Glutamine and its effects on the intestine

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The amino acid glutamine has become a subject of great interest in recent years because of its vital role in intestinal metabolism. Glutamine has been shown to reduce the effects of enterocolitis in experimental animal models (1-8). Due to its low stability in aqueous solution and relative insolubility, glutamine is not readily available in commercial enteral or parenteral nutrition solution preparations. This paradox of apparent need yet unavailability of glutamine is the impetus to the present review of glutamine and its effects on healing of intestinal injury.

BIOCHEMISTRY

Glutamine is detected in large quantities in many animal tissues. In addition to being a protein constituent, glutamine has several other functions (9-11). Glutamine’s classification as a nonessential amino acid may be misleading (12) in view of its diverse and important roles (10,11), especially in the intestinal tract (13-18). The degradation of glutamine to glutamate by glutaminase (Figure 1) has been studied in numerous tissues (19,20), and the properties of the enzyme have been reviewed (9,21). Glutamine is an energy source via the Kreb’s cycle, and a nitrogen donor for the urea cycle and for nucleotide synthesis. The metabolism of glutamine by the intestine has been comprehensively reviewed by Windmueller (20,22,23) and others (13-16). Energy production from

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partie dépendre de l'action de la glutaminase présente dans la muqueuse, et du transport de la glutamine par les entérocytes. Il est prouvé que la glutamine améliore la morphologie de l'intestin et l'évolution de l'entérocolite chez les modèles animaux. Elle pourrait jouer un rôle similaire en contribuant à la guérison des lésions intestinales chez les patients dont le régime alimentaire contient suffisamment de glutamine par rapport à ceux qui souffrent de carences. La glutamine aurait une action positive sur la fonction immunitaire du tissu lymphoïde associé à la muqueuse intestinale. Pour le moment, la glutamine ne fait pas partie des préparations nutritionnelles d'usage clinique courant; on a pourtant récemment établi qu'elle contribuait à maintenir un bilan azoté équilibré chez l'homme. A cause de l'instabilité et de la faible solubilité de la glutamine, on s'est penché sur les dipeptides. La L-alanyl-L-glutamine semble le précurseur le plus prometteur de la glutamine pour l'utilisation parentérale chez l'homme: elle est sûre et rapidement hydrolysée in vivo pour libérer la glutamine libre. Le rôle exact de la glutamine en tant qu'agent thérapeutique favorisant la santé de l'intestin reste encore à déterminer. Toutefois, les résultats préliminaires laissent entrevoir l'utilité de la glutamine dans divers tableaux cliniques.

Glutamine has been demonstrated in the intestine (24-26), lymphocytes (27), fibroblasts (28) and malignant cells (29,30).

INTERORGAN RELATIONSHIPS

Glutamine balance across various organs has been well documented (31-33). Glutamine synthesis occurs in the muscle of healthy individuals (34-36) and in the lungs of septic patients (15,37,38). The intestine is normally a glutamine consumer (39), as is the kidney during acidosis (31,40). Excretion of urinary ammonia is a reflection of the kidney's tendency to consume glutamine in the acidic state. The liver has a complex relationship with glutamine metabolism; on average it is in glutamine balance. The liver synthesizes glutamine to detoxify portal ammonia (41-44) and may consume glutamine under other circumstances (Figure 2).

GLUTAMINE AND THE INTESTINE: EXPERIMENTAL ASPECTS

Intestinal energy substrates: In 1965, Neptune (25) reported that glutamine was oxidized in preference to glucose in rabbit ileum. More detailed work on glutamine as an intestinal energy substrate was done in the 1970s by Windmueller and Spaeth (24,26,45) and others (46-49). The contribution of glutamine to respiratory carbon dioxide in the intestine is high (50), indicating glutamine's importance as an intestinal fuel. In addition to glutamine, ketone bodies have also been shown to act as fuels for the small intestine (51). In contrast, isolated colonocytes metabolize short chain fatty acids and ketone bodies preferentially as energy substrates (52). These nutrients are primarily derived in vivo from fermentation of luminal contents by endogenous microflora. The small intestine prefers glutamine for fuel, whereas the colon shows a preference for short chain fatty acids and ketone bodies (53-58). Human colonocytes preferentially metabolized short chain fatty acids over glutamine, especially those sampled from the distal colon (59). Glutamine metabolism was higher in proximal versus distal colonocytes.

CONSUMPTION

Windmueller and Spaeth (24) used isolated, vascularly perfused rat intestine to investigate the metabolic fate of glutamine and demonstrated the relative importance of the small bowel in glutamine metabolism. They then studied the in vivo metabolism of glutamine in a luminally perfused segment of rat intestine (45). Labelled carbon dioxide accounted for 60% of

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**Figure 1** The enzymatic degradation of glutamine to glutamate and ammonia. "Glutaminase"

**Figure 2** Interorgan relationships of glutamine. Bold arrows indicate predominant direction of glutamine transfer. (Adapted from reference 14)
the glutamine carbon recovered. The metabolic products recovered with respect to both glutamine nitrogen and carbon showed a profile strikingly similar to those of vascularly perfused segments. These results suggest that there is a common glutamine pool within mucosal cells consisting of both luminaly and arterially derived glutamine. The experiments achieved similar results using germ-free rats, indicating that intestinal microflora were not responsible for the breakdown of luminal glutamine and ammonia release. No $^{14}$C-glutamate was released in venous blood after glutamine luminal perfusion, indicating that the rate-limiting step in intestinal glutamine metabolism is the deamination of glutamine to glutamate.

Weber and Veach (60,61) studied ammonia production in the intestines of dogs. The small intestine was comparable to the uncleaned colon in terms of amount of ammonia released into portal blood. Ammonia from the small intestine was nearly fully accounted for by the breakdown of glutamine. In contrast, 51% of the ammonia released by the colon was due to metabolism of glutamine and urea; the remaining portion was presumably produced by intestinal flora. Glucose was infused to determine if an energy source alternate to glutamine would reduce the amount of ammonia released in portal blood. Instead, luminal infusion of glucose significantly increased mesenteric venous ammonia release and intestinal glutamine consumption. Hepatic synthesis of glutamine helps detoxify ammonia (42); the clinical implications of ammonia production and glutamine have been discussed by Souba (33).

Glutamine uptake by the gastro-intestinal tracts of splenectomized dogs nearly doubled by the second post-operative day compared to control animals in the face of reduced portal bloodflow and lower arterial glutamine levels (62). Glutamine consumption is probably up-regulated rather than determined solely by the quantity of glutamine presented to the gut. Conversely, rat intestinal glutamine extraction is decreased in sepsis (63,64) and diabetes (65) and soon after massive small bowel resection (66). Glutamine extraction in the small intestine of peak lactating rats was increased compared to nonlactating controls (67). Glutamine consumption was increased in vivo and in isolated enterocytes after rats were treated with dexamethasone (68). Similarly, gut glutamine uptake doubled in dogs receiving anabolic steroids compared to controls (69).

**Glutaminase**: Glutamine is used by the intestine whether supplied arterially or luminally. The metabolic fate of glutamine is similar for both modes of entry into the mucosal cell. Intestinal glutamine extraction is modified by many factors, probably through modulation of the rate-limiting step of glutamine metabolism: deamination by enterocyte glutaminase. High enzyme levels are found in the bowel mucosa of several species including rat, dog, hamster and monkey (20) but not guinea pigs or chickens (20). Lower blood glutamine levels and intestinal extraction were noted in the latter two species. Intestinal glutaminase is predominantly the phosphate-dependent type (20) and is located in the mitochondria of mucosal epithelial cells. Intestinal glutaminase has a high substrate affinity consistent with low mucosal cell concentrations of glutamine. Phosphate-independent glutaminase and other enzymes required for glutamine metabolism showed low activity.

It has been postulated that stimulation of intestinal glutaminase activity may be necessary for the gut to take full advantage of circulating glutamine (8). The well-being of the intestine may therefore depend on both the supply of glutamine and the activity of glutaminase. Glutaminase activity is affected by a number of factors.

Intestinal glutaminase activity was shown to decrease after 48 and 72 h of starvation in rats (54,70,71). Supplementation with glutamine (20) did not raise intestinal mucosal glutaminase activity compared to controls unless a period of prior fasting was employed (72). Dexamethasone increased glutaminase-specific activity in both jejunum and colon of rats two days after administration (73). The enzyme was unaffected by acidosis (20,70,74) or potassium deficiency (70). Another study showed a decrease in small intestinal glutaminase in chemically induced acidosis (75), with no change in the colonic enzyme. The effect of acidosis on intestinal glutaminase is clearly different from that on kidney glutaminase, where acidosis up-regulates renal glutaminase in order to promote urinary ammonia excretion (20). Septic rats have demonstrated decreased consumption of glutamine along with reduced glutaminase activity in small intestinal mucosal scrapings (64).

Streptozotocin-diabetic rats showed decreased glutaminase activity in both colonic mucosal scrapings and the whole colon (75). A substantial increase was observed in small intestinal glutaminase activity for the whole organ but not for mucosal scrapings (74,75). The increased enzyme activity paralleled the increase in intestine size which normally accompanies induced diabetes in rats. The higher total enzyme activity was accompanied by complete suppression of glutamine extraction measured by arteriovenous differences (74). This example in diabetic rats is an exception to the hypothesis that glutamine consumption is predominantly a function of glutaminase activity.

**Glutamine transport**: Brush border membrane glutamine transport occurs by two mechanisms (76,77). The primary route is via a sodium-depend-ent system. Glutamine is also transport-ed by a sodium-independent, carrier-mediated system. Both are saturable and inhibited by other amino acids. Glutamine transport occurred against a chemical gradient, evidenced by the ‘overshoot’ phenomenon prior to equilibration. Similar glutamine transport was documented in human brush border membrane vesicles (78). Brush border membrane transport of glutamine was increased in diabetic rats (79). This finding contrasts with decreased endogenous glutamine intestinal cell uptake (80), implying that in diabetes the enterocyte favours dietary rather than endogenous glutamine. This effect was reversed by insulin. Dietary glutamine
supplementation for four days after an equal period of fasting in rats resulted in a 75% increase in glutamine brush border membrane uptake over controls (76). The clinical relevance of enhanced intestinal glutamine transport is not yet clear, although it has been speculated that an impact on gut metabolism, structure and function may play a role (76).

Basolateral intestinal membrane transport of glutamine (which would occur from arterial blood during fasting) has also been characterized in rats (81-83). Glutamine transport was carrier-mediated and probably involved an exchange of extracellular glutamine for intracellular alanine. Alanine, a metabolite of glutamine (22), is a logical substance to exchange: as a non-energy byproduct of glutamine metabolism, it becomes available to the liver for gluconeogenesis as more glutamine enters the enterocyte for processing. Similar transport systems were reported for rat colonocytes (84) and human small intestinal basolateral membrane vesicles (85).

**Intestinal injury models and glutamine**: Baskerville (86) administered parenteral glutaminase to monkeys and other mammals in order to investigate its properties as an anti-tumour agent. All but the lowest doses were fatal within 10 days. All animals had vomiting, dysentery and diarrhea. Pathological features included lesions in liver, kidney and intestine, the most prominent being an acute necrotizing colitis. The colonic lesions were histologically similar to human ulcerative colitis, and a suggested mechanism is that of glutamine deficiency induced by the glutaminase enzyme (86).

Healthy rats (87) receiving parenteral nutrition had increased jejunal mucosal weight, DNA content and villus height when glutamine was added to the solution. Recovery including weight gain and intestinal adaptation after 60% bowel resection in rats was enhanced (88). Rats with intraperitoneal 5-fluorouracil-induced enterocolitis were given total parenteral nutrition with or without glutamine (1). After four days, the glutamine-supplemented rats had increased small bowel mucosal DNA content and villus height. There was a trend towards increased survival in glutamine-fed rats. Methotrexate-induced enterocolitis in glutamine-treated rats has also been studied (4,5). In these experiments, an enteral diet was supplemented with glutamine for the treatment group and with glycine for controls. A four day period of prefeeding was employed. Groups treated with glutamine had less protein and DNA loss in the colon and jejunum. Animals fed supplemental glutamine had improved survival curves and a significantly lower incidence of positive blood cultures.

Glutamine has also been shown to be beneficial in radiation enteritis models in rats (6-8). Bacterial translocation, indicated by culture positive mesenteric lymph nodes (7) and bloody diarrhea (8) was significantly decreased in the glutamine-fed irradiated group. Mucosal mass was maintained and weight loss less with glutamine treatment. Reasons postulated include protection of the gut mucosal barrier by glutamine as an energy substrate and as a nitrogen donor for nucleotide synthesis. Glutamine may also have enhanced lymphocyte function to decrease bacterial translocation. Arterial glutamine levels, intestinal glutamine extraction and intestinal glutaminase levels were all higher in the glutamine-fed irradiated animals. Arterial glutamine levels (2,7,8) and extraction (8) are significantly increased only when dietary supplementation is combined with intestinal injury. Glutaminase levels, however, have increased with dietary glutamine alone after an initial period of fasting (72). Glutaminase may help the animal to take advantage of circulating glutamine from endogenous sources (8).

Of the injury models discussed (5-fluorouracil, methotrexate and radiation), glutamine was administered through the gastrointestinal tract in all but one study (1). Most experiments had a period of prefeeding of glutamine prior to injury induction. The injury models examined glutamine’s effect on intestinal morphometrics predominantly, although bacterial translocation (5-7) and survival (1,5,8) were also considered. While these findings cannot be extrapolated to humans directly, they are suggestive of many possible clinical applications (89), and potentially demonstrate glutamine’s role in supporting gut mucosal structure and function.

**GLUTAMINE AND THE INTESTINE: CLINICAL ASPECTS**

**Starvation**: Significant alterations of glutamine metabolism occur during starvation (90-92). During the post absorptive state or early fasting, the intestine obtains most of its glutamine from the arterial blood. After four days, the intestine increased its uptake of glutamine from the circulation by 80%. This increase was accompanied by the liver switching to glutamine release. Intestinal uptake of glutamine is increased in starvation despite lower mucosal glutaminase levels and is therefore likely a reflection of increased supply from other endogenous sources.

**Surgical trauma**: Trauma and surgical stress result in nitrogen loss due to muscle catabolism. Elective cholecystectomy in man results in decreased plasma amino acid levels, including glutamine, in the early postoperative period (93). The negative nitrogen balance associated with surgery was significantly reduced in six patients receiving a glutamine-containing parenteral fluid undergoing elective resection of colonic carcinoma compared to controls (94). A similar benefit to postoperative nitrogen balance has been observed as a result of intravenous infusion of glutamine in humans (95,96). The gut uses substantially more glutamine after operative stress (18); this is probably partially mediated through glucocorticoids (97,98) and glucagon (99).

Soubat et al (100) studied the effects of enteroectomy on glutamine interorgan exchange in dogs. After 60% small bowel resection, the intestine entered near glutamine balance, compared to avid glutamine consumption in controls. Alanine was released from the intestine at a significantly lower rate in the resection group. The intestine uses glutamine in the postopera-
tive state, reflected by reduced total intestinal glutamine consumption in partially resected animals.

Alanine and glutamine are released from muscle in large quantities in healthy (34) and critically ill (101) patients. Alanine and glutamine were studied in healthy volunteers and critically ill patients who underwent laparotomy (101). These amino acids were significantly decreased in arterial blood in the patient groups. Two patients who underwent greater than 80% bowel resection had a markedly decreased extremity glutamine efflux. Both healthy volunteers and those who underwent laparotomy without enterectomy had normal glutamine mobilization. Thus, surgical stress resulting in catabolism and negative nitrogen balance is characterized by increased intestinal uptake of glutamine. Alanine and ammonia, released in portal blood, are converted to glucose and urea by the liver. Muscle provides glutamine to the gut; however, if no exogenous glutamine is available to the stressed patient, the circulating pool becomes depleted.

Sepsis: As with trauma, the septic patient displays increased mobilization of glutamine with an associated negative nitrogen balance. However, in sepsis, intestinal consumption of glutamine is decreased in both rats and humans (63,64). At the expense of intestinal uptake, both lymphocyte and hepatic consumption of glutamine increase (102). Additional glutamine is supplied to the circulating pool by the lungs (37).

A detailed study of a multiple trauma patient revealed a steady decrease in splanchnic uptake of both glutamine and alanine over time (103-105). Both alanine and glutamine uptake reached zero as the patient became systemically septic and began deteriorating on day 10. Although this observation does not distinguish between intestinal and hepatic consumption, the patient's poor condition was likely coincidental with decreased intestinal consumption of glutamine (for gut energy substrate) and reduced hepatic uptake of alanine (for gluconeogenesis). In the basal state, glutamine is used by the intestine as an energy substrate.

Malignancy: Glutamine is the principal amino acid consumed by malignant cells (29,30); therefore consideration must be paid to both the tumour and the intestine in the cancer patient. That tumour cells may compete with the intestine for use of the circulating glutamine pool was suggested by an experiment using tumour-bearing rats (106). Glutamine's benefit to the intestine in the radiation enteritis model (6-8) may also benefit a malignant tumour treated by radiation. Therefore, indiscriminate administration of glutamine as an intestinal protectant to a patient with a malignancy would not be prudent. Glutamine has been shown to support muscle without stimulating tumour growth in rats (107). It afforded more benefit to the intestine than to lymphocytes with 5-fluorouracil toxicity (1). Until a better understanding of glutamine's role in malignant disease is obtained, caution regarding glutamine supplementation is urged (108).

Glutamine and nutritional preparations: Changes in the gastrointestinal tract that occur as a result of parenteral feeding include mucosal hypoplasia, decreased carbohydrate transport and reduced enzyme activities (109,110). These changes do not occur with enteral feeding. It is suggested that enteral glutamine is especially important in maintaining healthy intestinal mucosa (110,111). For example, glutamine supplementation of an elemental enteral diet for rats resulted in increased jejunal mass and crypt mitotic rate over controls (112). Glutamine and fibre were added to diets of separate groups of rats and neither reduced bacterial translocation, although benefits to jejunal mucosal architecture were noted (113).

Glutamine is not presently available in total parenteral nutrition solution due to its low solubility, instability and potential release of toxic products (ammonia and glutamate). Parenteral nutrition supplemented with glutamine resulted in increased jejunal weight, protein, DNA, mucosal thickness and villus height in rats (87,114). A similar benefit was demonstrated in colon and stomach with enhanced jejunal disaccharide activity (115).

It has been suggested that glutamine may be beneficial to the immune system through support of gut-associated lymphatic tissue (116). Supplementation of total parenteral nutrition with glutamine significantly reduced positive bacterial cultures of mesenteric nodes in rats (117). This phenomenon may have been due to improved immune function as secretory IgA levels in bile were observed to be significantly higher than in rats receiving glutamine-deficient total parenteral nutrition. The beneficial effects of parenteral glutamine on intestinal morphology were enhanced by the addition of epidermal growth factor (118), supporting the general hypothesis that specific nutrients in combination with hormonal factors can provide maximal growth of tissue.

The safety of intravenous glutamine has been studied in healthy volunteers (119,120). There were no untoward side effects noted, nor signs of central nervous system toxicity. Plasma levels of ammonia and glutamate (two potential toxic metabolites of glutamine) were not significantly elevated. Amino acid levels, cortisol, insulin, glucagon, growth hormone values and routine chemistry analyses were all unchanged. Intravenous glutamine did increase insulin levels slightly in another study (121). There was a significantly higher rise in arterial glutamine from intravenous compared to oral glutamine, suggesting splanchnic uptake of glutamine in the orally administered group. Glutamine has also been given in an intravenous parenteral solution over four weeks to patients receiving bone marrow transplants (120). This was well tolerated with no untoward effects.

The use of dipeptides with glutamine and an additional amino acid has been considered (122). Three dipeptides containing glutamine were compared in conscious dogs (123). Alanyl and glycyl glutamine dipeptides were hydrolyzed in vivo rapidly, resulting in prompt elevation of arterial glutamine. In humans, L-alanyl-L-glutamine was hydrolyzed more rapidly than L-glycyl-L-glutamine (124). The mechanism suggested reflects both plasma and
extracellular cell membrane hydrolysis. Intravenous use of N-acetyl-l-glutamine was studied in 14 healthy humans (125); however, the kinetics of consumption of this dipeptide render it less than ideal for clinical use.

Clinical applications of glutamine dipeptides have been reviewed (126, 127). L-alanyl-l-glutamine seems to be the most practical glutamine precursor for use in humans. Its properties have been studied in dogs (128), rats (129) and humans (130). L-alanyl-l-glutamine is 16 times more soluble than glutamine alone and is stable under conditions of heat sterilization and storage (131). No side effects were noted in clinical trials (131), and the benefits of L-alanyl-l-glutamine have been noted in catabolic patients (94,132).

Glutamine dipeptides have been given intravenously to septic patients with no impairment of peptide metabolism (122); however, more study is required if they are to be used in patients with either hepatic or renal failure.

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CONCLUSIONS

Glutamine is an amino acid whose importance in clinical medicine is rapidly being elucidated. As glutamine is important to rapidly dividing cells, the intestine has been of central interest. Glutamine will likely benefit critically ill subjects, as it is these patients whose endogenous sources cannot meet increased demands during stress. The introduction of glutamine dipeptides in parenteral nutrition solutions will likely be clinically important.

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