Conditionally Indispensable Amino Acids
(Glutamine, Cyst(e)ine, Tyrosine,
Arginine, Ornithine, Taurine) in Enteral
Feeding and the Dipeptide Concept

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There is a long list of so called essential and conditionally indispensable nutritional substrates, mostly old or old but newly packaged. Certain amino acids, short-chain fatty acids, nucleotides and so on have been known to be essential for a very long time. What has changed is that their nutritional significance has been reconsidered, and the functional and physiological properties of certain nutrients under various pathological conditions have been reassessed [1]. ‘Old substrates with new specifications’, which include glutamine, cystine, arginine, short-chain fatty acids, nucleotides, ω-3 fatty acids, and so on, have been variously described as essential, indispensable, conditionally essential nutrients, functional nutrients, and acquired indispensable nutrients. With the discovery of common and specific mechanisms for alterations in substrate metabolism, unique opportunities have arisen to intervene in the disease process. Undoubtedly, the efficacy of providing functional substrates to the injured, immunocompromised, or malnourished host has caused a rebirth in the clinical application of dietary interventions in the treatment and prevention of disease [1]. Simultaneously a new class of definitions has entered the scientific and, especially, the medical literature. The task of this review is to propose an acceptable definition of an essential/indispensable amino acid, to summarize the potential applications of these nutrients when given enterally in experimental and clinical settings, and
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finally to anticipate their future importance in the developing field of clinical nutrition.

**Essential and Nonessential Amino Acids**

According to Rose’s classical definition, the adult human body can maintain nitrogen equilibrium on a mixture of eight pure amino acids as its sole source of nitrogen. By 1962, Mitchell had already pointed out, in the first volume of his treatise *Comparative Nutrition of Man and Domestic Animals*, that ‘an amino acid may be a dietary essential even if an animal is capable of synthesizing it, provided that the demand for it exceeds the capacity for synthesis’. The strict nutritional classification of the common amino acids formulated by Rose and later by Jackson, Chipponi and others is now no longer acceptable as we attempt to understand how dietary protein serves to meet our nutritional needs in health and disease [2].

Grimble [3] proposes that, regardless of the definition used, a final judgment about the usefulness of an essential amino acid will be on the grounds of clinical and nutritional efficacy. A more general proposition is that ‘a possible and useful direction might put more emphasis on metabolic control and its regulation of tissue and organ function and nutritional status’. This definition offers suggestions as to how certain shared metabolic characteristics might be used to differentiate the various nutritionally important amino acids. It also implies that the dietary ‘essentiality’ of a given amino acid is dependent on the ratio of supply to demand; the distinction between ‘essential’ and ‘nonessential’ largely disappears because it is dependent on conditions [1, 2].

This notion might be used when evaluating the significance of amino acids in clinical enteral nutrition. In recent years there have been two principal trends in the use of nutritional support: first, there has been a major shift from intravenous to enteral feeding; second, there have been important changes in the content and protein/energy mix of the formulations used [4]. The most striking new research findings, however, relate to the use of specific nutrient substrates to supplement standard enteral diets, both old substrates with new indications and truly new substrates. For example, the role of nucleic acids is currently being discussed because expression of nucleic acid-synthesizing enzymes in the *de novo* pathway is apparently impaired during catabolic stress [5]; arginine is claimed to be a potent immunomodulator during episodes of stress and in particular in cancer patients [6]; few of the commercially available enteral preparations contain sufficient amounts of glutamine, and it is questionable whether the contents of cysteine and tyrosine are adequate. These latter nutrients are either unstable or poorly soluble [1, 7, 8], but new insights into the efficient use of dipeptides may create the possibility of supplementing enteral preparations with cysteine, tyrosine, and glutamine in stable, soluble dipeptides [9, 10]. Their use might have advantages over the native substances.
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**Glutamine**

*Metabolism*

Glutamine is the most prevalent free amino acid in the human body. In skeletal muscle, it constitutes more than 60% of the total free amino acid pool [11]. It is a precursor which donates nitrogen for the synthesis of purines, pyrimidines, nucleotides, amino sugars, and glutathione, and is the most important substrate for renal ammoniagenesis (regulation of the acid-base balance). Glutamine serves as a nitrogen transporter between various tissues. Finally, glutamine represents the major metabolic fuel for the cells of the gastrointestinal tract (enterocytes, colonocytes) [12, 13] and many rapidly proliferating cells, including those of the immune system [14].

There is much evidence that hypercatabolic and hypermetabolic situations are accompanied by marked depression of muscle intracellular glutamine. This has been shown after elective operations, major injury, burns, infections, and pancreatitis, regardless of nutritional attempts at the time of repletion. Reduction of the muscle free glutamine pool (to about 50% of the normal level) thus appears to be a hallmark of the response to injury [7]. Furthermore, during catabolic stress or when tumors are proliferating, peripheral glutamine stores are rapidly diminished and the amino acid is preferentially shunted as a fuel source towards visceral organs or tumor tissue. This creates a glutamine-depleted environment, the consequences of which include enterocyte and immunocyte starvation [15].

*Glutamine and Experimental Disease Models*

There is a striking direct correlation between the muscle free glutamine concentration and the rate of protein synthesis. This suggests that maintenance of the intracellular glutamine pool may promote conservation of muscle protein during catabolic stress [16]. Thus glutamine supplements might be beneficial in the treatment of stressed and malnourished patients. Numerous experimental studies support this hypothesis. Glutamine-supplemented enteral or parenteral nutrition solutions cause an increase in intestinal mucosal thickness, DNA and protein content, reduced bacterial translocation after radiation [12], weakened adverse effects of experimentally induced enterocolitis [17], preserved intestinal mucosa during parenteral nutrition [18], and enhanced mucosal hyperplasia after small bowel resection in the rat [19]. In addition, glutamine supply prevents pancreatic atrophy and the development of fatty liver during elemental feeding [20], and supports muscle glutamine metabolism without stimulating tumor growth [21]. Further reports emphasize enhanced bowel absorptive capacity after major intestinal resection [22, 23], morphological preservation of bowel integrity [24], decreasing intestinal permeability and improving experimental pancreatitis [25], and promotion of liver regeneration after hepatectomy [26]. In addition, glutamine supplementation is reported to restore mucosal immunoglobulin A and enhance upper respiratory tract immunity [27], prevent gut-derived sepsis in obstructive jaundice [28], reverse
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gut-derived sepsis associated with prednisone administration [29], and enhance bacterial clearance in peritonitis [30]. In tumor-bearing animals, glutamine supplementation was shown to restore glutathione levels in normal tissues while reducing the antioxidant concentration within tumor cells [31, 32]. This increased tumor sensitivity to chemotherapy and radiotherapy while reducing host toxicity [33]. Immunologically, glutamine supplementation augments the cytotoxic activity of natural killer and lymphokine-activated killer cells [18, 34, 35].

Clinical Use of Enteral Glutamine

The first clinical trial with enteral glutamine in man concerned patients with septic ulceration [36]. Administration of a heat-labile substance (glutamine) in raw cabbage juice resulted in a better healing response than a lactose-based placebo. However, the results of subsequent enteral/oral glutamine nutrition studies have been controversial. The available results from enteral glutamine studies are summarized in Table 1.

In contrast to the beneficial results seen with parenteral supplemental glutamine [8], there was no improvement in nitrogen balance, protein synthesis, and other variables relating to nitrogen economy on administration of glutamine-enriched tube feeds in short-term trials in patients in intensive care units (ICUs) [37, 38]. On the other hand, oral glutamine supplementation slowed down the rate of whole body protein breakdown in children with Duchenne muscular dystrophy [39]. Enteral tube feeds supplemented with L-glutamine failed to increase or even to normalize plasma glutamine levels in adult or pediatric ICU patients [40, 41].

Oral glutamine supplementation in cancer patients did not prevent the occurrence of doxifluridine-induced diarrhea and had no impact on tumor response to chemotherapy [42]. The results are in accordance with those reported by Earl et al. [43], but are somewhat at variance with the findings of Conversano et al. [44], who reported a decrease in both duration and severity of gastrointestinal symptoms with oral glutamine supplementation.

It is apparently very important to consider therapeutic endpoints [45]. Small enteral/oral doses of glutamine have been useful in correcting altered intestinal permeability in patients with inflammatory bowel disease [46]. Pouchitis, which is an inflammation of the surgically fashioned ileal pouch often associated with pain and bloody diarrhea, responded beneficially to glutamine suppositories (2 g/day for 3 weeks) with no recurrence during treatment and one week after [47].

It is notable that enteral glutamine nutrition, which initially did not raise the blood glutamine concentration, has been shown to improve the outcome in premature infants; for example, the frequency of sepsis decreased and immunity increased following enteral supplementation of glutamine, 0.3 g/kg body weight [48]. These beneficial effects presumably reflect increased bowel maturation [48], indicating that enteral glutamine can act on the gastrointestinal tract.
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Table 1. Enteral glutamine–clinical studies

<table>
<thead>
<tr>
<th>Positive findings:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer–response to chemotherapy [44]</td>
<td>improved</td>
</tr>
<tr>
<td>Premature infants–outcome and immunity [48]</td>
<td></td>
</tr>
<tr>
<td>Peptic ulcer–healing [36]</td>
<td></td>
</tr>
<tr>
<td>Stomatitis–duration and severity [50, 51]</td>
<td>reduced</td>
</tr>
<tr>
<td>ICU–hospital costs [40, 58]</td>
<td></td>
</tr>
<tr>
<td>Morbidity in multiple trauma patients [40]</td>
<td></td>
</tr>
<tr>
<td>IBD–intestinal permeability [46]</td>
<td></td>
</tr>
<tr>
<td>Pouchitis [47]</td>
<td>beneficial</td>
</tr>
<tr>
<td>Chemotherapy–intestinal barrier function [49]</td>
<td></td>
</tr>
<tr>
<td>DMD–protein breakdown [39]</td>
<td>slowed down</td>
</tr>
<tr>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>Recovery of body Gln pool [60]</td>
<td>delayed</td>
</tr>
<tr>
<td>Cancer–response to chemotherapy [43]</td>
<td>not influenced</td>
</tr>
<tr>
<td>Premature infants [41]/adults [40]–plasma glutamine levels</td>
<td></td>
</tr>
<tr>
<td>Nitrogen economy [37, 38]</td>
<td></td>
</tr>
<tr>
<td>Mucositis [52, 53]</td>
<td></td>
</tr>
<tr>
<td>Morbidity/mortality [58]</td>
<td></td>
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</tbody>
</table>

without exerting direct systemic effects [45]. In adult patients glutamine has been shown to have a beneficial effect on intestinal barrier function when given orally (30 g/day) for several weeks following high-dose chemotherapy or radiotherapy for esophageal cancer [49].

In a pilot study, ‘swish and swallow’ therapy with 4 × 2 g glutamine/day for 28 days reduced the symptoms of severe mucositis following extensive chemotherapy [50]. Following this pilot study, a large randomized clinical trial was done on the effects of oral glutamine on mucositis in 195 bone marrow transplant patients [51]. Less mouth pain, less difficulty in eating, and a reduction in the use of opioids were found with a low dose of glutamine, and the 28-day survival rate was improved in the glutamine group. In contrast, a randomized, placebo-controlled, double-blind study in 24 mucositis patients showed no benefit of oral glutamine (16 g/day) over placebo [52]. Another study in 28 cancer patients receiving 5-fluorouracil showed no difference between the glutamine-treated group (16 g oral glutamine/day) and the placebo group [53].

The reasons for the unfavorable results with enteral glutamine supplementation are multifactorial. The presence of bacterial overgrowth in stressed patients might in part explain the observed low circulating glutamine concentrations, as it
is well known that bacteria readily consume glutamine as a preferred substrate. It is also possible that splanchnic glutamine utilization may contribute to the inability of glutamine-enriched enteral feeds to increase the plasma glutamine levels. Glutamine is absorbed in the upper part of the small intestine and subsequently metabolized in the liver, and thus it may not be available in sufficient quantity for the target mucosal tissue at the lower sites of the intestine. Another problem is that the amount of glutamine in available commercial enteral products is insufficient. Using a recently developed method [54], the content of protein- and peptide-bound glutamine could be measured in 15 commercially available diets (Table 2). The content in the protein-based preparations varied between 5.2 and 8.1 g/16 g nitrogen. In the peptide-based products, considerably lower glutamine contents were measured (1.3–5.6 g/16 g nitrogen). This daily amount might be satisfactory for healthy individuals but is certainly not sufficient for the adequate support of the stressed patient [55].

An impressive confirmation that enteral glutamine is effective in preventing infective complications has, however, been recently reported in 60 patients with severe multiple trauma [40]. In a randomized controlled study, enteral glutamine nutrition (25–30 g/day) was begun within 48 h of trauma by nasoduodenal tube for a minimum of 5 days [56]. There was a significant reduction (~50%) in the 15-day incidence of pneumonia, bacteremia, and severe sepsis. As a measure of the systemic inflammatory response, the glutamine group showed lower levels of soluble tumor necrosis factor (TNF) receptors. The strength of this study lies in the relatively homogeneous population of patients studied, and the fact that it did not suffer from the confounding factors present in multicenter studies [57]. The results of this fascinating study require early confirmation.

In a current study of a more heterogeneous group of ICU patients capable of tolerating enteral feeding [58], many of whom were already infected on admission, there was no suggestion of reduced mortality, but overall postintervention hospital costs were significantly reduced in both enteral and parenteral glutamine recipients. In a randomized, double-blind study oral and parenteral glutamine supplementation was evaluated in 66 bone marrow transplant patients. Unfortunately the investigators did not distinguish between enteral (oral) and parenteral treatment. Nevertheless there was a suggestion of a possibly improved long-term survival [59].

There is some evidence that the body glutamine pool is slower to recover when the same dose of glutamine is given enterally (orally) as opposed to parenterally [60]. The enteral route may be ideal when given early to the noninfected patient to improve gut-associated lymphoid tissue function and immune defense against infection, but for already severely stressed or infected ICU patients, enteral supplements alone may be inadequate, and parallel parenteral support is likely to be required. It has been clearly shown that during intensive care parenteral supplementation of enteral nutrition with glutamine does not increase the risk to the patients and may ensure a better overall outcome [61]. It should, however, be borne in mind that enteral supplementation with glutamine


### Table 2. Glutamine contents of selected enteral formulae [55]

<table>
<thead>
<tr>
<th>Product (Company)</th>
<th>Gln content g/16 g nitrogen, ± SD</th>
<th>mg/100 ml formula</th>
<th>Gln (g/day) per max. daily dosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrodrip® Standard® (Sandoz)</td>
<td>5.18 ± 0.56</td>
<td>189.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Impact® (Sandoz)</td>
<td>5.58 ± 0.44</td>
<td>312.3</td>
<td>6.3</td>
</tr>
<tr>
<td>Fresubin® (Fresenius)</td>
<td>7.89 ± 0.50</td>
<td>300.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Salvimulsion® (Clintec)</td>
<td>6.17 ± 0.31</td>
<td>292.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Biosorbin® MCT (Nutricia)</td>
<td>5.85 ± 0.88</td>
<td>292.4</td>
<td>5.9</td>
</tr>
<tr>
<td>Surogat D Milkfree (Nutricia)</td>
<td>5.26 ± 0.34</td>
<td>210.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Ensure® (Abbot®)</td>
<td>7.71 ± 0.54</td>
<td>323.7</td>
<td>6.5</td>
</tr>
<tr>
<td>Nutricomp® Intensive (Braun)</td>
<td>6.52 ± 0.26</td>
<td>390.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Peptisorb® Liquid (Nutricia)</td>
<td>1.34 ± 0.21</td>
<td>50.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Salvation® Liquid (Clintec)</td>
<td>5.58 ± 0.47</td>
<td>265.1</td>
<td>5.3</td>
</tr>
<tr>
<td>Survimed® OPD (Fresenius)</td>
<td>1.86 ± 0.02</td>
<td>83.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Nutricomp® Peptide (Braun)</td>
<td>2.87 ± 0.04</td>
<td>129.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Meritene® (Sandoz)</td>
<td>8.10 ± 0.52</td>
<td>1620.3/250 ml</td>
<td>4.9</td>
</tr>
<tr>
<td>Dilsana® (Milupa)</td>
<td>6.99 ± 0.14</td>
<td>789.7/50 g+150 ml</td>
<td>2.4</td>
</tr>
</tbody>
</table>

is a potential hazard as such formulations may form a vigorous culture medium for microorganisms if strict care is not taken [57].

### Cyst(e)ine

**Metabolism**

In healthy adults, the sulfur-containing amino acid cysteine can be synthesized from methionine using the liver-specific transsulfuration pathway. In liver tissue of fetuses and of preterm and term infants, the activity of cystathionase, a key enzyme in the transsulfuration pathway, is low or undetectable [62]. In liver disease, the cysteine requirements of the body cannot be met owing to the diminished transsulfurating capacity. Cysteine should be considered an essential amino acid in immature infants and a conditionally essential amino acid in liver disease. In both cases it should be provided exogenously [63]. The addition of cyst(e)ine to nutritional preparations might, however, cause problems. At neutral or slightly alkaline pH, cysteine rapidly oxidizes during storage to yield the dimer cystine, which itself is very poorly soluble and thus precipitates. Acidic conditions may lead to a reduction of the sulfhydryl group and the formation of H₂S.

Cyst(e)ine is a potent antioxidant. This property will be evaluated in detail elsewhere in this volume. Cysteine also enhances various lymphocyte functions, for example cytotoxic T-cell activity [64]. A high glutamate to cysteine ratio is associated with a low proportion of T-helper cells [65]. N-Acetylcysteine, reduced
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glutathione, and cysteine inhibit the expression of the nuclear transcription factor in stimulated T-cell lines [66, 67]. This might provide an interesting approach in the treatment of AIDS, as the transcription factor enhances HIV mRNA expression. In fact, in vitro studies show that the stimulatory effects of TNF, induced by free radicals, on HIV replication in monocytes can be inhibited by sulfur-containing antioxidants [68, 69]. These basic studies indicate that treatment of inflammatory diseases and AIDS with sulphydryl antioxidants may be beneficial, and powerful arguments have been advanced in favor of such treatment [65, 70]. Clinical studies using this strategy are not yet available. One reason for this might be the lack of suitable preparations. For example, N-Acetylcysteine cannot be used in human subjects because of the lack of tissue acylases, except in the kidney [7]; thus, the compound will accumulate and subsequently be excreted in the urine [7, 71]. The use of stable and highly soluble synthetic dipeptides might be advantageous (see below).

Tyrosine

Metabolism

The aromatic amino acid tyrosine has traditionally been considered a nonessential amino acid for adult humans. Tyrosine is synthesized exclusively from phenylalanine by hydroxylation; inclusion of tyrosine in the diet exerts a sparing effect on the dietary phenylalanine requirement. In premature infants, tyrosine is considered an essential amino acid; reduced endogenous tyrosine synthesis also may occur in full-term infants [72].

Clinical Use

In renal failure, the concentration of tyrosine and its ratio to phenylalanine is consistently low [73, 74]. These results have been repeatedly attributed to reduced oxidation of tyrosine from phenylalanine owing to a partial inhibition (perhaps by uremic toxins) of the enzyme phenylalanine hydroxylase. The low solubility of tyrosine in aqueous solution means that its concentration in amino acid solutions cannot exceed 0.4–0.5 g/l, an amount that may well be insufficient to meet tyrosine requirements in clinical situations. Highly soluble tyrosine-containing synthetic dipeptides are now available, enabling adequate tyrosine nutrition in clinical practice (see below) [1].

Arginine

Metabolism

Arginine is a dibasic amino acid which the body obtains from dietary sources and by endogenous synthesis through the urea cycle. Arginine is absorbed in the small intestine by the active transport system y+.
Arginine is a precursor of polyamine, histidine, and nucleic acid synthesis, a promoter of thymic growth, and an endocrinologic secretagogue stimulating release of growth hormone, prolactin, insulin, and glucagon [75]. It is metabolized within the enterocyte by the arginase pathway to ornithine and urea, and by the arginine deiminase pathway to citrulline [76]. Arginine metabolism in enterocytes may participate in the support of gut morphology and function by acting as a substrate for nitric oxide synthesis [77]. Inhibition of nitric oxide synthesis increases intestinal mucosal permeability in experimental models of ischemia/reperfusion intestinal injury [78] and acute necrotizing enterocolitis [79]. In addition, administration of L-arginine reverses the effect of nitric oxide synthase inhibition [78]. These results suggest that basal nitric oxide production is important in minimizing the mucosal barrier dysfunction in these models.

Arginine and Experimental Disease Models

In experimental colitis [80], supplemental enteral arginine increased mucosal inflammation, whereas administration of nitric oxide synthase inhibitors reduced intestinal inflammation in colitis and ileitis models [81]. In an experimental burn model [82, 83], supplemental enteral arginine reduced bacterial translocation and improved survival. In experimental sepsis, supplemental arginine improved survival in mouse [83] and rat [84] models, but not in a guinea pig model [85]. Additional animal studies have shown the protective effect of arginine against hepatic ischemia reperfusion injury [86] and its ability to attenuate intestinal bacterial translocation [87]. In contrast, other recent experiments have shown that arginine supplementation augments tumor growth in a murine breast cancer cell line [88].

These experimental studies have provided conflicting evidence on the effects of arginine on gut structure, function, and outcome. Enhancement of immune rather than gut function may explain the arginine induced exacerbation of experimental colitis [80], as well as the beneficial effects of arginine on bacterial translocation and survival in the experimental burn model [83].

One potential complication of arginine administration is its known competition with the essential amino acid lysine for tubular reabsorption [89]. Thus large doses of arginine may theoretically induce lysine deficiency by increasing its renal excretion. An additional potential complication relates to the risk of metabolic acidosis in patients receiving acidic arginine salts enteraly. In addition, the role of arginine as a substrate for nitric oxide synthesis and the observations implicating nitric oxide in the pathogenesis of septic shock syndrome [90] suggest that excess arginine supplementation could be hazardous in severely ill patients [77].

Clinical Use of Arginine

Clinical studies on enteral arginine administration have shown moderate net nitrogen retention and protein synthesis compared with isonitrogenous diets in critically ill and injured patients; and after surgery for certain malignancies
in elderly postoperative patients supplemental arginine (25 g/day) enhanced T-lymphocyte responses to phytohemagglutinin and concavalin A and increased the CD4 phenotype number [91]. Interestingly, insulin-like growth factor 1 levels were increased by about 50%, reflecting the growth hormone secretion induced by arginine supplementation. A large oral arginine intake (30 g/day) improved wound healing [92] and enhanced the blastogenic response to several mitogens [93]. On the other hand, in some of these studies there was also in vitro evidence of enhanced immunoactivity [94–97]. Overall, there was no improvement in patient outcome or length of hospital stay [98].

It is probable that the observed beneficial effects of these substrates reflected improvements in immune function rather than improved gut barrier function; in the largest study, clinical benefit was claimed for a subgroup of patients [99]. The methods used, however, have been seriously questioned [100].

**Ornithine**

Ornithine is a basic amino acid which is not a constituent of proteins but is a precursor of aliphatic polyamines and an endocrinological secretagogue [77]. Ornithine shares an active transport system (y+K) in the small intestine with arginine. Ornithine is metabolized to citrulline by the ornithine carbamoyltransferase pathway, to glutamate and proline by the ornithine aminotransferase (EC 2.6.1.13) pathway, and to putrescine, spermidine, and spermine by the ornithine decarboxylase pathway [77]. Enterocytes possess ornithine decarboxylase, ornithine carbamoyltransferase, and ornithine aminotransferase [77]. However, after enteral administration of 14C-labeled ornithine the radioactivity was only recovered in proline, glutamate, and polyamines [101]. It has been suggested that ornithine metabolism in enterocytes, by acting as a precursor for polyamine synthesis, may support gut morphology. Ornithine has been shown to increase nitrogen retention and to improve wound healing and thymic function in experimental animals [75].

**Ornithine α-Ketoglutarate**

The salt ornithine α-ketoglutarate (OKG) might exert a synergistic effect on both its constituents. Recent investigations suggest that following enteral OKG administration gut morphology and function improve, trauma-induced immune dysfunction is alleviated, and there are anabolic/anticatabolic actions on protein metabolism [102, 103]. The majority of these studies were performed in various experimental models. It is now necessary to confirm the postulated benefits in controlled clinical trials. Theoretically the properties of OKG should counteract the catabolic response that occurs during episodes of infection and after trauma and injury. Enteral administration has been proposed in various catabolic situations [103]. Favorable effects on muscle protein synthesis have been observed in trauma and burned patients after enteral administration [103, 104], and improved
N balance and protein synthesis have been found after intravenous administration [105]. Nevertheless, direct beneficial effects of ornithine or OKG on gut structure, function, or outcome have not been demonstrated [4]. Thus any clinical impact of the findings outlined above requires confirmation by further controlled studies [106].

**Taurine**

*Metabolism*

Taurine (2-aminoethane sulfonic acid) is the most abundant free amine in the intracellular compartment [11]. The major pathway for the biosynthesis of taurine is through cysteine sulfenic acid. Taurine seems to have a functional role in stabilizing the membrane potential in bile salt formation, growth modulation, osmoregulation, antioxidation, promotion of calcium transport and calcium binding to membranes, vision, and positive ionotropic effects on the heart, as well as having antiarrhythmic and antihypertensive effects. It is involved in many metabolic responses in the central nervous system, has an anticonvulsant action, may have an insulinogenic action, and is required for eye function [107, 108]. Taurine is capable of influencing programmed cell death in various cell types depending upon the initiating apoptotic stimulus [109], and of affecting Fas (CD95/APO-1)-mediated neutrophil apoptosis through the maintenance of calcium homeostasis [110].

*Is Taurine Conditionally Indispensable during Stress?*

There is some evidence that taurine might be indispensable during episodes of catabolic stress. We and others found low extracellular and intracellular taurine concentrations after trauma and infection [111, 112]. Low taurine concentrations in plasma, platelets, and urine have been described in infants and children and also in adult trauma patients undergoing taurine-free long-term parenteral nutrition [113–116]. Plasma taurine deficiency after intensive chemotherapy or radiotherapy is more severe in patients receiving taurine-free parenteral nutrition than in orally fed patients [117]. Low intracellular taurine concentrations in muscle are a typical feature in patients with chronic renal failure, probably because of impaired metabolic conversion of cysteine sulfenic acid to taurine [118, 119]. Intracellular taurine depletion may be associated with the well-known muscle fatigue and arrhythmic episodes that occur in uremia.

*Taurine–A Potent Antioxidant*

Taurine offers protection against oxidant damage in experimental lung inflammation [120]. Experimental depletion of tissue taurine concentrations, especially in the lung, produces inflammation; administration of pro-oxidants results in severe lung edema and interstitial fibrosis. Taurine administration ameliorates the symptoms [121]. The underlying mechanism of taurine action may be
its interaction with \( \text{H}_2\text{O}_2 \) and \( \text{Cl}^- \) in the myeloperoxidase reaction, thereby producing taurine chloramine, an oxidant with very low reactivity, partially quenching free radical generation [68, 69]. Taurine chloramine may exert a potent anti-inflammatory effect by suppressing TNF and nitric oxide production in endotoxin and interferon-\( \alpha \)-stimulated macrophage cell lines [122].

**Taurine Supplementation?**

Taurine has been characterized as a conditionally essential amide in preterm infants and neonates and is currently incorporated in most neonatal dietary regimens [108]. Our understanding of the role of taurine in various pathological functions is largely based upon animal studies. More recently, however, there have been a few human studies that provide some insight into possible therapeutic applications. Taurine is obviously important in several medical conditions such as sepsis, ischemia-reperfusion states, postoperative states, pulmonary fibrosis, cardiac failure, and so on [108].

The question arises as to whether taurine supplementation could be beneficial in chronic renal failure, during episodes of catabolic stress, and in other conditions in which it might have a beneficial effect on morbidity or outcome. Free crystalline taurine is available for inclusion in intravenous or enteral preparations. However, we hypothesize that the extremely high intracellular to extracellular transmembrane gradient (250:1) might limit the cellular uptake of taurine. We proposed a novel binding of taurine to a suitable amino acid carrier in the form of a synthetic taurine conjugate [10]. Experimental data strongly suggest improvement in transmembrane transport and intracellular utilization with this conjugate [123].

Taurine might possess biological properties that enable it to act as a potent molecule in the regulation of inflammatory and immunological processes as well as serving as a powerful antioxidant. It is worthwhile considering taurine as a future important member in the growing family of pharmacological nutrients.

**The Dipeptide Concept—True New Substrates in Protein Nutrition**

The obvious limitations to the use of glutamine, tyrosine, and cysteine as free \( \text{L} \)-amino acids have initiated a research program to combine the synthesis and characterization of glutamine-, tyrosine-, and cysteine-containing short-chain peptides or taurine conjugates with investigations of their \( \text{in vivo} \) uptake and subsequent tissue utilization in animal models, healthy human subjects, and patients with various disorders. Synthetic dipeptides are highly soluble in water (Table 3) and they are thermostable during sterilization procedures.

Basic experimental and human studies with various synthetic glutamine-, tyrosine- and cysteine-containing dipeptides and taurine conjugates provide convincing evidence that these new substrates are well utilized in man and experimental animals.
**Table 3.** Chemical/physical characteristics of selected free amino acids and synthetic short-chain peptides

<table>
<thead>
<tr>
<th></th>
<th>Solubility g/l H₂O at 20°C</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystine</td>
<td>0.1</td>
<td>yes</td>
</tr>
<tr>
<td>Cysteine-HCl</td>
<td>252.0</td>
<td>no</td>
</tr>
<tr>
<td>Bis-L-alanyl-L-cystine</td>
<td>&gt;500.0</td>
<td>yes*</td>
</tr>
<tr>
<td>Bis-glycyl-L-cystine</td>
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<tr>
<td>Tyrosine</td>
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</tr>
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<td>Glutamine</td>
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</tr>
<tr>
<td>Glycyl-L-glutamine</td>
<td>154.0</td>
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* Sterile filtration

Clinical studies are providing overwhelming evidence that the currently applied concept of dipeptide nutrition is beneficial in supplying patients with the conditionally indispensable amino acids, which are otherwise difficult to deliver. Indeed, the provision of glutamine, cystine, and tyrosine should be considered as eliminating a deficiency rather than as supplementing currently provided intakes. It is conceivable that the beneficial effects observed with dipeptide nutrition result simply from the correction of inadequate conventional nutrition [89, 124]. The availability of stable dipeptide-containing preparations will certainly improve amino acid nutrition in the routine clinical setting and represents a new dimension in clinical nutrition.

**Conclusions**

The main focus of this chapter has been on the evaluation of enteral provision of certain amino acids with selective actions on the immune system, the ability to maintain gut mucosal integrity, and the capacity to reduce morbidity and mortality. From the experimental and clinical studies to date, the strongest candidate would appear to be glutamine. Enteral arginine supplementation, although its immunological benefit is proven, has no direct protective effect on mucosal integrity. High doses induce urinary loss of lysine and might be associated with the formation of nitric oxide. At present, there is insufficient evidence that ornithine or OKG supplementation could improve immunity or intestinal structure and function. Cystine and taurine are highly interesting substrates with several new functional properties. The growing body of evidence
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from experimental studies should be critically evaluated in future clinical investigations.

This review serves to illustrate how far we have advanced in our knowledge of the importance of substrates, both old and new, in modern clinical enteral nutrition. I have also made an attempt to highlight areas that hold promise for the use of conditionally essential amino acids in patient care. There is little question that efforts to modify the response to disease by nutritional means will be rewarded by improved patient outcome.

References

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**Discussion**

*Dr. Silk:* It seems to me there are a number of problems with these new substrates. One is the poor quality of controlled clinical trials in terms of design and endpoints. Another relates to the problem of multiple substrates, where investigators have decided to put them all in the pot and then draw what conclusions they wish, depending on which substrate they have an interest in. I think the scientific and clinical community has got to do a great deal better. We’ve made a start: some very helpful information is starting to come out of these studies and we’ve got to learn from them – for example, the brilliant trial you quoted from Holland indicating a potential benefit of glutamine on infection. But I fail to see how you can even expect to show a benefit of any of these compounds in patients undergoing massive chemotherapy. They’ve got malignant disease, they’re malnourished, and serious chemotherapy these days is about the biggest insult the human organism can handle. Yet people are putting such patients in these trials and measuring every single endpoint they can imagine, hoping that one or two will come up and forgetting that they’re bound to, because that’s what a 5% chance is. So I’m somewhat skeptical about these new substrates, and I’m not at all surprised that you had a ‘for’ slide and an ‘against’ slide. Though much of the data you have discussed has potential, one has to decide on the endpoints and what to expect of them, and then test one substrate at a time.

*Dr. Fürst:* First, I would like to return the ball to the clinician. I have synthesized the dipeptides, performed some animal studies, carried out rat N-balance studies and, together with other investigators, made more or less important studies and the product finally came to the clinic after 8–10 years. Probably no substance has been investigated as much as glutamine, but despite that, people are still asking questions and being suspicious. If it was a pharmaceutical drug, no such questions would be raised; people accept the findings about an antibiotic, or a hypotensive agent. This probably reflects the fact that on the one hand we are speaking of a drug and on the other hand of a nutrient. Please try, therefore, to accept these substances as pharmaceuticals and judge them on that basis.

Second, if the quality of trials is poor, that is the clinician’s business, though I agree some of the trials are very poorly constructed.

Third, I agree with you that we need clear evidence that glutamine or other substances affect morbidity and mortality, these are the true endpoints. We have now four studies showing very interesting effects on patients suffering from critical illness [1–4].

Finally, about controversial results: controversial results have been obtained with respect to the enteral studies, not the parenteral. Actually, 92% of the parenteral data indicate benefit. The controversial enteral data might be partly due to the bacterial overgrowth, bacteria might consume 30% of the glutamine provided. Another reason might be that enterally administered glutamine will be absorbed in the upper part of the small intestine and, thus, won’t be available for the real intestinal target. It is absolutely clear, that in many of the studies, including those in which glutamine was beneficial, plasma glutamine concentrations were not increased, so this means that the beneficial effect
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observed, for instance, in very low birth weight infants [5, 6] is not dependent on plasma concentration but on some other mechanism.

In order to promote clinical nutrition with new substrates, one should make all efforts to perform well-designed clinical studies, instead of overcriticism and scepticism.

Dr. Silk: I’m not arguing with you personally; I’m actually arguing against my own clinical research colleagues, who I don’t think overall during the last 20 years have designed spectacularly good trials. And indeed the quality of the trials has been considerably less in my opinion than most of the controlled clinical trials designed to test pharmacological compounds, which have defined endpoints. One of the problems in this field is defining endpoints.

Dr. Young: What endpoints would you propose?

Dr. Fürst: Morbidity and mortality, no more, no less. None of the other measures are of any real interest, such as plasma amino acids, tissue amino acids, nitrogen balance, and so on.

Dr. Hunter: When we first started using enteral feeding in Crohn’s disease back in the early 1980s, we didn’t give glutamine because it wasn’t stable in the solutions we used. Nevertheless they worked beautifully. When we started to learn about glutamine we added it and the results were no better. It seems to me that we should perhaps be considering separate indications for the use of enteral feeds: short-term conditions, such as postoperative patients and those with critical illnesses, and long-term illnesses, such as Crohn’s and other conditions one can treat enterally. Maybe glutamine is very useful in one situation and not so important in the other.

Dr. Fürst: I think that’s a very important comment. I would warn you against giving glutamine in inflammatory bowel disease. Glutamine promotes mucosal proliferation and that’s just what you don’t want in Crohn’s disease.

Dr. Barbul: I agree with all your comments but I think you’re being too restrictive about endpoints. There are some wonderful biological endpoints one can look at, such as wound healing or the immune response, which if looked at in a focused way may be just as beneficial as overall outcome. There are now two meta-analyses, for example, on the combined cocktail of arginine, fish oil, and so forth, that show great reductions in length of stay, great reductions in infection, great reductions in days of ventilation, but no difference in mortality at all [7, 8]. This leaves us with the conundrum that, if we have all these wonderful effects, why is there no impact on mortality? It is very reminiscent of the 1970s and the whole TPN issue. The problem with most of the older literature, as you say, is that it has been using pharmacologic doses of these compounds but looking at nutritional effects. But I wouldn’t be so restrictive about endpoints as you.

Dr. Fürst: I agree about wound healing, but I am more hesitant about immunity, because my gut feeling is that improved immunity should be associated with reduced morbidity. Also it is extremely difficult to appropriate measure immunity.

Dr. Millward: It seems to me that at some stage we have got to be very pragmatic and make a decision as to whether we are going to use glutamine as standard therapy. I think the real question is, what are the contraindications to the use of glutamine in both enteral and parenteral nutrition? Maybe we’ve reached the stage where we should say, there’s still a lot of work to do to understand what’s going on, but we have sufficient evidence of potential benefit and insufficient evidence of any contraindication, so we should go ahead and start using it.

Dr. Fürst: I don’t know of any situation in which glutamine is harmful. It has been given in very high doses, up to 80 g [9] without any problems, although I would be careful in inflammatory diseases as glutamine promotes proliferation and that might be undesirable.

Dr. Wernerman: I think the difficulty in doing clinical trials is to define which leg to stand on: is there a deficiency of glutamine or is it working as a drug? To define glutamine
deficiency is in my view very difficult, because if you starve the level goes down – the concentration in muscle, for example, goes down by half – but it is restored immediately when you start to eat again. Postoperatively it goes down, and then it’s restored over the next 2 weeks. In intensive care it declines to <25%, but a proportion of these patients can still synthesize a lot of glutamine if we measure A-V difference or export from the periphery, though some can’t. So just to measure the intracellular concentration will not be enough to define glutamine depletion. The next step is a clinical study. We include a lot of ICU and postoperative patients, and some of these are really depleted while some are not. From this mixture we try to determine clinical outcome which is difficult. So my question is, have you any idea how we should define glutamine depletion?

Dr. Fürst: Unfortunately that’s very difficult. There can be a considerably decreased muscle intracellular content of glutamine that is not reflected in the plasma. I have collected data from the available literature and it appears that even a 50% reduction in intracellular glutamine can be associated with normal plasma concentrations. The only exception is the critically ill patient with severe sepsis showing low plasma concentrations. I don’t have any tool to monitor glutamine depletion at present.

Dr. Roessle: The dipeptide concept has been developed to overcome various technical problems and to allow amino acids to be included in parenteral nutrition. In oral and enteral nutrition you have at least the possibility of adding free amino acids just before giving the enteral feeds to the patient. Do you see any advantages of peptides or dipeptides over free amino acids in enteral and oral nutrition?

Dr. Fürst: Free amino acids can be kept satisfactorily as powder or freeze-dried, but at some point you must dissolve them, and then the problem starts. At 20°C there is no real decomposition of glutamine, but at 30°C there is considerable decomposition. So the question arises as to what is practicable.

References