids are absorbed by one of the active L-amino acid transporters (step 4).

Proton dependence provides a key for dissecting the peptide transporter. Small intestine uptake of cephalosporin antibiotics, which contain a peptide bond, occurs via the dipeptide transporter (114). An outstanding series of studies by Kramer and colleagues has identified and isolated a 127 kDa membrane protein, using photolabile ^{3}H-benzylpenicillin as an affinity-probe (117). This protein was incorporated into synthetic liposomes, which possess all the characteristics of dipeptide uptake (saturable pH-sensitive transport). Moreover, these synthetic liposomes can be inhibited by antisera to the 127 kDa protein (115, 116), which is either the entire peptide transporter system or a part of it.

These antibiotic studies indicate the promiscuity of peptide transport in regard to its substrate requirements. Although there is a strong relationship between hydrophobicity and transport affinity for neutral amino acids (2), no such relationship is observed when the same amino acids are presented to the mucosa in the form of homologous dipeptides (130). Whether a peptide carrier

of broad specificity, allosterically modified by more hydrophobic peptides, or multiple carriers (83) were present remained unclear. Kinetic analysis of uptake of many model peptides by the high-affinity system of renal BBMV (46) has defined the structural requirements of the di- or tripeptide substrate as a free terminal α-NH_2 and α-COOH group and amino acids linked by a *trans-* rather than a *cis*-peptide bond. L-amino acid isomers at both termini yield the highest rates of uptake, although hydrophobic D-amino acids are acceptable at the N-terminus.

The amino acids of intact peptides will ultimately efflux from the enterocyte basolateral membrane after hydrolysis by cytoplasmic peptidases to free amino acids or, if the peptides are particularly resistant to hydrolysis (e.g. proline peptides), as intact peptides. Luminal or arterial leucine concentration may upregulate amino acid efflux (38), but the exact mechanism of basolateral membrane uptake or efflux of dipeptides remains unclear (34, 51).

Solvent Drag and Peptide Uptake

Peptide absorption in the intestine may occur via the tight junction between enterocytes. Fisher was the first to note that glucose-stimulated water uptake by preparations of rat small intestine was associated with uptake of small molecules such as urea. This process, often called solvent drag, has been invoked as the predominant mechanism for glucose uptake in the small intestine (154). Furthermore, Atisook & Madara suggested that glucose-elicited tight-junction dilatation may provide a control mechanism for solvent drag uptake of significant amounts of peptides in the intestine (17).

However, this hypothesis is highly unlikely because postprandial luminal free glucose concentrations rarely exceed 50mM l^{-1} and will not saturate the transporter (56), even though earlier estimates of luminal glucose concentrations appeared to be much higher than the capacity of the glucose/Na^+ cotransporter (154). This observation confirms previous human perfusion studies performed in our laboratory (181). The kinetic characteristics of the glucose transporter therefore parallel its postprandial substrate concentration. This finding does not mean that paracellular passage of small molecules (e.g. dipeptides, glucose) cannot occur, merely that it is not nutritionally significant. Luminal loads of hyperosmolar glucose certainly increase the permeability of the small intestine to lactulose (118) but decrease that of the poorly hydrolyzed dipeptide carnosine (70). Paracellular absorption of amino acids and urea is not significant in the renal tubule (111), nor have we been able to discern any relationship between net transport of water and urea in the perfused human jejunum (81). The negligible rates of intestinal D-amino acid uptake (91) add weight to this analysis. This point has been labored because current knowledge of the kinetics of intestinal peptide uptake leaves few unexplained gaps. The high capacity of the system resides in the downhill transmembrane concentra-

tion gradient, which is maintained by continuous removal of transported peptides by intracellular peptidases. Even free intestinal amino acid transport, often considered concentrative and uphill, is downhill postprandially (8) and may contain significant contributions from passive diffusion (78).

CLINICAL APPLICATIONS OF INTESTINAL PEPTIDE TRANSPORT

Digestive and Absorptive Capacity

The use of peptide diets for malabsorbing patients remains controversial. The original elemental or chemically defined diets for such patients (178) contained glucose, amino acids, and small amounts of fat in accordance with intestinal physiology as it was understood in the 1950s and 1960s. Excluding one specific clinical application [i.e. Crohn's disease (27)], these diets are still prescribed to patients with severely impaired gastrointestinal function.

The use of special diets depends on the degree of impairment of absorptive function, which has been most closely studied in relation to the malabsorption of untreated cystic fibrosis (50). Digestive and absorptive mechanisms of the small intestine present a formidable array (reserve capacity or safety margin) to any polymeric nutrient. In small animals, total intestinal absorptive area accommodates the metabolic mass of each species because it is modulated by the formation of intestinal folds, villi, and microvilli, all of which amplify the nominal surface area (55). Reserve capacity can be upregulated, as suggested by one study of hyperphagic, cold-adapted mice (201). When these data are extrapolated to humans, the corresponding safety margin is two to three times total energy expenditure (ca. 4500–6000 kcal d^{-1}), an enteral load that, according to our experiments, can be efficiently assimilated (166).

One should bear this finding in mind when considering studies that show biochemical impairment of digestive or absorptive processes. Few of these studies actually address the question of whether this impairment in turn gives rise to clinically significant impairment of nutrient uptake. Because of the overlap in digestive systems, malabsorption of some nutrients may take place only if 90% impairment of organ function has occurred [e.g. exocrine pancreatic disease (31, 48)].

Peptides and Reduced Absorptive Function

STUDIES IN HEALTHY VOLUNTEERS Studies showing faster absorption rates for peptide-based protein hydrolysates (54, 77, 169, 177) provide a rational basis for diets designed for patients with markedly impaired absorptive capacity who can utilize the potential of both L-amino acid and peptide transport systems. The term "protein hydrolysate" or "peptide-based diet" covers a

Table 2 Peptide chain-length profile of several protein hydrolysates used in enteral diets

Diet	Peptide Chain-Length Profile (%)									
	L-amino acids	2	3	4	5	6	7	8	9	10
Survimed[a]	18.0	5.0	5.0	1.0	1.0	24.0	10.0	2.0	17.0	17.0
Travasorb[b]	20.0	15.0	5.0	1.0	1.0	29.0	29.0	—	—	—
Amirige[c]	15.0	5.0	21.0	12.0	12.0	1.0	2.0	2.0	16.5	16.5
Pepti 2000[c]	17.0	11.0	16.0	14.0	14.0	13.0	15.0			
Peptisorb[d]	28.0	11.0	17.0	11.0	12.0	1.0	10.0	10.0	—	—
Steraldiete	25.0	1.0	28.0	18.0	17.0	11.0	—	—	—	—
Reabilan[f]	2.0	11.0	21.0	1.0	17.0	17.0	15.0	16.0		
Tipeptid[g]	8.0	34.0	35.0	16.0	7.0	—	—	—	—	—
Tolerex[h]	100.0								—	—

Note: Peptide profile measured by Cu(II)-sephadex chromatography (171). Some of these diets are no longer available o may be marketed under other names in various countries. [a] Fresenius, [b] Clintec, [c] Nutricia, [d] E Merck Pharmaceuticals [e] Dubenard, [f] Roussel, [g] Laboratoires Roger Bellon, [h] Norwich Eaton Pharmaceuticals.

multitude of different peptide size distributions (Table 2). Although the starter protein and method of hydrolysis govern absorptive characteristics, we investigated peptide-chain length as the most significant variable. Hydrolysates prepared from lactalbumin, ovalbumin, and casein showed increased amino acid absorption in humans when the proportion of di- and tripeptides was increased (77, 81, 82). Similar studies in animals or humans using protein hydrolysates (60, 96) or tetrapeptides (9, 33) led to the conclusion that in the absence of luminal pancreatic enzymes, brush-border hydrolysis of tetra- and pentapeptides is the rate-limiting step in uptake of nitrogen. Thus, in order to maximize nitrogen assimilation in patients with marked impairment of gut absorptive capacity, the ideal protein hydrolysates should comprise only di- and tripeptides and free amino acids.

CLINICAL AND ANIMAL STUDIES Clinical and animal studies, on the other hand, have not yielded such clear results. Animal comparisons of casein (102) or whey-hydrolysate (79) with equivalent amino acid mixtures revealed that all promote similar body and organ growth (79). Long-term feeding trials in healthy volunteers showed that the degree of hydrolysis of lactalbumin did not affect nitrogen balance (142).

Impaired absorptive function When GI function is moderately impaired, protein hydrolysates have no nutritional advantages over whole protein (168). The same holds true when 60–150 cm of the jejunum remain after small bowel resection (132). We found that even when absorptive surface was severely reduced (50–80 cm jejunum), a peptide-based diet had no effect on whole-body protein kinetics compared with an equivalent amino acid–based diet (167).

However, comparable data on short-chain hydrolysates are lacking because the nitrogen source in both of these studies contained mainly tetra- and pentapeptides, the assimilation of which may have been rate limited.

Impaired digestive function Patients with total pancreatectomy assimilated 60% of intact lactalbumin compared with 91% of a lactalbumin hydrolysate (196). The remarkable feature of this study is the absence of measurable luminal pancreatic enzymes, which suggests that the brush-border peptidase capacity of the entire small intestine is high and has considerable versatility toward oligopeptides released by gastric digestion.

EFFECTS OF FEEDING SCHEDULES ON PEPTIDE UTILIZATION One animal study revealed that a peptide diet can significantly increase net protein utilization (140). This study is significant because nutrients were administered daily as two rapid gastric boluses, which accentuated the more rapid aminoacidemia following peptide administration (176, 177). Increased insulin secretion (140) may thus provide a basis for metabolic benefits of cyclical nutrition (100, 216). It may also explain why postsurgical patients utilized peptide-based diet better than they did the equivalent whole-protein diet (222). These investigators discontinued the feed for several hours on 4 of 18 postoperative days. The stimulus of restarting the diet may have accentuated the absorptive differences between the two nitrogen sources. Cyclical enteral feeding and the absorptive advantage of peptides may act synergistically and should be investigated further.

PEPTIDES IN SURGICAL PATIENTS AND THE CRITICALLY ILL A second major issue concerning efficacy of peptide diets is the prevention of diarrhea in enterally fed patients. By increasing blood albumin and thereby reducing small intestinal water and electrolyte secretion (73), two small trials have shown that these diets may be effective (30, 136). The design of the animal study is open to criticism, and recent trials in critically ill patients have not been clinically confirmed (19, 107, 143). To consider albumin status alone may be fruitless since concurrent antibiotic therapy (24) and perhaps nasoenteral feeding–induced colonic water secretion are dominant etiological factors (28).

Another proposed benefit of peptide diets is their ability to resist coagulation in the acid environment of the stomach, which can lead to feeding tube blockage (128) or even to esophageal obstruction (203). Two trials reported a higher incidence of gastric residuals with milk-protein diets (210, 222), but this hypothesis has not been confirmed in critically ill patients (143). Overall, therefore, some aspects of the clinical efficacy of peptide diets remain open for speculation.

INTRAVENOUS PEPTIDES IN CLINICAL NUTRITION

The Current Situation

Routine use of i.v. peptides has never lapsed. Protein hydrolysates are currently used in the Commonwealth of Independent States (the former Soviet Union), and I would like to thank the anonymous sender of some samples—a gesture of perestroika in the late 1980s. Until recently, all amino acid infusions were mixtures of free L-amino acids, but the wheel may be turning full circle for the reasons summarized in Table 3. In brief, the criteria for successful use of i.v. peptides are that they should be nontoxic, soluble, stable, amenable to quality control, and have good utilization and be clinically effective, all at reasonable cost. A rationale for peptides hinges on whether parenteral intake of conditionally essential amino acids can be increased (with clinical benefit) using the peptide route (75, 83). Current standard total parenteral nutrition (TPN) formulations lead to cysteine imbalance in the newborn (95) because of poor conversion of methionine to cysteine, secondary to low tissue levels of cystathionase (72). Indeed, the inflammatory response may increase sulphur amino acid requirements (86). Similarly, conversion of phenylalanine may be limited in neonates (62). Glutamine represents a special case, which is described in more detail below.

Routes of Metabolism of Intravenously Infused Dipeptides

The similarity in mechanisms for removal of peptides from the intestinal and renal-tubular lumen extends to their brush-border peptidases (108), to peptide uptake by BBMV prepared from either source (67), and to kinetic uptake of maltose and peptides by individual microperfused renal tubules (174). The alternative pathway for salvage of filtered oligopeptides and proteins is via luminal endocytosis (163), which is influenced by peptide modifications such as N-terminal residue cyclization (163) and renal disease (15). On this basis, one would expect the kidney to play a major role in the disposal of i.v. dipeptides merely by salvaging amino acids. However, this analysis excludes the possibility of organ targeting of amino acids with specific peptides.

Table 3 Rationale for i.v. peptides

Free L-amino acid	Problems
Glutamine	Stable in short-term (1 month) but not sufficient for long shelf-life i.v. product
Tyrosine	Relatively insoluble (0.4 g l^{-1})
Cysteine	Relatively insoluble (cystine—0.1 g l^{-1}) and unstable in presence of O_2
Tryptophan	Relatively unstable in presence of O_2/carbohydrate

Synthetic Dipeptides as Prodrugs

Matthews & Payne coined the phrase "smugglins" to describe modified peptides that could be used to smuggle an antibiotic or another antimetabolite across the bacterial wall and thus cheat the permeability barrier (131, pp. 428–29). This description was prophetic because they stated that "The biological potential of smugglins cannot be overemphasised" in relation to antibacterial therapy. Neuroactive peptides have been delivered across the impermeable blood-brain barrier by attaching lipophilic or other moieties that allow membrane permeation and intracellular release of the active peptide by hydrolases (20, 182). Bai & Amidon used a similar approach to protect oral peptide drugs from brush-border hydrolysis (18). Synthetic, i.v. dipeptides also held promise because of their ability to increase pools of their constituent amino acids in different tissues (10). If intact peptide uptake had been shown to occur in liver or in muscle, for example, then sequence changes in i.v. dipeptides could modulate organ targeting of amino acids. It was therefore a great disappointment when dipeptide hydrolysis was shown to precede free amino acid uptake by the liver or muscle plasma membrane (124, 165). Nevertheless, i.v. α-linked peptides are prodrugs because they are efficient nutritional amino acid precursors.

General Considerations for Use of Parenteral Peptides

The criteria for applying small peptides to parenteral nutrition are also summarized in Table 3. Generally speaking, free L-amino acids are more soluble if incorporated into dipeptides and have a higher pK_a (159) as confirmed for specific dipeptides containing glutamine (C-terminal), tyrosine, and cysteine, which are resistant to heat sterilization (186). Quality control techniques such as high-performance liquid chromatography (HPLC), isotachophoresis, nuclear magnetic resonance (NMR), and mass spectrometry (MS) have been applied to ensure purity (186, 191). Protein hydrolysate toxicity and composition as well as peptide-chain length profile and composition have been determined by standard in vitro/in vivo testing (112, 206), amino acid analysis, Cu(II)-sephadex chromatography (Table 2), peptide sequencing (173), gas chromatography(GC)/MS (57) or HPLC/MS (120), and capillary electrophoresis (76). Cost is a contentious issue, but one comparison showed i.v. protein hydrolysates to be approximately half the price of similar L-amino acid solutions (204).

STRUCTURE-ACTIVITY RELATIONSHIPS FOR INTRAVENOUS DIPEPTIDES

Furst suggested that peptides rapidly hydrolyzed by extrarenal tissues spare a filtered load to the renal tubule (62). The N- and C-terminal amino acids

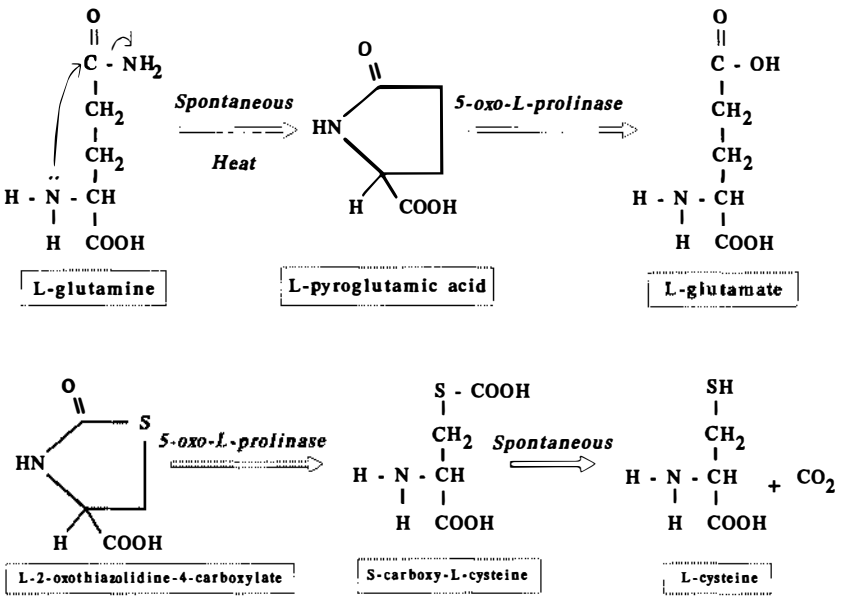

Figure 2 **Pathways of formation and metabolism of pyroglutamic acid and OTCA.**

influence this process, as shown by one study in which baboons infused for up to 14 days with complete mixtures of glycyl-dipeptides (195) excreted between 0.6 (glycyl-L-methionine) and 4.1% (glycyl-proline) of the load. Later papers from this group discuss structure-activity relationships in some detail (3,97), but proline-containing dipeptides will clearly be poorly utilized (98) because only renal dipeptidyl peptidase IV can hydrolyze prolyl-peptide bonds (139), whereas the other peptidases have rather broad specificity (108, 200). Peptide-chain length also influences renal utilization (10), a process analogous to peptide absorption in the small intestine (9). Nevertheless, in the baboon TPN studies (4, 5), tripeptide mixtures were extensively metabolized (>95%). The cost of synthetic tripeptides is prohibitive but does not exclude the use of i.v. protein hydrolysates containing mainly di- and tripeptides.

Cysteine Peptide Prodrugs

N,N-bis-L-alanyl-L,L-cystine and N,N-bis-L-glycyl-L,L-cystine are rapidly utilized after bolus injection in the rat (162, 187). Their relative plasma half-lives correlate with plasma peptidase specificity in humans, which suggests that this is a major site of i.v. peptide hydrolysis; indeed, calculated human plasma peptidase capacity toward cystine dipeptides is ~ 10.4 g d^{-1} (189), without

taking into account the activity of membrane peptidases of liver and muscle. Meister suggested an alternative pathway for cysteine provision, the glutathione cycle (217). A thio-analog of pyroglutamic acid, L-2-oxo-thiazolidine-4-carboxylic acid (OTCA) contains an internal peptide bond that can be hydrolyzed to cysteine by 5-oxo-prolinase (Figure 2), an enzyme with wide tissue distribution and high activity (198). OTCA can therefore protect mice against lethal doses of paracetamol (217), and it restored growth in TPN-fed rats receiving a cysteine-deficient diet (23). As a prodrug, its peptide bond and site of hydrolysis (intracellular) direct cysteine to tissues with high rates of glutathione production. Another i.v. cysteine prodrug, N-acetyl-L-cysteine, is primarily filtered by the kidney and hydrolyzed by brush-border N-acylases (52). The liberated amino acid is reabsorbed in rats (145) and humans (126), but significant urinary excretion of the intact molecule has been demonstrated, which indicates that renal N-acylase capacity may be limited.

Tyrosine Peptide Prodrugs

At high infusion rates (2 mmoles kg^{-1}), L-alanyl-L-tyrosine in rat TPN regimes has been efficiently metabolized (45). Glycyl-L-tyrosine has been infused in TPN regimes in healthy subjects (13) or in patients with acute pancreatitis (197) or renal disease (49) to similar effect, namely maintenance of plasma tyrosine concentration and rapid plasma clearance. However, clearance of L-alanyl-L-tyrosine was more rapid for glycyl-L-tyrosine in renal patients, which suggests that impaired renal metabolism of the glycyl peptide is important (49). In contrast, N-acetyl-L-tyrosine was poorly metabolized in renal patients (49), neonates (94), and healthy subjects (126).

The γ-glutamyl cycle (134) has also been exploited as a means of delivering tyrosine into tissues. It was proposed that membrane-bound γ-glutamyl transpeptidase will accept an amino acid (AA) by reaction with glutathione to produce a γ-glutamyl-AA dipeptide. This dipeptide can be absorbed and hydrolyzed to the free amino acid and pyroglutamic acid. Pyroglutamic acid is hydrolyzed to glutamic acid, which may reenter the glutathione synthesis pathway. Thus, 50% substitution of phenylalanine in TPN regimes with γ-glutamyl-tyrosine dipeptide normalized plasma and brain levels of tyrosine and phenylalanine/tyrosine ratios in rats (164). However, the authors acknowledged that they lacked definitive proof of intact uptake of γ-glutamyl-tyrosine, which was undetectable in brain tissue, at the level of sensitivity of their HPLC technique (<2 nmoles g^{-1}). This report is fascinating for two reasons: (*a*) The dipeptide was heat stable (164); and (*b*) in view of the wide selectivity of γ-glutamyl transpeptidase (135), other γ-glutamyl dipeptides, including γ-glutamyl-L-glutamine, could be considered. The latter may be a particularly effective means of organ targeting glutathione synthesis.

Glutamine Peptide Prodrugs

CLINICAL AND METABOLIC RATIONALE FOR GLUTAMINE DIPEPTIDES Three lines of evidence suggest that glutamine may become conditionally essential under certain clinical conditions. First, surgery (160) or acute septic pancreatic disease (170) markedly reduces plasma and muscle concentrations of glutamine. Muscle intracellular glutamine concentration correlates closely with muscle protein synthesis (103). Second, glutamine is a major intestinal respiratory fuel (180), and luminal and arterial supplies are readily metabolized (218). At supraphysiological intakes, glutamine reverses the intestinal mucosal atrophy consequent on TPN feeding in the rat (99) and can improve the survival of rats made septic (101) or that have lethal methotrexate-induced enterocolitis (58). Small bowel transplants in the rat have markedly improved morphology and biochemical status (59), and bacterial translocation across the mucosal barrier is reduced in septic states (106) with glutamine supplementation. Third, intermediary metabolism of cultured cells is adapted to high rates of glutamine catabolism (133, 149). These cells require an adequate supply of exogenous glutamine to maintain de novo synthesis of nucleic acid precursors (199) as well as rates of RNA and protein synthesis (see 75). The metabolic characteristics of enterocytes and cells of the immune system may require adequate circulating levels of glutamine for optimal function in the stressed patient. These data have therefore led to the general hypothesis that glutamine is a gut-specific nutrient that can be of specific benefit in clinical situations in which barrier function of the intestine is impaired.

NEED PEPTIDE-BOUND GLUTAMINE BE USED? This question naturally leads to speculation as to whether manufacturing synthetic glutamine dipeptides is necessary since we (90) and others (109, 223) have shown that hospital pharmacy production of ultrafiltered sterile 2.5% solutions of L-glutamine is not only feasible, with excellent stability over 7 days (degradation % d^{-1} at 4° C storage), but can also be safely administered to patients as part of an all-in-one TPN regime (223). One could also argue that supplementing TPN regimes with up to 40 g of glutamine in dipeptide form presents an excessive nitrogen load from the extra alanine or glycine moieties. In reality, however, this controversy relates to hospital pharmacy practice, not to issues of toxicity of the breakdown products of L-glutamine (188). Pyroglutamic acid (Figure 2) is not a neurotoxin (74) but rather an intermediary of the γ-glutamyl cycle that is efficiently metabolized (134), as the data on its thiol analog, OTCA (see above), suggest. Could the mood enhancement experienced by patients receiving L-glutamine-supplemented TPN (221) result from the anxiolytic effects (21, 25) of pyroglutamic acid present in the solution? L-glutamine is completely degraded to pyroglutamic acid and NH_4^+ by heat sterilization and, because the

amino acid is less soluble than either L-alanyl-L-glutamine or glycyl-L-glutamine, large i.v. amounts cannot be given to fluid-restricted patients. Although hospital manufacturing pharmacies may formulate L-glutamine solutions subject to normal pharmaceutical safeguards (74), commercial solutions of terminally sterilized, glutamine dipeptide–supplemented amino acids solutions with good long-term stability will eventually be made available (191).

METABOLIC UTILIZATION OF GLUTAMINE DIPEPTIDES Glycyl- and L-alanyl-glutamine have been extensively investigated in addition to N-acetyl glutamine, which is poorly utilized in rats (146), dogs (1), and humans (127). Clearance of an i.v. bolus of L-alanyl-L-glutamine by rats or dogs was rapid (half-life 3.8 min), with little urinary excretion (64). Plasma glutamine concentration was increased (192), and the glutamine moiety of L-alanyl-L-[U-^{14}]-glutamine was rapidly incorporated into tissue glutamine pools (193). Similar studies in fed or starved healthy volunteers yielded essentially the same results for glycyl-L-glutamine (7, 123, 125) and L-alanyl-L-glutamine (12, 13). One can therefore conclude that both dipeptide forms of glutamine behave in much the same metabolic fashion as parenteral L-glutamine.

CLINICAL STUDIES OF GLUTAMINE DIPEPTIDES Vinnars and colleagues have contributed greatly to our understanding of postoperative metabolism by repeating the same TPN study in patients undergoing cholecystectomy, a standard surgical operation. They defined the postsurgical time course of reductions in muscle intracellular glutamine concentration (160) and in the number of muscle ribosomes and their recruitment into protein synthesis as polyribosomes (161). In all studies, a portion of the amino acids in the TPN regime for one group of patients was replaced with glutamine or its analogs. Having established that TPN feeding alone did not restore muscle glutamine, polyribosomes, or nitrogen balance to preoperative levels (16, 214), Vinnars and colleagues showed that ornithine α-ketoglutarate (119, 209, 213), α-ketoglutarate (88, 212), L-glutamine (87), and L-alanyl-L-glutamine (89) all normalize these parameters of injury response. A common feature of these studies is that each substrate augmented concentrations of glutamine in plasma and skeletal muscle. Similarly, after major colonic surgery, nitrogen balance and muscle glutamine concentrations were improved in patients receiving L-alanyl-L-glutamine-supplemented TPN (194), and short-term infusions have increased rates of muscle protein synthesis in postsurgical patients (22). In critically ill patients, 20 g L-alanyl-L-glutamine (13 g glutamine) infusion had no effect on muscle glutamine but significantly improved nitrogen balance (63). In relation to gut function, L-alanyl-L-glutamine (202) or glycyl-L-glutamine (207) supplementation of TPN regimes resulted in improved absorptive function (xylose test) or permeability (lactulose:mannitol ratios), respectively. Taken together with the most recent trial of L-glutamine-supplemented TPN (221), these data

indicate that glutamine in dipeptide form is clinically indistinguishable from L-glutamine.

COMPLETE DI- AND TRIPEPTIDE MIXTURES

Clinical Rationale

Thus far the discussion has focused on dipeptides as a stable vehicle for specific amino acid residues. Can the same technology be applied to complete mixtures containing all amino acids? The primate studies of Adibi et al (5, 195) show that such an application is feasible, but at a cost. However, there is a specific indication for such mixtures as an amino acid source for peripheral parenteral nutrition. Complete mixtures of di- and tripeptides would reduce osmolarity because equal solution weights of di- or tripeptides have one half and one third the osmolarity of free amino acids, respectively. At present, most hypertonic, hypercaloric TPN courses are infused into a central vein, where the high blood flow will rapidly dilute the infusate. This has been standard practice since the 1960s. Complications of central venous feeding are few (<5%) but carry serious morbidity (157), even in experienced hands. In contrast, the only complication of peripheral vein infusion is peripheral vein thrombophlebitis (PVT), which requires re-siting of the infusion cannula. A reduction in TPN solution osmolarity is one of several therapeutic maneuvers that can minimize PVT (157), but with current regimes, this reduction will in turn lead to a reduction in nutrient intake, even if all-in-one TPN regimes are used. Use of mixtures of di- and tripeptides would allow a significant decrease in solution osmolarity without reductions in amino acid intake. Indeed, the general utility of peripheral parenteral nutrition has been suggested by two National Clinical Nutrition Surveys (155, 156), which showed that although the total annual number of courses of TPN administered to patients did not change between 1988 and 1991, the proportion of these patients fed by the peripheral venous route increased from 7 to 15%.

This result suggests a growing need for special, low-osmolarity TPN regimes of which di- and tripeptide mixtures could form a part. A further advantage of these hypothetical peptide mixtures is that the solubility of constituent tyrosine and cysteine peptides would resolve two formulation problems of the insolubility of the free L-amino acids. This goal could be economically achieved with protein hydrolysates comprising di- and tripeptides. Although this may at first seem to be a "low-tech" solution to the problem, it soon becomes evident that this hypothetical preparation is the same as the equivalent mixture of synthetic di- and tripeptides.

Comparison of Methods for Large-Scale Production of Peptides

The fundamental constraints preventing large-scale peptide production are the purity, stereospecificity, and scale-up costs of the peptide product (Table 3).

Classical chemical synthesis can produce N^2-L-Tyr-N6-L-Tyr-L-Lys, L-Ala-L-Gln (190), L-Ala-L-Cys-L-Cys, Gly-L-Cys-L-Cys, L- Cys-L-Ala-L-Ala, and L-Cys-Gly-Gly (186). A more promising alternative is enzymic synthesis with proteases (in solution or immobilized), which uses appropriately blocked amino acid substrates to suppress ionization of the α-NH_2 and α-COOH groups (65, 141, 186). Another interesting option is the use of bacterial aminoacyl-tRNA synthetases in preparative peptide synthesis (144). Neither technique is stereospecific, since D-amino acid can be incorporated from enantiomers in the substrate, albeit at low rates (65, 144). The issue was raised following reports of eosinophilic-myalgia syndrome (EMS) among patients with self-prescribed L-tryptophan (1–3 g d^{-1}) as treatment for insomnia, depression, or premenstrual syndrome. A slight change in the manufacturing process resulted in trace amounts of a dimer of L-tryptophan linked by acetaldehyde that may have been the active agent (150). This finding highlights the importance of the purity of feedstock chemicals.

Although protein hydrolysis produces only L-amino acid–containing peptides, processing technology is less accessible because many of these techniques are commercially sensitive and thus have not been published. However, several guidelines could be applied (Table 4). Food-grade pancreatic enzymes (trypsin and chymotrypsin) have been widely used (11, 110) but are not suitable as primary hydrolytic enzymes because they release large quantities of free L-tyrosine (47) that must be removed by activated charcoal filtration (110). Specific amino acid losses during this processing step were probably responsible for the reports of amino acid inadequacy in some i.v. protein hydrolysates (e.g. see 205), even though their long-term stability in complete TPN formulations was good (61, 83, 113). More recently, bacterial proteases with broad specificity have been used (36, 37). Initial hydrolysis with a protease (alkaline pH optimum) was allowed to proceed for a preset time, where-

Table 4 Technical considerations for i.v. protein hydrolysate manufacture

Starter protein	Fermentation technology	Final product
Good biological value	Nontoxic, food grade enzymes	Nonantigenic
Balanced amino acid profile	Temperature tolerant proteases/ endopeptidases	High di- and tripeptide content
Known primary sequence(s)	Enzymes with different pH optima	Low free L-amino acid content
Little source variance	Differing chain-length specificity	Salt-free
No irreversible posttranslational modifications	Will not release glutamine N-terminus peptides	Economy
Nontoxic	Prolyl peptidase activity	
Inexpensive	Little tyrosine release	
	Ultrafiltration and deionization	

upon the pH of the incubation was reduced and a second protease (neutral pH optimum) added. Larger peptides in the final product were clipped with pancreatic endopeptidases (36, 37). This process preserved the amino acid composition of the starter protein without the need for charcoal filtration. Ion-exchange purification increased the di-, tri-, and tetrapeptide content of the product from 75 to 95.5% of total amino acids. Free amino acid content was 3% (36). We have used this product as a starting point to investigate i.v. utilization of di- and tripeptide-enriched protein hydrolysates (76, 80, 84, 85). This example does not exclude the many other bacterial proteases and peptidases with different bond specificity that have been used in the manufacture of modified food protein (14) or in protein sequencing (147). The ability to predict protein cleavage sites will ultimately lead to improvements in the biotechnology of hydrolysate production.

Clinical and Experimental Studies with Protein Hydrolysates

Reviews of the historical development of i.v. nutrition (121, 219) may lead the reader to assume that crystalline L-amino acid solutions were a major advance over preexisting protein hydrolysates, which had been used safely for 40 years. This conclusion is understandable in the light of such statements as "Amino acid preparations have several advantages over hydrolysates: defined formulations, flexibility and avoidance of loss of peptides in urine." (121). Synthetic L-amino acids or peptides are attractive because they can be defined chemically, as described above. Although the same degree of precision is lacking for protein hydrolysates, a combination of analytical techniques nevertheless can be used to define them quite closely, as discussed above (e.g. see 57, 120). Moreover, blending hydrolysates of different protein or supplementation with L-amino acids confers flexibility. Avoidance of peptiduria should not be overstated since it occurs with synthetic dipeptides, depending on amino acid sequence (195). Indeed, the scale of peptiduria in TPN studies with L-alanyl-L-tyrosine (45) led the authors to conclude that it was no worse than that found with the use of protein hydrolysates (184). The term "protein hydrolysate" is merely generic, in the same way that "lipid emulsion" or "dextran" is used to denote i.v. solutions with a wide range of properties. Thus, not all protein hydrolysates showed the same efficacy (40, 41, 104); the best was Swedish Aminosol®, with a biological utilization of 91% (122). Comparisons of amino acid and protein hydrolysates in surgical patients (158, 204, 205, 208) or infants (92, 93, 184, 219) have shown that both can maintain similar nitrogen balance or growth. Excess urinary excretion of nonmetabolizable Maillard products formed during heat sterilization in the presence of glucose is an avoidable problem that affects synthetic amino acids and peptides (185) as well as hydrolysates (43, 183).

We have therefore reinvestigated the use of short-chain i.v. protein hydrolysates, which comprise mainly di- and tripeptides. When complete TPN regimes were infused in healthy volunteers, total amino acid excretion (free and peptide bound) accounted for 4% of total load during amino acid infusion and rose to 10% during peptide infusion (80). There were no differences in the evolution of plasma amino acid profiles, and capillary electrophoretograms of urine showed little relationship between infused peptides and excreted peptides, which contained significant amounts of larger peptides. A later study of an ovalbumin and of casein hydrolysates (or their L-amino acid controls) in TPN-fed rats (85) revealed that all groups of animals grew and were maintained in positive nitrogen balance, but peptide-nitrogen excretion in the peptide groups increased. The peptide load stimulated growth of liver and kidney (major organs of i.v. peptide disposal), but we could detect no adaptation in specificity of peptidases, since urinary capillary electrophoretograms at the start and end of the study were identical. We and others (4, 195) have therefore concluded that if >90% of i.v. nitrogen intake is provided in the form of small peptides, it will be efficiently metabolized.

CONCLUSIONS AND PERSPECTIVES

This paper has reviewed whole-body metabolism of small peptides. The last five years have witnessed major advances in applications of peptide metabolism to clinical nutrition (7, 62, 83), ruminant nutrition (211), and organ-specific drug delivery (18). The widespread distribution of tissue mechanisms of peptide uptake or metabolism is now more apparent. Several developments will likely take place in the future. First, one can take advantage of the rapid kinetics of peptide absorption in the enterally fed patient when diet administration more closely resembles normal eating patterns (e.g. cyclical feeding). Intravenous peptides can deliver amino acid residues, which may limit clinical recovery or growth in current amino acid formulae (Figure 3). Recent attempts to manipulate the γ-glutamyl cycle (Figure 3) show that smugglins or prodrugs may prove useful for site-specific delivery of amino acids. Quantitative aspects of peptide metabolism will also be bought into sharper focus. Adult humans possess sufficient peptidase activity to clear small peptides from the circulation. Placental peptide transport suggests that the child in utero may have already adapted, through exposure to small peptides, to metabolism of peptide-based TPN regimes suitable for neonates. Finally, we will see further advances in synthesis and manufacture of peptides for nutrition at reasonable cost. Such improvements represent a major challenge for the future and should enable investigators to meet the goals of adequate nutrition (i.e. amino acid quality) and safer nutrition (e.g. peripheral parenteral nutrition).

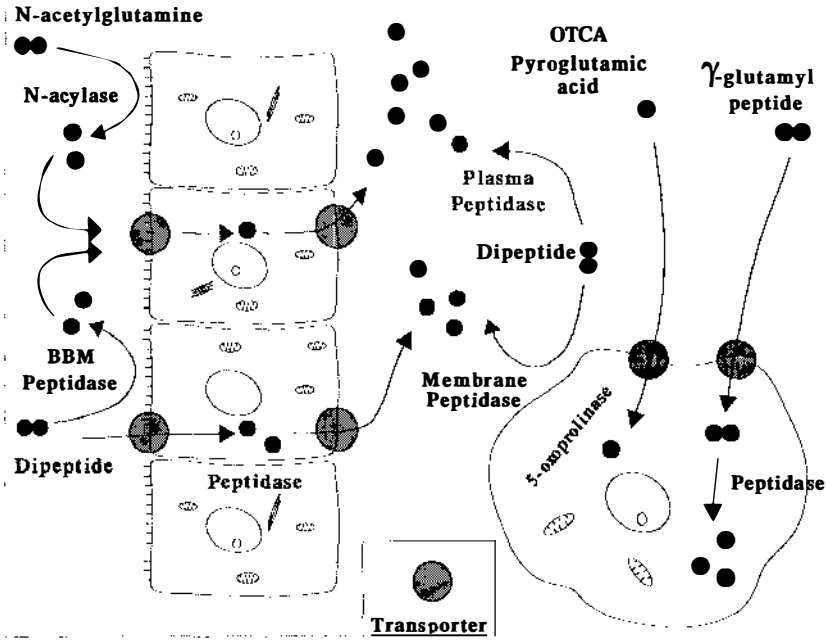

Figure 3 Metabolism of i.v. peptides. The two routes of dipeptide metabolism are shown. The shaded area on the left shows metabolism of filtered dipeptides and N-acetylglutamine within the renal tubule. The area on the right shows metabolic disposal of the different prodrugs discussed in the text.

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