NOVEL SUBSTRATES TO MAINTAIN GUT INTEGRITY

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Numerous scientific studies in the previous 20 years have documented the value of nutrient support in the management of patients with critical illness. In recent years there have been two major trends in the use of nutritional support. Primarily, there has been a major shift from intravenous towards enteral administration. Several large studies have established the advantages (reduced septic morbidity, and reduced cost) of early enteral (compared to parenteral) feeding. Secondly, there have been major changes in the content and protein/calorie mix of the formulations used. The most striking findings relate to the use of specific nutrient substrates to supplement standard enteral diets. Glutamine, arginine, branched chain amino acids, n-3 fatty acids, ornithine and nucleotides have attracted most attention. They produce their beneficial effects in several ways: support of the systemic immune response, enhancement of gut mucosal barrier function, modulation of the neuroendocrine and cytokine responses and of Kupffer cell function.

This article will critically review the use of novel substrates which have a perceived role in the maintenance of gut integrity. We will assess their roles in health and disease, the rationale for their clinical use, their mechanism of action and supporting evidence from clinical and animal studies of their efficacy.

The intestinal tract is usually thought of primarily as a digestive and absorptive organ. However, it also has a major barrier function, protecting the body from potentially harmful intraluminal pathogens and large antigenic molecules (Saadia et al. 1990), and additionally plays a pivotal role in the metabolic processing of glutamine (Soubra et al. 1985). The gut mucosal barrier comprises both non-immunological and immunological components (Langkamp-Henken et al. 1992; Table 1). Normal epithelial cell structure prevents transepithelial migration of particles from the gut lumen, and tight junctions between cells prevent movements through the paracellular channels (van Leeuwen et al. 1994).

**EVIDENCE FOR GUT MUCOSAL BARRIER DYSFUNCTION**

Many studies investigating the intestinal barrier have examined nutritionally induced changes in intestinal weight or structure (villous height, crypt depth) or mucosal content of protein or DNA as indicators of intestinal atrophy. The demonstration of intestinal atrophy...
Table 1. Components of the gut mucosal barrier

<table>
<thead>
<tr>
<th>Non-immunological</th>
<th>Immunological</th>
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<tbody>
<tr>
<td>Salivary secretions (lactoferrin, lysozyme)</td>
<td>Gut-associated lymphoid tissue</td>
</tr>
<tr>
<td>Intraluminal gastric pH</td>
<td>Secretory immunoglobulin</td>
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<tr>
<td>Proteolysis</td>
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<tr>
<td>Intestinal bile salts</td>
<td></td>
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<tr>
<td>Peristalsis</td>
<td></td>
</tr>
<tr>
<td>Mucus coat</td>
<td></td>
</tr>
<tr>
<td>Microvillus membrane</td>
<td></td>
</tr>
<tr>
<td>Commensal bacteria</td>
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</table>

has often been assumed to indicate impaired intestinal barrier function. However, animal studies of prolonged protein malnutrition have shown no direct correlation between the development of intestinal atrophy and the loss of barrier function (Deitch et al. 1990a).

Dysfunction of the mucosal barrier is thought to result from an imbalance of aggressive and defensive factors acting on the gastrointestinal mucosa (Sartor, 1990). Genetic and environmental factors may modify the response of the gastrointestinal mucosa to proinflammatory factors or may modify the protective factors. Increased gastric acid production, colonization with *Helicobacter pylori*, ingestion of non-steroidal anti-inflammatory analgesics and the use of broad spectrum antibiotics predispose the gastrointestinal mucosa to ulceration (Berg, 1981). Mucosal blood flow (Fink, 1991), oxidative fuel supply (van der Hulst et al. 1993) and a normal intestinal bacterial flora (Barber et al. 1991) protect against barrier dysfunction.

Failure of the intestinal mucosal barrier results in permeation of microbial and dietary antigens across the intestinal wall. The transmural migration of enteric bacteria or bacterial endotoxins to extraintestinal sites has been termed translocation (Berg & Garlington, 1979; van Leeuwen et al. 1994). Bacterial translocation is assessed by quantitative and qualitative culture of samples of blood, mesenteric lymph nodes, liver and spleen or by the detection of radioactivity in extraintestinal tissues following the oral administration of bacteria labelled with $^{14}$C or fluorescein isothiocyanate (FITC). Translocation of endotoxin into the portal or systemic circulation can be assessed using the chromogenic *Limulus* assay or by measurement of plasma radioactivity after administration of labelled $[^{3}$H$]$, $[^{125}$I$]$- or $[^{14}$C$]$endotoxin. There is growing evidence from both *in vivo* (Alexander et al. 1990; Wells et al. 1990) and *in vitro* (Wells et al. 1993; Cruz et al. 1994; Go et al. 1995) studies that transmucosal passage of intestinal organisms occurs transcellularly.

The term ‘permeability’ is used to describe the passive penetration of the intestinal barrier by non-charged and usually non-absorbable macromolecules (Travis & Menzies, 1992). An increase in the permeability of the intestinal wall to macromolecules in comparison to normal intestine is thought to result from migration through the junctional complex (paracellular route), through ulcerations or across extrusion zones at the villous tip. The degree of intestinal permeability is usually assessed by measuring the urinary excretion of orally administered non-metabolized macromolecules such as sugars (lactulose, mannitol, rhamnose, cellobiose), inert polymers (polyethylene glycol, FITC-dextran) or radiolabelled compounds ($[^{51}$Cr$]$EDTA, $[^{99m}$Tc$]$diethylenetriaminepenta-acetic acid (DTPA); Travis & Menzies 1992).

The evidence that bacterial translocation occurs transcellularly and that permeability is related to molecular penetration of the tight junction suggests that these measures of barrier function may be unrelated. Indeed, bacterial translocation is a much more complex
phenomenon than permeability, depending on intestinal resistance to bacterial adherence and the gut-associated lymphoid tissue, in addition to the mechanical barrier of the intestinal mucosa (Deitch, 1990a). Thus increased translocation reflects a failure of one or more components of the mucosal defense system and not necessarily damage to the epithelial barrier per se (Fink, 1991).

However, increases in both permeability and translocation have been observed in animal models of gut atrophy induced by elemental diets, lack of dietary fibre or total parenteral nutrition (TPN), sepsis and haemorrhagic shock (Deitch et al. 1990b, 1991, 1995; Spaeth et al. 1994). Rats that are nourished by TPN alone develop progressive gut atrophy with loss of mucosal thickness and villous height (Levine et al. 1974; Koga et al. 1975; Eastwood, 1977), a significant increase in intestinal permeability to lactulose within 48 h (Helton et al. 1991) and translocation of enteric bacteria after 7–10 d (Alverdy et al. 1990). In contrast, Illig et al. (1992) did not find any correlation between intestinal permeability to lactulose and translocation of bacteria in TPN induced intestinal atrophy.

CLINICAL SIGNIFICANCE OF GUT MUCOSAL BARRIER DYSFUNCTION

Enhanced uptake of macromolecules and bacteria has been demonstrated where the intestinal mucosa is damaged by inflammation (Gardiner et al. 1995), infection (Brock-Utne & Gaffin, 1989), neoplasia (Lescut et al. 1990) or trauma (Kelley et al. 1985). In addition, systemic endotoxaemia, bacterial translocation and increased intestinal permeability to macromolecules have been demonstrated in patients with rheumatic diseases (Busch et al. 1988), haemorrhagic shock (Roumen et al. 1993), burns (Deitch, 1990b; LeVoyer et al. 1992), burn sepsis (Ziegler et al. 1988), major trauma (Rush et al. 1988; Roumen et al. 1993), following chemotherapy or radiotherapy (Parrilli et al. 1982), and in experimental endotoxaemia in human volunteers (O'Dwyer et al. 1988) in the absence of macroscopic intestinal disease. However, translocation of endotoxin or bacteria have not been commonly found in patients after major trauma (Rush et al. 1988; Moore et al. 1991; Hoch et al. 1993; Roumen et al. 1993) or burns (Deitch, 1990b).

The clinical significance of intestinal mucosal barrier dysfunction is more difficult to determine. In patients with inflammatory bowel disease, translocation of endotoxin and bacteria may explain systemic immune activation, disturbances in hepatic function, the pathogenesis of abscesses and fistulas, extraintestinal manifestations, and the high incidence of sepsis following elective surgery (Eade & Brooke, 1969; Ambrose et al. 1984; Wellmann et al. 1986). Evidence to support this hypothesis comes from studies demonstrating positive correlations between clinical and laboratory measures of disease activity and intestinal permeability to hydrophilic probes $[^{51}\text{Cr}]\text{EDTA}$ and $[^{99m}\text{Tc}]\text{DTPA}$ (Murphy et al. 1989; Pironi et al. 1990) and systemic endotoxin concentrations (Wellmann et al. 1986; Gardiner et al. 1995) in both ulcerative colitis and Crohn’s disease. In addition, the incidence of positive portal blood cultures in patients with ulcerative colitis submitted to colectomy was found to be associated with disease severity (Eade & Brooke, 1969).

In patients with intestinal obstruction, a significantly higher incidence of septic complications was seen in those with bacterial translocation (28%) compared with those without (11%), but mortality was unaffected (Sedman et al. 1994). However, the organisms cultured from the operative samples rarely correlated with those causing postoperative sepsis. There are two possible explanations: either septic complications were causally related to bacterial translocation, or both the septic complications and bacterial translocation were separate manifestations of impaired immunity (Sedman et al. 1994).
In critically ill patients, there is circumstantial evidence linking intestinal barrier failure to the hypermetabolic response and multiple organ system failure associated with major trauma, burns and sepsis (Carrico et al. 1986; Saito et al. 1987a; Wilmore et al. 1988; Saadia et al. 1990). Infections acquired by intensive care patients were found to be similar to bacterial cultures from the proximal gastrointestinal tract (Marshall et al. 1988). Enteric microorganisms were responsible for the majority of infective complications in a group of 206 critically ill trauma patients (Tran et al. 1993). Enteric organisms have also been demonstrated in the peripheral blood in patients who are immunocompromised (Tancrede & Andremont, 1985) or traumatized (Rush et al. 1988). In the leukaemic patients, Gram-negative bacteraemia was due to the dominant faecal strain of Enterobacteriaceae or Pseudomonas aeruginosa in 82% of episodes (Tancrede & Andremont, 1985). More direct evidence was provided by Ziegler et al. (1988) who found that an increase in intestinal permeability to lactulose was associated with infection in burn patients. LeVoyer et al. (1992) were able to go further by establishing a positive correlation between intestinal permeability to lactulose and the later development of septic complications in patients with burns. In addition, Oudemans found a clear relation between translocation of endotoxin from the gut and postperfusion syndrome in patients having cardiopulmonary bypass (van Leeuwen et al. 1994).

Clinical studies have therefore shown that intestinal barrier dysfunction is a frequent occurrence in patients with intestinal inflammation or obstruction and can also occur during critical illness. Against this background, it has been hypothesized that translocating endotoxin and bacteria activate a systemic inflammatory cascade and promote multiple organ dysfunction. However, more evidence is required before a direct causal link can be established (Sedman et al. 1994).

GUT DERIVED SEPSIS: THERAPEUTIC STRATEGIES

Maintenance of intestinal epithelial cell structure, balanced luminal microbial populations and gut associated lymphatic tissue have all been considered necessary to prevent translocation of bacteria and toxins from the intestinal lumen to the bloodstream and other organs (Deitch 1991). On this basis, several strategies have been devised to prevent gut origin septic complications in critically ill patients (Table 2).

A wide variety of antibiotics has been successfully used to suppress the faecal Gram-negative aerobic bacilli in healthy volunteers, immunosuppressed patients, critically ill patients and experimental animals (Dekker et al. 1981; Edlund et al. 1987; Rozenberg-Arska & Dekker, 1987). Modification of the intestinal flora is associated with a decreased incidence of complicating infections, including Gram-negative bacteria in various organs and also a decrease in Gram-negative bacteraemia (Dekker et al. 1981; Rozenberg-Arska & Dekker, 1987; Blair et al. 1991).

Enteral diet, in comparison with TPN was found to reduce septic complications, improve nutritional status and improve survival in burned children, injured adults, intensive care unit patients and animals with experimental haemorrhagic shock (Alexander et al. 1980; Border et al. 1987; Moore et al. 1989; Zaloga et al. 1991; Kudsk 1994). In addition to the route of administration, the composition of the enteral diet is also important. Polymeric enteral diets are superior to liquid elemental diets in preventing atrophy of the intestinal mucosa, maintaining mucosal protein and DNA content, secretory immunoglobulin A production, a normal balance of intestinal microflora and mucosal barrier function (Alverdy et al. 1990; Barber et al. 1990; Shou et al. 1991; Deitch et al. 1993, 1995; Spaeth et al. 1994).
Table 2. Strategies to prevent gut origin sepsis

<table>
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<tr>
<th>Strategy</th>
<th>Method</th>
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<tr>
<td>Selective digestive decontamination</td>
<td>Use of antibiotics to eliminate target organisms (enteric Gram-negative bacilli and fungi) without affecting non-pathogenic organisms</td>
</tr>
<tr>
<td>Immunomodulation</td>
<td>Administration of substrates (arginine, nucleotides, n-3 fatty acids) to enhance the immune response to bacteria</td>
</tr>
<tr>
<td>Mucosal enhancement therapy</td>
<td>Promotion of intestinal epithelial cell proliferation, differentiation and function</td>
</tr>
<tr>
<td>(a) Enteral nutrition</td>
<td></td>
</tr>
<tr>
<td>(b) Selective intestinal nutrients</td>
<td>Deitch 1991</td>
</tr>
<tr>
<td>(c) Growth factors</td>
<td>Jacobs et al. 1988</td>
</tr>
<tr>
<td>(d) Trophic gut hormones</td>
<td>Evers et al. 1990</td>
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</table>

**NOVEL SUBSTRATES AND GUT INTEGRITY**

Several strategies combining early nutrition and eradication of bacteria/endotoxin have been suggested for preventing gut failure and subsequent sepsis syndrome or multiple organ failure. However, there has been considerable interest in the utilization of specific substrates (selective gut nutrition) to maintain intestinal mucosal integrity as a barrier to bacteria and endotoxin (Deitch 1991; Elsen & Bistrian, 1991). Substrates suggested as capable of maintaining intestinal integrity include amino acids (glutamine, arginine and ornithine), fatty acids (short chain and n-3 polyunsaturated) and nucleotides.

**GLUTAMINE**

Glutamine is a neutral amino acid which is obtained directly from the diet or indirectly by conversion from α-ketoglutarate or transamination of other amino acids. Glutamine was originally classified as non-essential but is now considered to be conditionally essential in many pathological states (Wilmore et al. 1988; Souba et al. 1990a).

*Metabolism of glutamine*

Glutamine is actively transported across the intestinal brush border by system B transporters (Souba & Copeland, 1992). Glutamine is metabolized in the urea cycle, protein synthetic pathways and the Krebs cycle for energy and for production of citrate, lactate and glucose. It acts as a vehicle for nitrogen transfer between tissues, as a substrate for renal ammoniagenesis and as a precursor for nucleotides. Glutamine is important in the regulation of glycogen synthesis and protein turnover (Smith & Wilmore, 1990). It is also an important metabolic substrate for enterocytes, lymphocytes and many cell culture systems (Smith & Wilmore, 1990).

*Glutamine and the gut mucosa*

Glutamine is the principal respiratory fuel of the small intestine (Windmueller 1982; Souba et al. 1985) and also provides a major portion of the energy required by colonocytes (Ardawi & Newsholme, 1985). It stimulates protein synthesis in isolated enterocytes (Higashiguchi et al. 1993) and is essential for the proliferation and differentiation of
enterocytes (Beaulieu & Calvert 1985). Supplementation of standard chow with glutamine increased blood flow to stomach, small intestine and colon in healthy rats (Houdijk et al. 1994). Intestinal uptake of glutamine from both the intestinal lumen and mesenteric circulation falls during sepsis and endotoxaemia (Souba et al. 1990b; Salloum et al. 1991). It has been suggested that this impairment in gut glutamine uptake may result in ultrastructural changes in the small intestine and failure of the gut mucosal barrier (Wilmore et al. 1988).

**Glutamine and experimental disease models**

Experimental glutamine deficiency results in diarrhoea, villous atrophy, mucosal ulceration and intestinal necrosis (Baskerville et al. 1980). As the administration of either TPN or of an elemental diet as the sole energy source is associated with intestinal mucosal atrophy and barrier dysfunction, the effects of glutamine supplementation will be considered in these two situations. The effects of supplementation with novel substrates will be evaluated under four headings: (a) intestinal morphology (mucosal thickness and villus height); (b) biochemical parameters (mucosal protein and DNA content); (c) function (digestion, absorption, metabolic activity, barrier function); and (d) outcome.

**Glutamine supplemented TPN and experimental disease models**

In starvation-induced gut atrophy (Inoue et al. 1993b), glutamine supplementation (a) improved intestinal morphology; and (c) increased mucosal activity of sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20).

In a model of short bowel syndrome (Wang et al. 1988; Jiang et al. 1993), glutamine supplementation (a) improved intestinal morphology.

In TPN induced gut atrophy (Grant & Snyder, 1988; Jacobs et al. 1988; Burke et al. 1989; O'Dwyer et al. 1989; Tamada et al. 1992; Jiang et al. 1993; Platell et al. 1993; Burrin et al. 1994; Li et al. 1994), provision of glutamine as either a free amino acid or in dipeptide form (a) improved small and large intestinal morphology (exceptions—jejunal (Babst et al. 1993; Spaeth et al. 1993); colonic (Platell et al. 1993)); (b) increased mucosal DNA and protein contents (exception—Burrin et al. 1994); and (c) increased mucosal enzyme activity, decreased bacterial translocation (exception—Spaeth et al. 1993) and prevented an increase in intestinal permeability (Li et al. 1994).

In a small bowel transplantation model (Frankel et al. 1993), supplemental glutamine (a) improved intestinal morphology; and (c) improved intestinal monosaccharide absorption.

In experimental sepsis (Ardawi, 1991; Yoshida et al. 1992; Inoue et al. 1993a; Kaneko et al. 1993; Chen et al. 1994), supplemental glutamine (a) improved intestinal morphology; (b) increased intestinal mucosal protein content; (c) increased mucosal glutaminase (EC 3.5.1.2) activity and reduced bacterial translocation; and (d) improved survival.

In a rat abdominal radiation model (Scott & Moellman, 1992), glutamine supplementation had no effect on (a) intestinal morphology; and (b) mucosal DNA content.

**Glutamine supplemented enteral nutrition and experimental disease models**

In experimental haemorrhagic shock, suffusion of the small intestinal mucosa with isotonic glutamine attenuated the shock induced impairment in intestinal blood flow (Flynn et al. 1992). Bark et al. (1995) reported that in a rat model of non-lethal haemorrhage a glutamine-supplemented enteral diet (c) had no effect on bacterial translocation.

Pretreatment with oral glutamine decreased the severity of aspirin induced gastric ulcerations (Okabe et al. 1975).
In starvation induced gut atrophy (Salloum et al. 1989), a glutamine supplemented elemental diet (a) improved intestinal morphology; and (c) increased mucosal glutaminase activity and decreased bacterial translocation.

In elemental diet induced gut atrophy (Barber et al. 1990; Deitch et al. 1993; Wusteman et al. 1995), supplemental glutamine (a) improved intestinal morphology; (b) increased mucosal protein content; and (c) had no effect on bacterial translocation. However, rats given Vivonex TEN, which has a high content of free glutamine, had decreased bacterial translocation compared with two other elemental diets (Alverdy et al. 1990).

In a cyclosporin treated model of small bowel transplantation in rats (Zhang et al. 1995), supplemental glutamine (a) improved intestinal morphology; and (c) reduced bacterial translocation.

In experimental sepsis (endotoxaemia), glutamine supplementation of an elemental diet (c) had no effect on bacterial translocation; and (d) had no effect on survival (Barber et al. 1990).

Prophylactic enrichment of an elemental diet with glutamine protected the gut mucosa from radiation injury (Klimberg et al. 1990b) by (a) improving intestinal morphology; but (b) had no effect on mucosal DNA or protein content; and (c) had no significant effect on bacterial translocation.

In radiation induced enterocolitis (Klimberg et al. 1990a; Souba et al. 1990c; Karatzas et al. 1991; Jensen et al. 1994), supplemental glutamine (a) diminished bloody diarrhoea and the incidence of bowel perforation, and improved intestinal morphology; (c) increased intestinal glutaminase activity and decreased systemic endotoxaemia and bacterial translocation; and (d) improved survival.

In a study on experimental colonic anastomoses (McCaulley et al. 1991), supplemental glutamine had no effect on colonic healing.

In a mouse burn model (Gianotti et al. 1995), glutamine supplementation (c) reduced bacterial translocation; and (d) increased survival.

In a rat methotrexate treated sarcoma model (Klimberg et al. 1992), supplemental glutamine (b) increased jejunal DNA content; (c) reduced bacteraemia; and (d) reduced mortality.

In chemotherapy induced enterocolitis (Jacobs et al. 1987; O’Dwyer et al. 1987; Fox et al. 1988a, b; Shou et al. 1991), supplemental glutamine (a) promoted repair of intestinal mucosa; (b) increased jejunal and colonic mucosal protein and DNA content; (c) decreased bacterial translocation (exception Shou et al. 1991) and decreased systemic endotoxaemia; and (d) increased survival (exception Shou et al. 1991).

In summary, glutamine supplementation of TPN or of elemental enteral nutrition improves intestinal structure, function and outcome in most of these experimental models. However, two important points should be remembered. Firstly, while TPN administration is associated with intestinal atrophy and bacterial translocation in the rat, there is no evidence of intestinal atrophy on TPN either in the mouse (Sitren et al. 1992) or in humans (Guedon et al. 1986) and no increased incidence of TPN induced bacterial translocation in patients (Sedman et al. 1993). Secondly, while glutamine supplementation of elemental nutrition was beneficial in these models, a normal or polypeptide diet was well tolerated and even more beneficial in the chemotherapy induced enterocolitis models (Harvey et al. 1987; Shou et al. 1991).

Rationale for clinical use of glutamine

Stress, surgery and sepsis result in an increased demand for glutamine as a substrate by enterocytes both as a fuel and as a precursor for purines, pyrimidines and other nitrogen-containing compounds (Souba et al. 1990a). Increased glutamine demand without
increased supply will result in efflux of glutamine from skeletal muscle and may lead to structural and functional intestinal disturbances (Souba et al. 1990a). Exogenous supply of glutamine or glutamine-containing dipeptides may therefore be beneficial to the intestinal mucosa in patients with injury and infection by stimulating enterocyte metabolism and maintaining intestinal mucosal integrity, thereby reducing bacterial translocation.

Clinical use of glutamine

In pre-operative patients with inflammatory bowel disease or cancer (van der Hulst et al. 1993), glutamine supplemented TPN (a) preserved duodenal mucosal morphology; and (c) prevented deterioration in intestinal permeability.

In the critically ill (Tremel et al. 1994) and in those with short bowel syndrome (Byrne et al. 1992), glutamine supplemented TPN (c) improved intestinal absorption of nutrients.

In patients undergoing bone marrow transplantation, glutamine supplemented TPN (c) reduced microbial colonization (Ziegler et al. 1992); and (d) reduced length of hospital stay (Schloerb & Amare, 1993).

In premature infants (Wilmore, 1994), glutamine supplemented TPN (d) reduced length of time on mechanical ventilation and on TPN.

Glutamine supplemented TPN had no effect on gastrointestinal toxicity in patients undergoing chemotherapy for haematological malignancies (van Zaanen et al. 1994).

Taken together, these studies suggest that glutamine supplementation of TPN is superior to standard TPN in the maintenance of intestinal structure and function in patients (Wilmore, 1994). There is also some evidence that these benefits translate into reduced hospital costs (MacBurney et al. 1994).

ARGININE

Arginine is a dibasic amino acid which the body obtains from dietary sources and by endogenous synthesis via the urea cycle. Arginine is essential for growth in cats, conditionally essential in mice and rats but non-essential in man (Grimble, 1993). There is evidence that arginine becomes indispensable for adequate nitrogen balance under severe stress due to sepsis, trauma or nitrogen overload (Kirk & Barbul, 1990; Gonce et al. 1990).

Metabolism of arginine

Arginine is absorbed by the small intestine by active transport system y+. Both the liver and the kidney can synthesize arginine from citrulline and ornithine via the urea cycle (Barbul, 1986). Arginine is a precursor of polyamine, histone and nucleic acid synthesis, is a promoter of thymic growth and is an endocrinological secretagogue stimulating release of prolactin, insulin and glucagon (Barbul, 1986).

Arginine and the gut mucosa

Arginine is metabolized within the enterocyte via the arginase (EC 3.5.3.1) pathway to ornithine and urea and via the arginine deiminase (EC 3.5.3.6) pathway to citrulline and nitric oxide (Blachier et al. 1991). Arginine metabolism in the gut protects this amino acid from excessive degradation in the liver (Cynober, 1994). In addition, arginine metabolism in enterocytes may participate in the support of gut morphology and function by acting as a substrate for nitric oxide synthesis (Cynober, 1994). Inhibition of nitric oxide synthesis increased intestinal mucosal permeability in experimental models of ischaemia–reperfusion intestinal injury in the cat (Kubes, 1993) and acute necrotizing enterocolitis in the rabbit (Miller et al. 1993b). In addition, administration of L-arginine reversed the effect of nitric oxide synthase inhibition (Kubes, 1993). 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 (Neill et al. 1993), supplemental enteral arginine (a) increased mucosal inflammation, whereas administration of nitric oxide synthase inhibitors reduced intestinal inflammation in rat colitis and rabbit ileitis models (Miller et al. 1993a; Neilly et al. 1995).

In an experimental burn model (Saito et al. 1987b, Gianotti et al. 1993), supplemental enteral arginine (c) reduced bacterial translocation; and (d) improved survival.

In experimental sepsis, supplemental arginine (d) improved survival in mouse (Gianotti et al. 1993) and rat (Madden et al. 1988) models but not in a guineapig model (Gonce et al. 1990).

Rationale for clinical use of arginine

As a result of its actions as a precursor of polyamine synthesis and protein synthesis and as a secretagogue, it has been suggested that arginine might be a substrate capable of supporting gut function (Gianotti et al. 1993; Cynober 1994).

However, a large fraction of metabolized arginine is transported out of the enterocyte as ornithine and citrulline, suggesting that arginine metabolism may not be responsible for substantial intestinal polyamine synthesis. The experimental studies reviewed above provide conflicting evidence on the effects of arginine on gut structure, function or outcome. Enhancement of immune rather than gut function may explain the arginine induced exacerbation of experimental colitis (Neill et al. 1995) as well as the beneficial effects of arginine on bacterial translocation and survival in the experimental burn model (Gianotti et al. 1993). 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 (Petros et al. 1991) suggest that excess arginine supplementation could be potentially hazardous in severely ill patients (Cynober, 1994).

Clinical use of arginine

In combination with nucleotides and n-3 polyunsaturated fatty acids, arginine (Impact) has been administered to patients with gastrointestinal cancer and critical illness (Cerra et al. 1990; Daly et al. 1992, 1995; Bower et al. 1995). No effect on intestinal morphology, biochemical parameters or function has been reported. Patients administered this triple therapy had reduced infectious and wound complications and reduced length of hospital stay. It is probable that the observed beneficial effects of these substrates were due to improved function of the immune system rather than improved gut barrier function.

ORNITHINE

Ornithine is a non-essential basic amino acid obtained from the diet or by synthesis from arginine via the urea cycle. It is not a constituent of proteins but is a precursor of aliphatic polyamines and an endocrinological secretagogue (Cynober, 1994). In addition, in the form of the a-ketoglutarate salt, ornithine generates glutamine which plays a key part in the control of protein metabolism (Cynober et al. 1990). Ornithine has also been shown to increase nitrogen retention, and to improve wound healing and thymic function in experimental animals (Barbul, 1986).

Metabolism of ornithine

Ornithine shares an active transport system (y') in the small intestine with arginine. Ornithine is metabolized to citrulline via the ornithine carbamoyltransferase (EC 2.1.3.3) pathway, to glutamate and proline via the ornithine aminotransferase (EC 2.6.1.13)
pathway and to putrescine, spermidine and spermine via the ornithine decarboxylase (EC 4.1.1.17) pathway (Cynober, 1994).

Ornithine and the gut mucosa

Enterocytes possess ornithine decarboxylase, ornithine carbamoyltransferase and ornithine aminotransferase (Cynober, 1994). However, after enteral administration of [14C]ornithine, only [14C]proline, [14C]glutamate and [14C]polyamines are detected (Vaubourdolle et al. 1989). It has been suggested that ornithine metabolism in enterocytes, by acting as a precursor for polyamine synthesis, may support gut morphology (Cynober, 1994).

Ornithine and experimental disease models

In a rat starvation model (Cynober, 1994) supplementation with ornithine α-ketoglutarate (a) increased intestinal crypt and villous height; and (c) increased brush border enzyme content.

Rationale for the clinical use of ornithine

As with arginine, ornithine is a precursor of polyamine synthesis and an endocrinological secretagogue and might therefore be important in supporting gut function (Cynober, 1994). There is one experimental study, described above, to support this hypothesis (Cynober, 1994).

Clinical use of ornithine α-ketoglutarate

As with glutamine, supplemental ornithine α-ketoglutarate improved nitrogen balance and prevented reduction in muscle free glutamine in patients undergoing elective surgery (Stehle et al. 1989; Hammarqvist et al. 1990). The mode of action of ornithine α-ketoglutarate in the prevention of skeletal muscle breakdown is probably as a precursor of glutamine. A direct beneficial clinical effect of ornithine or ornithine α-ketoglutarate on gut structure, function or outcome has not yet been shown in clinical studies.

SHORT CHAIN FATTY ACIDS (SCFA)

SCFA (acetic, propionic and butyric acids) are among the major anions in colonic contents. They are found to a limited extent in the diet but are primarily produced in the proximal colon by anaerobic bacterial fermentation of dietary carbohydrates such as pectin (Cummings & Branch, 1986).

Metabolism of SCFA

SCFA are rapidly absorbed in the colon, mostly by non-ionic transcellular diffusion (Scheppach, 1994), and metabolized within the colonocyte via a β-oxidation pathway requiring the presence of coenzyme A, to produce energy (D’Argenio et al. 1994). Apart from the colonic mucosa, the liver is the main site of SCFA metabolism (Rémésy et al. 1980; Koruda et al. 1990; Scheppach, 1994). Acetate is also used as a fuel source by skeletal and cardiac muscle (Scheppach et al. 1991).

SCFA and the gut mucosa

SCFA are the preferred fuel source for colonocytes (Roediger, 1982). Their presence in the colonic lumen stimulates mucosal cell proliferation (Sakata, 1987; Kripke et al. 1988), and increases mucosal blood flow (Kvietys & Granger, 1981) and intestinal motility.
Colonic infusion of SCFA increases colonic ornithine decarboxylase activity, DNA content and weight in the rat (Reilly et al. 1995). Caecal infusion of SCFA in the rat also increases jejunal DNA content, villous height, surface area and crypt depth (Frankel et al. 1994). It is thought that butyrate contributes to the maintenance of intestinal integrity by suppressing the secretion of urokinase by colonic crypt cells (Gibson et al. 1994). In addition, acetacetate and 3-hydroxybutyrate stimulate protein synthesis in isolated enterocytes (Higashiguchi et al. 1993).

As instillation of SCFA in the colon has a trophic effect, it seems reasonable to infer that fibre or non-digested carbohydrates which are broken down to SCFA in the colon should also have this effect (Elsen & Bistrian, 1991). Indeed, pectin and guar have been found to increase mucosal DNA content in the rat colon (Jacobs & Lupton, 1984).

**Fibre or SCFA and experimental disease models**

In a rat model of the short bowel syndrome (Koruda et al. 1986, 1988), enteral pectin or intravenous short chain fatty acids (a) increased mucosal mass; and (b) increased DNA, RNA and protein content.

In rat models of TPN induced colonic atrophy (Kripke et al. 1988; Spaeth et al. 1990; Friedel & Levine, 1992), colonic SCFA or enteral cellulose (a) increased mucosal height; (b) increased mucosal DNA content; and (c) reduced bacterial translocation.

In experimental colitis (Rolandelli et al. 1988; D’Argenio et al. 1994), enteral pectin or butyrate enemas (a) reduced colonic inflammation.

In a study on experimental colonic anastomosis (Rolandelli et al. 1986a,b), enteral administration of pectin or intracolic infusion of SCFA enhanced anastomotic healing (bursting strength).

**Rationale for the clinical use of SCFA**

It has been proposed that during critical illness the colonic mucosa may be nutritionally deficient and that SCFA administration may enhance function, including water and sodium resorption, and decrease bacterial translocation (Frankel et al. 1994). In addition, low coenzyme A levels have been found in the colonic mucosa of patients with ulcerative colitis which may reduce the efficiency of SCFA oxidation to carbon dioxide and ketone bodies (Ellestad-Sayed et al. 1976). Indeed, decreased faecal concentrations of SCFA have been demonstrated in patients with ulcerative colitis and in those with pouchitis after restorative proctocolectomy (Vernia et al. 1988; Wischmeyer et al. 1993).

It has been suggested that the increased secretion of urokinase (EC 3.4.21.31) observed in inflammatory bowel disease (Elliott et al. 1987; De Bruin et al. 1988) may lead to increased urokinase activity at intercellular junctions and result in increased intestinal permeability (Gibson et al. 1994). Butyrate causes a concentration-dependent suppression of urokinase secretion by colonic crypt cells in vitro (Gibson et al. 1994) and therefore may be beneficial to patients with inflammatory bowel disease by improving intestinal barrier function.

**Clinical use of SCFA**

In patients with a defunctioned rectum (Harig et al. 1989; Roediger, 1990; Mortensen et al. 1991) or distal ulcerative colitis (Breuer et al. 1991; Scheppach et al. 1992) intrarectal administration of SCFA increases mucosal blood flow and (a) improves rectal mucosal morphology.

In patients after right hemicolecotomy (Scheppach et al. 1989), SCFA administration accelerated intestinal adaptation by (c) stimulating sodium and water absorption in the left hemicolon.
Experimental and clinical studies therefore show that administration of SCFA has a beneficial effect on colonic inflammation owing to defunction (colonic starvation owing to lack of luminal SCFA) or inflammatory bowel disease (impaired absorption of luminal SCFA) and has potential as a modulator of gut barrier function.

**n-3 POLYUNSATURATED FATTY ACIDS**

Polyunsaturated fatty acids are classified into two groups (n-3 and n-6) on the basis of the location of the first double bond counting from the methyl end of the molecule (Simopoulos, 1991). Neither class of fatty acid can be synthesized by humans and must be obtained from the diet. n-6 Fatty acids are found in the seeds of most plants whereas n-3 fatty acids are found in green leafy vegetables and in fish oils.

Metabolism of polyunsaturated fatty acids

While both n-3 and n-6 fatty acids are metabolized to eicosanoids, the prostaglandins and leukotrienes formed have different biological properties (Simopoulos, 1991). When fish oils are ingested, n-3 fatty acids replace n-6 fatty acids (especially arachidonic acid) in the membranes of platelets, erythrocytes, neutrophils, monocytes and hepatocytes. As a result, there is a decrease in the production of prostaglandin E₂ metabolites, thromboxane A₂ and leukotriene B₄ with an increased synthesis of prostacyclin PGI₂, thromboxane A₃ and leukotriene B₅ (Simopoulos, 1991). The finding of reduced neutrophil and monocyte functions following chronic intake of n-3 fatty acids suggests that they may have anti-inflammatory properties (Lee et al. 1985).

Eicosanoids and the gut mucosa

Eicosanoids are mediators of both defensive and inflammatory processes of the gut mucosa (Hawkey & Rampton, 1985). There is evidence that altered eicosanoid generation is a major pathophysiological feature of inflammatory bowel disease (Hawkey & Rampton, 1985). Patients who have active colitis have increased concentrations of leukotriene B₅ and prostaglandin E₂ in rectal biopsies and dialysates (Sharon et al. 1978; Lauritzen et al. 1984). When a patient goes into remission, the concentration of these eicosanoids falls (Sharon et al. 1978; Lauritzen et al. 1984).

n-3 Polyunsaturated fatty acids and experimental disease models

In experimental colitis (Vilaseca et al. 1990; Guarner et al. 1992), dietary supplementation with n-3 fatty acids, when compared with n-6 fatty acids, (a) reduced severity and duration of colitis in association with reduced luminal release of eicosanoid mediators.

In experimental sepsis, n-3 fatty acid supplementation significantly improved intestinal perfusion (Pscheidl et al. 1992) and (d) improved survival (Mascioli et al. 1989).

Rationale for clinical use of n-3 polyunsaturated fatty acids

Disturbances in colonic mucosal eicosanoid synthesis in ulcerative colitis and evidence that n-3 fatty acid dietary supplementation can influence eicosanoid production led to the hypothesis that supplemental fish oil would reduce the inflammatory response in patients with ulcerative colitis (Ross, 1993).

Clinical use of n-3 polyunsaturated fatty acids

In patients with inflammatory bowel disease initial encouraging reports on uncontrolled trials of fish oils (McCall et al. 1989; Salomon et al. 1990) have been followed by controlled crossover trials (Stenson et al. 1990; Tobin et al. 1990; Aslan & Triadafilopoulos, 1992).
Patients with ulcerative colitis given a dietary supplement of fish oils have been shown to increase weight gain, improve colonic histology, reduce disease activity and reduce LTB₄ in rectal dialysates (Stenson et al. 1990; Tobin et al. 1990; Aslan & Triadalilopoulos, 1992). There are other studies of fish oil supplementation which showed less promising results (Hawthorne et al. 1990). Ross (1993) has concluded that dietary fish oils provide modest benefits to patients with ulcerative colitis.

In patients with burns (Gottschlich et al. 1990) dietary supplementation with n-3 fatty acids or fish oils reduced infectious complications and hospital stay.

Studies describing the use of n-3 fatty acids in combination with arginine and nucleotides (Impact) in the critically ill and in patients with gastrointestinal cancer have been discussed under arginine.

NUCLEOTIDES

Nucleotides are ubiquitous in cells in either monomeric or polymeric form, serving as the structural units for DNA and RNA synthesis and participating (ATP) in intracellular metabolic reactions (van Buren et al. 1994). Nucleotides may be obtained from dietary sources, synthesized de novo or salvaged by intracellular degradation (van Buren et al. 1994).

**Metabolism of nucleotides**

Dietary nucleotides are degraded in the intestinal lumen to nucleosides by alkaline phosphatase and nucleotidases, and may be further broken down by nucleosidases to produce purine and pyrimidine bases (Carver, 1994). Enterocytes, however, have a limited capacity to absorb nucleosides or purine and pyrimidine bases. When purines are administered enterally, only adenine is incorporated into the nucleotide pool of the enterocyte and the remainder are degraded to uric acid or allantoin (Rudolph et al. 1990). Although some dietary pyrimidines and bases are absorbed, a considerable amount is catabolized and excreted (Iijima et al. 1993). The major source of nucleotides is, therefore, de novo synthesis from amino acids and sugars in the liver and other organs or salvage by degradation of intracellular polymeric nucleotides.

**Nucleotides and the gut mucosa**

The gut has a limited capacity for de novo synthesis of purine and pyrimidine bases and is dependent on the liver for the supply of nucleotides (Savaiano & Clifford, 1981; LeLeiko et al. 1983). Administration of nucleotides increases gut wall thickness and protein content in weanling mice (Carver et al. 1993) and the rate of intestinal maturation and growth in young rats as assessed by villous height, intestinal mass, RNA, DNA and protein concentrations and activity of brush border enzymes (Uauy et al. 1990). In vitro nucleotide supplementation enhanced the proliferation of a rat small intestinal crypt (IEC-6) cell line but did not affect proliferation and differentiation of a human colon tumour (Caco-2) cell line (He et al. 1993).

**Nucleotides and the gut flora**

Nucleotide supplemented commercial milk formulas have been shown to promote the growth of bifidobacteria in the faeces of infants (Gil et al. 1986).

**Nucleotides and experimental disease models**

In experimental models of intestinal ischaemia–reperfusion injury, administration of nucleotides, especially adenosine, has been shown to increase intestinal blood flow (Sawmiller & Chou, 1991) and to attenuate the increase in leukocyte extravasation.
In a lactose induced model of chronic diarrhoea in the weanling rat (Nuñez et al. 1990; Bueno et al. 1994), nucleotide supplementation (a) improved intestinal morphology; (b) increased intestinal protein content; and (c) increased brush border enzyme activity.

In a TPN model of gut atrophy in the rat (Iijima et al. 1993), nucleosides alone or in combination with glutamine (a) improved intestinal morphology; (b) increased intestinal mucosal protein and RNA content; and (c) increased brush border enzyme (maltase) activity.

In protein deficient mice (Adjei et al. 1994; Adjei & Yamamoto, 1995), intraperitoneal or oral administration of a nucleoside–nucleotide mixture (a) improved intestinal morphology; and (c) reduced bacterial translocation.

**Rationale for clinical use of nucleotides**

Enterocytes appear to have a limited ability to synthesize nucleotides de novo (Iijima et al. 1993). Under normal circumstances, enterocytes depend on the liver and intracellular salvage to provide sufficient monomeric nucleotides to facilitate protein synthesis and cellular proliferation. It has been proposed that these sources may become inadequate, during severe metabolic stress such as trauma, burns and sepsis, to sustain optimal growth of the intestinal mucosa (Uauy et al. 1990). Consequently gut mucosal atrophy may develop with resultant dysfunction of intestinal absorptive and barrier functions (Iijima et al. 1993; Grimble, 1994).

Nucleotide supplementation can improve intestinal morphometry, biochemical parameters and function in experimental models. It has therefore been suggested that intestinal structure and function is nucleotide limited during critical illness and exogenous nucleotide supplementation may prevent gut derived septic complications (Grimble, 1994).

**Clinical use of nucleotides**

In infants of low socioeconomic class in Chile (Brunser et al. 1994), a nucleotide supplemented formula was found to decrease the incidence of diarrhoeal disease. It was suggested that the nucleotides may act by stimulating the growth of bifidobacteria which are known to exert a protective effect against intestinal colonization by enteropathogens.

Studies describing the use of nucleotides in combination with arginine and n-3 fatty acids (Impact) in the critically ill and in patients with gastrointestinal cancer have been discussed under arginine. Beneficial effects of nucleotide supplements on gut mucosal structure or function during critical illness remain to be demonstrated (Grimble, 1994).

**CONCLUSIONS**

As reviewed above, there is experimental and clinical evidence to support the hypothesis that intestinal barrier dysfunction occurs in critically ill patients and that gut derived bacteria and endotoxin may activate and lead to derangements of the systemic immune system, thus promoting multisystem organ dysfunction. There have been encouraging results from the use of enteral nutrition in experimental models and in patients with trauma or sepsis (Moore et al. 1992). Overall, there is convincing evidence that enteral nutrition is associated with improved immune function and reduced septic complications in severely injured humans when compared to parenteral nutrition (Moore et al. 1992).

The main focus of this review concerns the evaluation of individual nutrients as having a selective action in the maintenance of gut mucosal integrity (selective gut nutrients). However, evidence that such a substrate improves the gut mucosal barrier and increases
survival does not necessarily imply that restored barrier function and improved survival are causally linked. It may in fact be that the substrates are exerting their beneficial effects by improving nitrogen balance and metabolism or by enhancing immune function (enhanced clearance of translocated bacteria). From the experimental and clinical studies to date, the strongest candidates as selective gut nutrients would appear to be glutamine for the enterocyte and short chain fatty acids for the colonocyte.

While glutamine supplementation of TPN or of elemental diets improves intestinal morphology and barrier function in experimental models, it is important to note that in many of these models complete recovery of intestinal structure and function was produced by a standard oral diet. Therefore a standard oral or polymeric enteral diet is still preferable when clinically feasible and safe. Even when patients are unable to tolerate their full energy needs via the enteral route, enteral administration of 0.6 g nitrogen/kg daily is sufficient to maintain gut integrity and immune function (Moore & Cerra, 1991). When enteral nutrition is not possible at all, there is experimental and clinical evidence that glutamine supplemented TPN conveys advantages from the gut point of view over standard TPN.

Both short chain fatty acids and n-3 polyunsaturated fatty acids have been shown to have modest beneficial effects in patients with ulcerative colitis. Specific arginine supplementation, despite its proven immunological benefit, has no direct mucosal enhancing properties and may be unwise in critical (septic) illness owing to its role as a precursor of nitric oxide synthesis. At present, there is insufficient evidence from clinical studies to suggest that either ornithine or nucleotide supplementation will improve intestinal structure or function. Continued research into these and other novel substrates for the intestine, it is hoped, will lead to improvements in the support of intestinal function during catabolic states and in overall outcome.

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