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Glutamine and Cancer

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Objective

This overview on glutamine and cancer discusses the importance of glutamine for tumor growth, summarizes the alterations in interorgan glutamine metabolism that develop in the tumor-bearing host, and reviews the potential benefits of glutamine nutrition in the patient with cancer.

Summary Background Data

Glutamine is the most abundant amino acid in the blood and tissues. It is essential for tumor growth and marked changes in organ glutamine metabolism are characteristic of the host with cancer. Because host glutamine depletion has adverse effects, it is important to study the regulation of glutamine metabolism in cancer and to evaluate the impact of glutamine nutrition in the tumor-bearing state.

Methods

Data from a variety of investigations on glutamine metabolism and nutrition related to the host with cancer were compiled and summarized.

Results

Numerous studies on glutamine metabolism in cancer indicate that many tumors are avid glutamine consumers *in vivo* and *in vitro*. As a consequence of progressive tumor growth, host glutamine depletion develops and becomes a hallmark. This glutamine depletion occurs in part because the tumor behaves as a "glutamine trap" but also because of cytokine-mediated alterations in glutamine metabolism in host tissues. Animal and human studies that have investigated the use of glutamine-supplemented nutrition in the host with cancer suggest that pharmacologic doses of dietary glutamine may be beneficial.

Conclusions

Understanding the control of glutamine metabolism in the tumor-bearing host not only improves the knowledge of metabolic regulation in the patient with cancer but also will lead to improved nutritional support regimens targeted to benefit the host.

Glutamine is the most abundant amino acid in the body.[1] It circulates in the mammalian bloodstream at a concentration of 0.6–0.9 mmol/L, and its concentration in some tissues may be as high as 20 mmol/L. Glutamine also has two nitrogen side chains (an amino and an amide group), and therefore, it is the most important circulating "nitrogen shuttle," accounting for 30% to 35% of all amino acid nitrogen transported in the blood.[2] In this capacity, glutamine serves as a vehicle for transporting ammonia in a nontoxic form from peripheral tissues to visceral organs where the ammonia can be excreted as ammonium (kidneys) or converted to urea (liver).

The circulating concentration of glutamine is main-

tained at a fairly constant level and is dependent on the relative rates of net glutamine uptake and release by the various organs in the body. The small intestine is the principal organ of glutamine uptake in the postabsorptive state.[3] The liver can behave as a net glutamine producer or consumer, depending on prevailing metabolic pressures. The kidneys also exhibit net glutamine uptake in the postabsorptive state, but renal glutamine consumption only becomes appreciable during acidosis, when additional circulating glutamine is needed to support renal ammoniagenesis. By contrast, net glutamine release occurs from skeletal muscle, which has a considerable capacity to synthesize glutamine *de novo* from glutamate and ammonia.[4,5]

Tumors cells are major glutamine consumers, and they compete with the host for circulating glutamine.[6] As a consequence, marked changes in interorgan glutamine metabolism resulting in host glutamine depletion develop with progressive tumor growth. Because glutamine is essential for tumor growth and host glutamine depletion has adverse effects, it is important to study the regulation of glutamine metabolism in cancer. This overview on glutamine and cancer will (1) discuss the importance of glutamine for tumor growth, (2) summarize the alterations in host glutamine metabolism that develop as a consequence of progressive tumor growth, and (3) discuss the potential benefits of glutamine nutrition in the host with cancer. Understanding the control of glutamine metabolism in the patient with cancer not only improves our knowledge of metabolic regulation but also will lead to improved nutritional support regimens targeted to benefit the host.

GLUTAMINE AND TUMOR METABOLISM

A substantial body of experimental evidence indicates that glutamine is the major respiratory fuel for tumor cells.[7-9] Glutamine has been shown to be an unusually good substrate for oxidation by tumor cell mitochondria; predictably, tumor glutaminase activity is relatively high. Phosphate-dependent glutaminase converts glutamine to glutamate and ammonia and is the first step in a series of reactions that generate the metabolic intermediates required for cell growth (Fig. 1). Glutaminase activity correlates well with tumor glutamine consumption and growth rates,[10,11] and the low intracellular glutamine

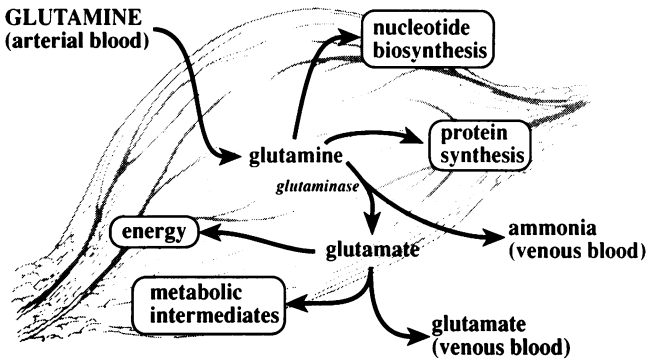

Figure 1. Pathways of glutamine metabolism in tumor cells. High rates of glutaminolysis explain the hyperammonemia observed with advanced malignant disease. Glutamine uptake by malignant cells provides substrate for nucleotide and protein biosynthesis, for energy production, and for the generation of key metabolic intermediates.

concentrations[12] have been attributed, in part, to an increase in the activities of glutamine-using nucleotide biosynthetic enzymes and glutaminase (Fig. 2). Physiologic concentrations of circulating glutamine are required for optimal growth of malignant cells in culture, although many cancerous cells do not have an absolute requirement for glucose.

In a series of elegant *in vivo* studies, the rate of glutamine uptake was quantified using implanted hepatomas attached to a surgically prepared vascular pedicle.[13,14] Glutamine was consumed at a rate faster than that of any other amino acid, and its uptake was proportional to its supply. Interestingly, tumor glutamine use was more efficient in tumor-bearing rats that were fasted. Consistent with these studies is another report that demonstrated that fast-growing fibrosarcomas are also avid glutamine consumers.[15] Glutamine extraction by this tumor has been quantified and may be as high as 45%, greater than the rate of glutamine extraction for any organ under conditions of health. The tumor thus behaves a "glutamine trap." The high rates of intracellular glutaminolysis are evident by the enormous release of ammonia into the venous effluent (Fig. 1).

As a general rule, malignant cells transport glutamine across their plasma cell membrane at a faster rate than do their nonmalignant counterparts. For example, human hepatoma cells consume glutamine at a rate five- to tenfold faster than do normal hepatocytes.[16] Because solid tumors are poorly vascularized, it has been suggested that they are endowed with efficient transport systems to compete with the host for glutamine.[6] Glutamine is transported into cells principally by the sodium ion-dependent systems A and ASC; in liver system N mediates glutamine uptake from the sinusoidal blood.[17,18] Although system A is normally repressed, malignant cells exhibit system A derepression,[19] an adaptive response that augments glutamine transport into the cell. After

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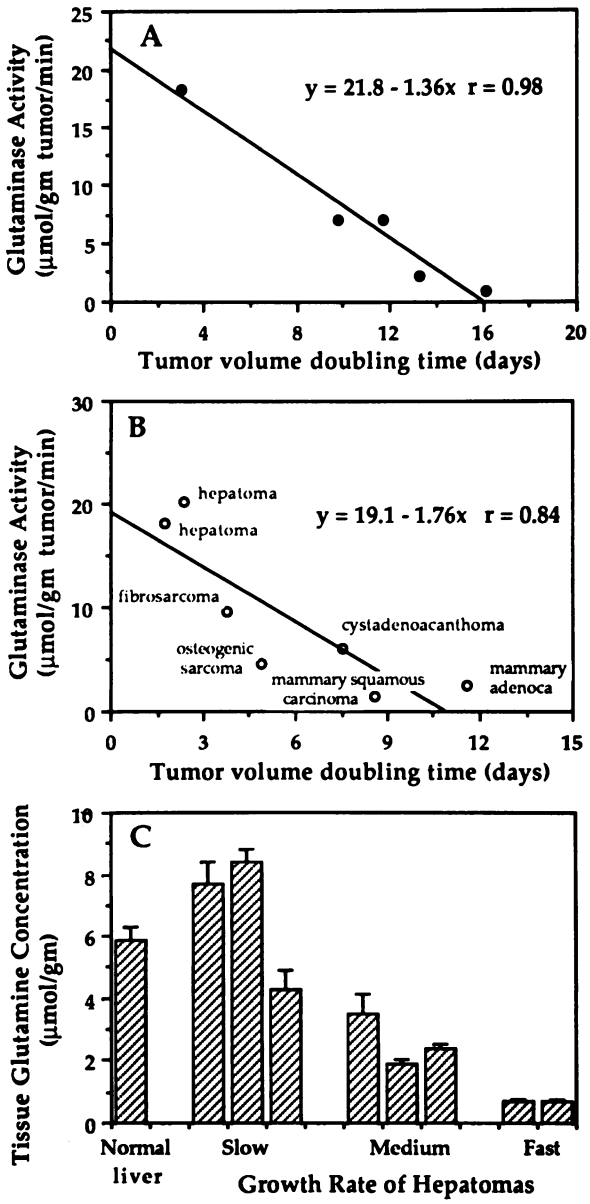

Figure 2. Correlation between tumor growth and the rate of intracellular glutamine metabolism. (A) Relationship between phosphate-dependent glutaminase activity and tumor doubling time in five different hepatomas; data modified from Linder-Horowitz et al.[10] (B) Positive correlation between phosphate-dependent glutaminase activity and tumor doubling time in seven solid malignancies of different tissue origin; data modified from Knox et al.[11] (C) Negative correlation between tumor glutamine concentration and cell proliferation (growth rate) in hepatomas; data modified from Sebolt et al.[12]

glutamine gains access to the cytoplasm, it must be transported into the mitochondria before it can be hydrolyzed by phosphate-dependent glutaminase. It is difficult to differentiate between glutamine transport and metabolism in isolated mitochondria, but it appears that glutamine transport in mitochondria is a carrier-mediated event.[20] Given the importance of glutamine for the survival of cancer cells, the regulation of the altered membrane glutamine transport that develops with spontaneous malignancy or cellular transformation is likely to be a fruitful area of research.

It is unclear why malignant cells consume such large amounts of glutamine. Neither energy expenditure nor biosynthetic requirements can explain the high rates of glutaminolysis found in rapidly proliferating cells. Simultaneously, there is high rate of glycolysis that generates large amounts of lactate and results in futile cycling. This apparent wasting has been theoretically justified on the basis of quantitative principles of metabolic control.[21] It has been suggested that high rates of glycolysis and glutaminolysis are necessary in malignant cells to allow sensitive and precise control of the pathways that generate metabolic intermediates for macromolecular biosynthesis.

GLUTAMINE METABOLISM IN THE HOST WITH CANCER

In the majority of patients with cancer, glutamine depletion develops with time, both from the disease process itself and from the catabolic effects of antineoplastic therapies. Although it is known that malignant lymphocytes from patients with leukemia exhibit extremely high rates of glutamine consumption,[22] organ metabolism has not been well studied, in part, because of the invasive nature of such investigations. One group measured amino acid flux across the extremities of malnourished patients with cancer but did not report glutamine exchange because glutamine and glutamate concentrations were reported together.[23]

Studies during the late 1950s in rats with large malignancies demonstrated a reduction in plasma and hepatocyte intracellular glutamine concentrations compared with nontumor-bearing controls.[24,25] Studies on skeletal muscle glutamine metabolism in rats bearing Walker carcinosarcomas in the thigh found glutamine uptake by the leg containing the tumor, although the contralateral leg demonstrated glutamine release at an accelerated rate.[24] A fall in muscle glutamine concentrations was detected in the tumor-bearing host.[26] These observations are consistent with the presence of a tumor-derived signal that results in accelerated skeletal muscle glutamine release, perhaps to maintain the blood glutamine concentration to supply the tumor. Some studies have shown that the circulating glutamine concentration rises within days after tumor cell inoculation;[27] others indicate a progressive fall in blood glutamine levels later in the course of the disease.[28,29]

The methylcholanthrene-induced sarcoma (MCA tumor) model has been used by several investigators to study the influence of cancer on interorgan glutamine

metabolism.[5,28–32] The tumor was first induced in rats by subcutaneous injection of the carcinogen MCA and subsequently was successfully transplanted in Fischer 344 rats.[33] The clinical relevance of this rat tumor model can be questioned because, like many animal tumor models, the MCA tumor grows to a size not observed clinically. Nonetheless, important information regarding host–tumor glutamine interactions has been acquired. This tumor is locally aggressive but rarely metastasizes, causing death (from inanition) 5 to 6 weeks after tumor implantation, at which time the size of the tumor may account for nearly one half of the animal's total body weight. The tumor grows most effectively in the Fisher 344 rat and has several advantages over murine models. The larger size of the rat makes the technical skills involved in regional flux measurements easier to acquire. Moreover, the larger organs in the rat often do not require pooling of tissues from several animals to do metabolic studies, and the volume of blood that can be sampled for repeated analysis is greater. Because a portion of the tumor-induced cachexia in this model is the result of a fall in voluntary food intake (tumor-induced anorexia), this must be taken into account when trying to differentiate between derangements in interorgan glutamine metabolism as a consequence of simple starvation as opposed to abnormalities that are secondary to the growing tumor. Therefore, control nontumor-bearing rats should be pair fed to carcass weight to control for simple starvation effects.

Predictably, both the magnitude and direction of glutamine flow in tumor-bearing rats changes during the course of the disease process. The changes that occur appear to be designed, in part, to maintain the blood glutamine concentration as the tumor grows and uses more glutamine. In rats bearing the MCA tumor, there is a progressive fall in circulating glutamine concentrations as the tumor grows (Fig. 3).[29,31] This reduction in blood glutamine level occurs despite an accelerated muscle glutamine release[5] and is consistent with marked glutamine use by the tumor.

A cardinal feature of the host response to the growing cancer is the development of muscle glutamine depletion.[5] This depletion becomes detectable early in the course of the disease process when the animal appears healthy and has a normal appetite. When the tumor comprises approximately 10% of total body weight, there is a 20% fall in the muscle glutamine concentration, which is associated with an accelerated glutamine efflux from the hindquarter (Fig. 4). This increase in muscle glutamine release is not secondary to an increase in regional blood flow but, instead, is the result of a twofold increase in the fractional release rate of glutamine. Simultaneously, the specific activity of the glutamine synthetase (GS) enzyme increases as does the quantity of GS

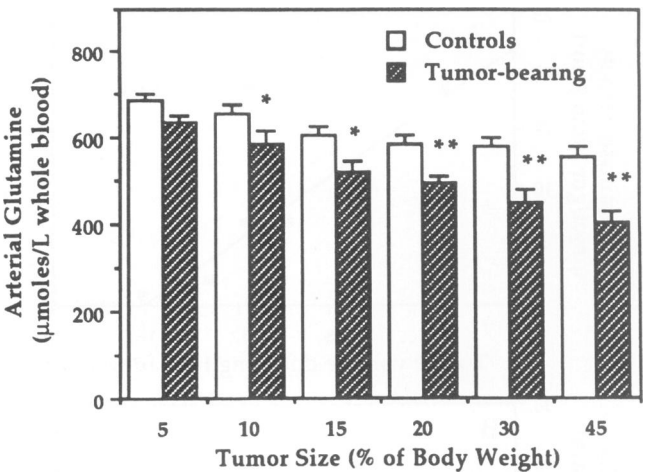

Figure 3. Changes in the circulating glutamine concentration as a function of progressive growth of the MCA tumor in the rat. A reduction in circulating glutamine is apparent when tumors are relatively small and before the host becomes clinically ill. The control animals are pair fed to similar carcass weights. *$p < 0.05$, **$p < 0.01$ *vs.* controls. Data compiled from Souba et al.,[29] Chen et al.,[5,28] Dudrick et al.,[40] and from unpublished observations.

messenger RNA in the muscle.[5] This may be viewed as an adaptive response whereby muscle is attempting to maintain its own glutamine stores by increasing intracellular glutamine biosynthesis. With time, the glutamine depletion becomes severe. and late in the course of the disease, muscle may become "exhausted." Based on the relationship between muscle glutamine concentrations and muscle protein synthesis,[34] a hypothesis could be put forth proposing that the progressive glutamine depletion that develops in the tumor-bearing host plays an etiologic role in the pathogenesis of tumor-induced cachexia.

At the same time that alterations in muscle glutamine metabolism are occurring, there are changes in glutamine use taking place in the small intestine.[29] Intestinal glutamine extraction falls as the tumor grows, an alteration that is not solely related to the reduction in circulating glutamine. With time, the tumor becomes the major organ of glutamine uptake in the body, "stealing" as much as 50% of glutamine from the circulating pool.[15,32] This fall in gut glutamine extraction is associated with a marked fall in mucosal glutaminase activity (Fig. 5), the major enzyme of glutamine hydrolysis in the gut.[35,36] As the tumor grows, the incidence of bacterial translocation increases,[36,37] suggesting a defect in the gut mucosal barrier or in gut immune function.

In response to the diminished extraction of circulating glutamine, there is an increase in the uptake of glutamine from the lumen. One group studied the effects of progressive malignant growth on the activities of several amino acid transport systems in the small intestinal brush border at various stages of tumor growth.[30] The

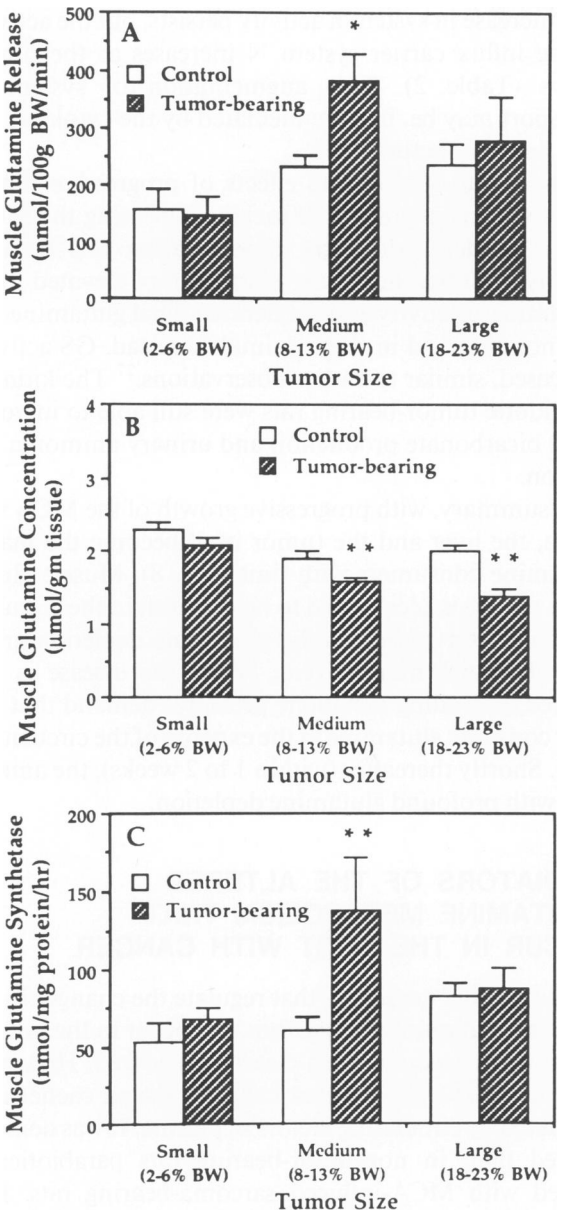

Figure 4. Skeletal muscle glutamine metabolism in the tumor-bearing rat. (A) Muscle (hindquarter) glutamine release as a function of tumor size. Advanced malignant disease may be associated with impaired muscle glutamine efflux. (B) Muscle glutamine concentrations as a function of tumor size. (C) Muscle glutamine synthetase specific activity in the tumor-bearing rat. Data = mean ± the standard error of the mean, *$p < 0.05$, **$p < 0.01$ *vs.* controls. Data adapted from Chen et al.[5]

rate of glutamine uptake by brush border membrane vesicles from tumor-bearing rats was significantly greater than that in controls, regardless of tumor size (Fig. 6). This augmented uptake was not observed for other amino acids. The increase in transport activity was caused by an increase in maximal transport velocity, consistent with an increase in the number of functional transporters in the brush border. Regardless of the mechanism(s) involved, it appears that maintenance of glutamine transport activity is a major synthetic priority that is preserved even when severe cachexia is present. Providing luminal nutrition at a time when glutamine transport activity is increased may be a biochemical rationale for the use of glutamine-enriched nutrition in certain patients with cancer.

The hepatic response to the growing cancer is somewhat different than that observed in skeletal muscle and in muscle. Early in the course of the disease, the liver switches from an organ of glutamine uptake to an organ of net glutamine release[29] (Fig. 7). This switch to net release may be related to an increase in the intracellular–circulating glutamine concentration gradient.[28] More importantly, however, carrier-mediated glutamine transport out of the hepatocyte, which is controlled by the sodium ion-independent system n,[38] is enhanced. Studies in rats with small tumors have demonstrated that the maximal transport velocity of system n is increased nearly threefold.[31] Thus, the accelerated net hepatic glutamine efflux that develops in rats with relatively small

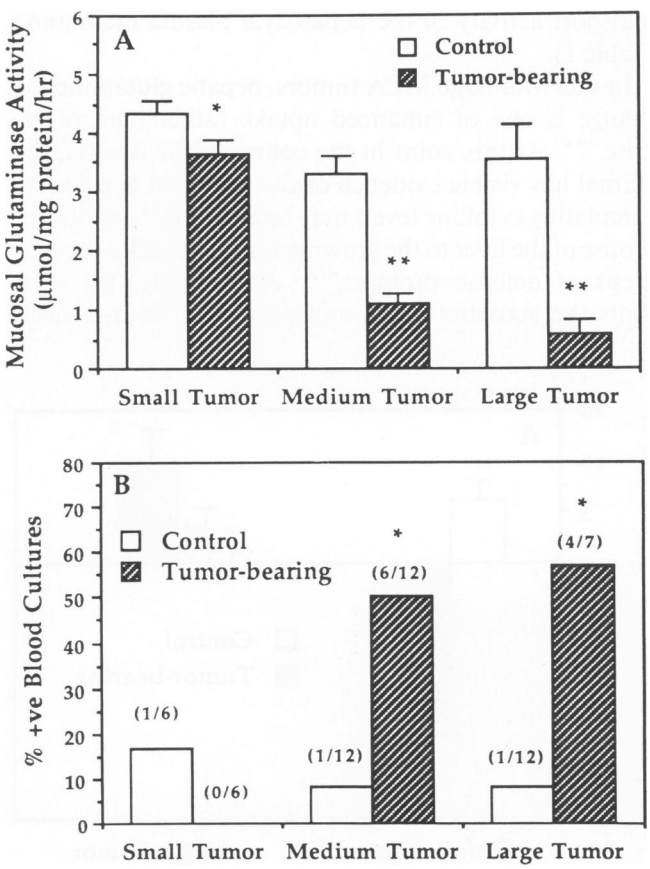

Figure 5. Intestinal glutamine metabolism and mucosal barrier function in the tumor-bearing rat. (A) Jejunal mucosal glutaminase specific activity as a function of progressive tumor growth. (B) The impaired intestinal glutamine metabolism may be associated with the development of positive blood cultures. *$p < 0.05$, **$p < 0.01$ *vs.* control. Data adapted from Souba et al.[36] and Salloum et al.[35]

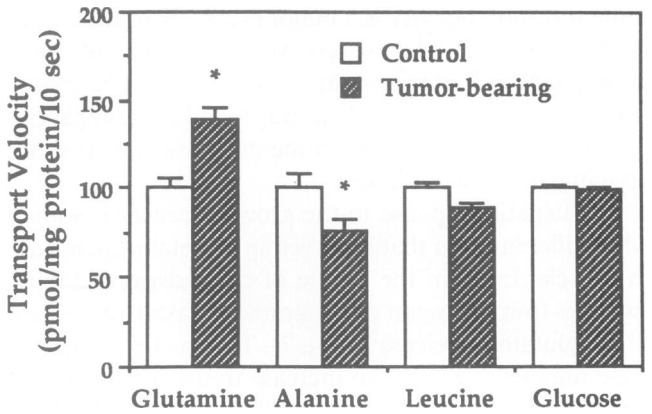

Figure 6. Sodium ion-dependent glutamine transport across the jejunal brush border in control (pair-fed) and tumor-bearing rats. Glutamine transport was increased, whereas other substrates exhibited no change (leucine and glucose) or a fall (alanine) in uptake. Data modified from Salloum et al.[30]

tumors is a function of both the mass-action effect of an increased glutamine gradient from the liver to blood and a stimulation of the sodium ion-independent glutamine transport activity of the hepatocyte plasma membrane (Table 1).

In rats with large MCA tumors, hepatic glutamine exchange is one of enhanced uptake rather than of release.[39,40] At this point in the course of the disease, the animal has visible evidence of cachexia and appears ill. Circulating cytokine levels may be elevated,[41] and the response of the liver to the growing tumor includes the synthesis of defense proteins.[42,43] Accordingly, the liver shifts the activities of its transporters to favor uptake.

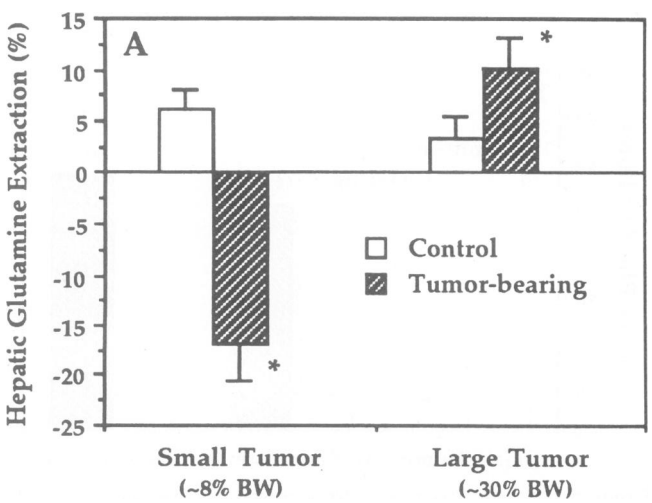

Figure 7. Hepatic glutamine extraction in tumor-bearing rats. In animals with small tumors, the liver released glutamine into the circulation. By contrast, when the cancer was large, the liver exhibited net glutamine consumption, exceeding the rate observed in control animals. Data adapted from Souba et al.[29] and Dudrick et al.[39,40]

The increase in system n activity persists, but the activity of the influx carrier system N increases as the tumor grows (Table 2). This augmentation of system N transport may be, in part, mediated by the cytokine, tumor necrosis factor.[44]

One group studied the effects of progressive tumor growth on renal glutamine metabolism using the MCA tumor model.[5] Although tumor-bearing rats became slightly acidotic, the classic adaptation of elevated renal glutaminase activity and accelerated renal glutamine use was not observed in these animals. Instead, GS activity increased, similar to earlier observations.[27] The kidneys of acidotic tumor-bearing rats were still able to increase renal bicarbonate production and urinary ammonia excretion.

In summary, with progressive growth of the MCA sarcoma, the liver and the tumor itself become the major glutamine consumers with time (Fig. 8). Muscle glutamine release is accelerated to help maintain the circulating pool, and consequently, glutamine depletion gradually becomes more severe. When the disease is advanced, prevailing metabolic pressures demand that the liver consume glutamine at the expense of the circulating pool. Shortly thereafter (within 1 to 2 weeks), the animal dies with profound glutamine depletion.

MEDIATORS OF THE ALTERED GLUTAMINE METABOLISM THAT OCCUR IN THE HOST WITH CANCER

The specific mediators that regulate the changes in interorgan glutamine metabolism that occur in the tumor-bearing rat have not been clearly elucidated. However, there is good evidence that cancer-induced cachexia is mediated by circulating factors in plasma. It was demonstrated that, in nontumor-bearing rats parabiotically united with MCA-induced sarcoma-bearing rats, features of cachexia developed that were consistent with the release of factors by the tumor or by the host in response to the tumor, which gained access to the nontumor-bearing animal through the circulatory bridge.[45]

It is likely that cytokines play an important role in mediating the altered glutamine metabolism that is characteristic of progressive tumor growth. Elevated levels of tissue and circulating cytokines have been demonstrated in the host with cancer, and these polypeptide molecules have been shown to induce many of the metabolic alterations that occur in the tumor-bearing host when they are administered to healthy animals.[46] Incubation of hepatocytes from normal rats with serum from tumor-bearing rats increases glutamine transport,[40] indicating that a circulating factor(s) is involved in the response. The most direct evidence for the role of cytokines are studies in which tumor-bearing rats have been treated

Table 1. ESTIMATED PHYSIOLOGIC HEPATIC GLUTAMINE TRANSPORT RATES IN CONTROL AND SMALL TUMOR-BEARING RATS*

Study Group	System "N"	System "n"	Rate of Glutamine Diffusion	Net Vectorial GLN Movement
Control	450	−125	−150	+175 (uptake)
Tumor-bearing (tumor ~8% BW)	450	−300	−250	−100 (release)

Transport rates are derived from vesicle studies and are expressed in pmol/mg protein/time. A minus sign indicates release. System N is Na^+-dependent and mediates the transport of glutamine into the hepatocyte. System n is Na^+-independent and mediates glutamine transport out of hepatocytes. Glutamine normally diffuses out of the liver into the blood because of the intracellular/extracellular glutamine gradient (10:1). Changes in this gradient will alter the diffusion rate. The switch from hepatic glutamine uptake in controls to glutamine release by the liver of rats with small tumors is consistent with *in vivo* extraction data (Fig. 7). GLN = glutamine, BW = body weight.

* Data are compiled from Pacitti et al.[31] and from unpublished results from the author's laboratory and represent approximate hepatic transport rates for glutamine at physiologic cytoplasmic and circulating glutamine concentrations.

with an antibody to tumor necrosis factor and hepatic glutamine transport measured. There was a marked reduction in hepatic glutamine transport in the tumor-bearing rats that received the antibody (Souba WW, et al., unpublished data). Similarly, treatment of healthy nontumor-bearing rats with tumor necrosis factor stimulates hepatic glutamine transport *in vivo*.[44]

GLUTAMINE ANALOGUES AS ANTICANCER AGENTS

Several glutamine analogues have been studied as possible chemotherapeutic agents in animals and patients.[47] One of the early agents used was the enzyme glutaminase, which converts glutamine to glutamate and ammonia. The logic behind the potential use of this compound was that its infusion into the bloodstream would diminish blood glutamine levels and, thereby, decrease the availability of glutamine to the tumor. Unfortunately, this kind of therapy was associated with intolerable side effects. In several species that received glutaminase, blood glutamine levels fell to near-undetectable levels, and the animals had diarrhea, mild villous atrophy, mucosal ulcerations, and intestinal necrosis.[48] This emphasizes the importance of glutamine for the gut and points out some of the difficulties with nonspecific therapies.

Two glutamine analogues that compete with glutamine in replicating cells are L-DON (6-diazo-5-oxo-L-norleucine) and acivicin (α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid).[47] Their structural similarity to glutamine is shown in Figure 9. The keto acid L-DON is an antitumor antibiotic isolated from Streptomyces that inhibits a number of biochemical reactions requiring glutamine.[49] In particular, L-DON inhibits glutamine-using enzymes by irreversible alkylation of susceptible L-cysteinyl residues, a mechanism it shares with acivicin. DON is active against the L1210 leukemia tumor, the CD8F1 mammary tumor, and the colon 38 carcinoma implanted in mice.[6,50] Clinical investigations with DON have been disappointing and have been limited by side effects, which include nausea, mucositis, and pancytopenia.

Acivicin also inhibits glutamine-requiring enzymes, especially the rate-limiting enzymes of *de novo* purine and pyrimidine biosynthesis.[6,47] Using total parenteral nutrition (TPN) regimens that ordinarily stimulate tumor growth, a reduction in tumor growth was demonstrated when acivicin was simultaneously adminis-

Table 2. ESTIMATED PHYSIOLOGIC HEPATIC GLUTAMINE TRANSPORT RATES IN CONTROL AND LARGE TUMOR-BEARING RATS*

Study Group	System "N"	System "n"	Nonsaturable Release	Net Vectorial GLN Movement
Control	400	−150	−150	+100 (uptake)
Tumor-bearing (tumor ~30% BW)	780	−300	−200	+280 (accelerated uptake)

Transport rates are from vesicle studies and are expressed in pmol/mg protein/time. A minus sign indicates release. The accelerated hepatic glutamine uptake by the liver of rats with large tumors is consistent with *in vivo* extraction data (Fig. 7). GLN = glutamine, BW = body weight.

* Data are compiled from Pacitti et al.,[31] Dudrick et al.,[40] and from unpublished results and represent approximate hepatic transport rates for glutamine at physiologic cytoplasmic and circulating glutamine concentrations.

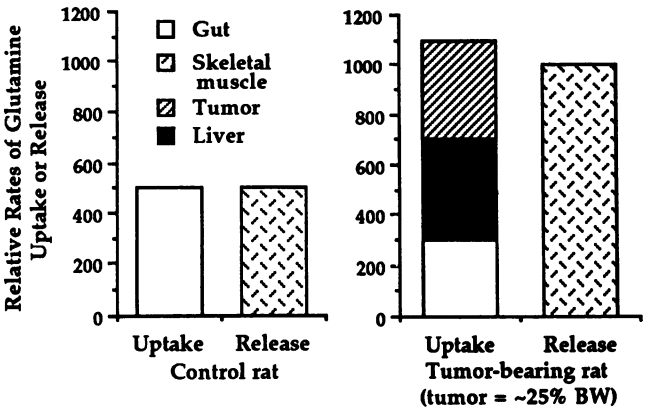

Figure 8. Major organs of net glutamine uptake and release in control and MCA tumor-bearing rats. When the disease was advanced, the tumor and the liver accounted for the majority of glutamine uptake. Data compiled from Souba et al.,[29] Klimberg et al.,[54] Chen et al.,[32] Dudrick et al.,[40] and from unpublished observations.

tered.[51] When insulin was added to the regimen, tumor growth ceased, carcass weight gain was observed, and muscle mass was preserved.[52] Unfortunately, phase I clinical trials have been disappointing and limited by toxicities similar to those elicited by DON administration.

GLUTAMINE NUTRITION IN THE TUMOR-BEARING HOST

Marked glutamine depletion develops in the host with cancer. This may be the result of alterations in host glutamine metabolism secondary to the presence of the tumor, to glutamine use by the tumor, or to the catabolic effects of antineoplastic treatments. This depletion is most obvious in skeletal muscle, which serves as the major "glutamine repository" in the body. This depleted state may have a negative impact on the function of host

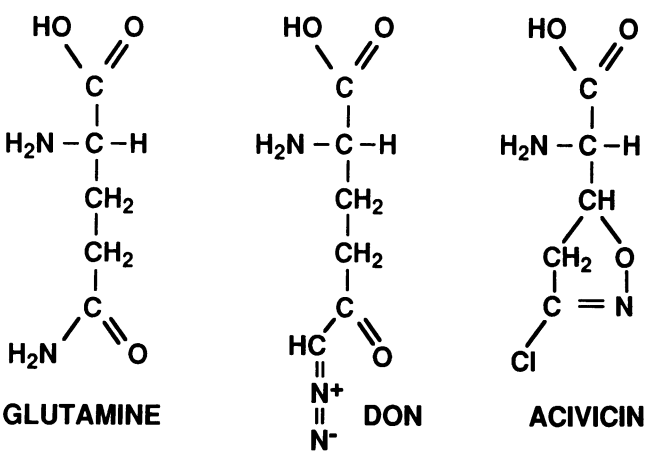

Figure 9. Glutamine analogues with antitumor activity.

tissues that require glutamine (*e.g.*, intestinal epithelial cells, lymphocytes, and endothelial cells) if glutamine availability in the blood becomes rate limiting. "Second hits" (*i.e.*, immunosuppression and sepsis) that further worsen the glutamine depletion are poorly tolerated. Although there is abundant evidence that glutamine occupies a key position in the maintenance of metabolism, structure, and function in several organs, the exact role that glutamine plays as a dietary supplement in the overall care of patients with cancer is only now being addressed.

The classification of glutamine as a nonessential or nutritionally dispensable amino acid implies that, in its absence from the diet, it can be synthesized in adequate quantities from other amino acids and precursors. For this reason, and because of its relative instability and short shelf-life compared with other amino acids, it has not been considered necessary to include glutamine in nutritional formulas. Glutamine has been eliminated from TPN solutions, and with few exceptions, glutamine is present in oral and enteral diets only at the relatively low levels that are characteristic of its concentration in most dietary proteins.[53] Based on our knowledge of the changes in glutamine metabolism that are characteristic of the host with cancer, this categorization of glutamine as a nonessential amino acid may be misleading (Table 3). It is therefore prudent to review some of the studies that have evaluated the effects of glutamine-enriched diets in the host with cancer.

GLUTAMINE NUTRITION AND SKELETAL MUSCLE

The marked skeletal muscle glutamine depletion that is characteristic of advanced malignant disease has stimulated several investigators to study the effects of glutamine-enriched diets on muscle glutamine metabolism. One group demonstrated that providing glutamine orally to the tumor-bearing rat helps replete muscle glutamine stores.[54] This partial restoration of the intracellular glutamine concentration was accompanied by an increase in the activity of GS, which catalyzes *de novo* glutamine biosynthesis in muscle.[5] Repletion of glutamine stores in muscle was associated with a rate of hindquarter glutamine release that was similar to that observed in the healthy postabsorptive rat.[54] Others studied the effects of glutamine-enriched TPN in tumor-bearing rats.[55] Providing 20% of TPN protein as glutamine produced a significant increase in the arterial glutamine level and maintained the skeletal muscle intracellular glutamine concentration. Concurrently, hindquarter glutamine fractional release increased nearly threefold in the glutamine-supplemented group.

Table 3. IS GLUTAMINE A CONDITIONALLY ESSENTIAL AMINO ACID IN THE HOST WITH CANCER?

Definition: A conditionally essential amino acid is one that is nonessential during health but is required in the diet in certain pathophysiologic states because tissue utilization exceeds the capacity for endogenous biosynthesis. Provision of the nutrient (glutamine) in the diet during the disease state (cancer) improves tissue metabolism, structure, and function.

Hypothesis: In the host with cancer, glutamine is a conditionally essential amino acid. Its provision counteracts the glutamine depletion that develops with progressive tumor growth and it also attenuates host tissue injury associated with tumor growth and antineoplastic therapy.

Evidence that glutamine may be conditionally essential in the tumor-bearing host

Required Effect/Criteria	Evidence/Examples
Decrease in blood and tissue glutamine concentrations	Tumor-bearing animals develop glutamine depletion in blood[5,28,29] and skeletal muscle.[5,26]
Atrophy or dysfunction of a specific tissue(s)	Tumor-bearing rats exhibit impaired intestinal glutamine metabolism,[35] villous atrophy,[29] and bacterial translocation.[36,37]
	Rats treated with radiation therapy or chemotherapy develop bacteremia,[58,63] mucosal atrophy,[59,60] or mucosal damage[61,63,64] when no glutamine is provided in the diet.

Effects of conditionally essential nutrient (glutamine) repletion

Required Effect/Criteria	Evidence/Examples
Correct tissue glutamine depletion	Glutamine-enriched diets restore muscle glutamine in tumor-bearing rats.[54,55]
Enhance cellular utilization	Feeding glutamine-enriched diets to rats receiving whole abdominal radiation increases gut uptake of circulating glutamine.[63]
Improvement in tissue morphology and function	Glutamine-enriched diets increase intestinal villous height in the tumor-bearing rat (M. Torosian, University of Pennsylvania, personal communication).
	Glutamine-enriched TPN increases gut mucosal glutathione levels in the tumor-bearing rat.[55]
	Glutamine-enriched enteral diets improve recovery and enhance mucosal healing after chemotherapy or radiation therapy.[58,61,63,64]
	Glutamine-enriched enteral diets increase carcass weight in tumor-bearing rats.[54]
Improvement in protein economy	Glutamine nutrition improves nitrogen balance in bone marrow transplant patients.[74]
Improvement in outcome	Glutamine nutrition decreases infections and shortens hospital stay in bone marrow transplant patients.[74]

IMPACT OF GLUTAMINE NUTRITION ON THE INTESTINAL MUCOSA AND ON GUT IMMUNE FUNCTION

The gut has received the most attention with regard to studies designed to evaluate the impact of glutamine nutrition, but most of this work has been done in nontumor models. A recent study by D. Bartlett and M. Torosian (Department of Surgery, University of Pennsylvania, personal communication of unpublished data) demonstrated the trophic effects of a glutamine-supplemented enteral diet on the small bowel in tumor-bearing rats. Rats were implanted subcutaneously with mammary carcinoma tumors and randomly assigned to receive standard enteral diets supplemented with either 3% glutamine or 3% glycine (control). Small bowel mucosa in the glutamine-fed group showed an increased mucosal DNA content (Fig. 10). In other studies, intestinal glutathione levels were higher in tumor-bearing rats nourished with glutamine-supplemented TPN.[55]

Bacterial translocation develops in the tumor-bearing rodent,[36,37] but the impact of glutamine nutrition on gut barrier function in the host with cancer requires further study. In nontumor-bearing rats, glutamine-enriched TPN results in decreased bacterial translocation compared with standard TPN formulas.[56] This decrease in translocation is associated with a normalization of biliary secretory immunoglobulin A levels and a decrease in bacterial adherence to enterocytes, suggesting that glutamine-supplemented TPN may enhance gut immune function.[57]

Other investigators have shown that providing glutamine-supplemented nutritional support may accelerate healing of the intestinal injury that occurs secondary to chemotherapy or radiation therapy. The addition of glutamine to an elemental, enteral diet resulted in a significant reduction in the severity of methotrexate-induced enterocolitis, as reflected by improved morphometric parameters.[58] Providing glutamine reduced the incidence of bacteremia and improved survival (Fig. 10). Similar improvements in jejunal villous height were

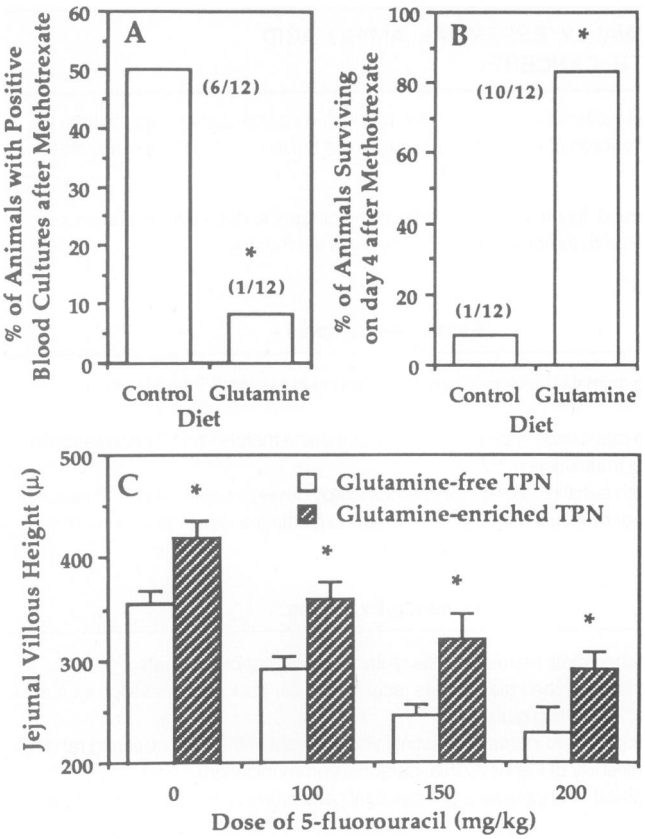

Figure 10. Benefits of glutamine nutrition in animals receiving chemotherapy. (A) Impact of a glutamine-enriched elemental diet on the incidence of bacteremia after a single dose of methotrexate (modified from Fox et al.[58]). (B) Impact a of glutamine-enriched elemental diet on survival after methotrexate (modified from Fox et al.[58]). (C) Effects of glutamine-supplemented TPN on jejunal villous height in rats treated with escalating doses of 5-fluorouracil (5-FU) (data modified from Smith[77]).

noted in rats receiving 5-fluorouracil and glutamine-enriched TPN.[59,60] More recent studies indicate that providing dietary glutamine to tumor-bearing rats receiving methotrexate enhances the tumoricidal effectiveness of the drug while reducing the morbidity and mortality rates of the chemotherapy.[61] When animals receiving methotrexate were randomized to receive identical glutamine-supplemented elemental diets that were administered orally or intravenously, enteral administration was associated with a significant decrease in the incidence of positive cultures in the spleen and a marked improvement in survival.[62] Therefore, the enteral route of feeding appears to be preferable to the intravenous route, even when glutamine is added to the diet.

Glutamine feeding has been shown to diminish the intestinal injury associated with whole abdominal radiation. Providing oral glutamine after abdominal irradiation improves mucosal morphometrics and decreases the morbidity and mortality associated with abdominal radiation (Table 4).[63] Feeding glutamine-enriched oral diets before abdominal radiation was equally effective in exerting a radioprotective effect.[64] Thus, providing glutamine to patients undergoing abdominal or pelvic irradiation may protect the intestinal mucosa from injury, accelerate healing of radiated bowel, and possibly, attenuate the long-term sequelae of radiation-induced enteritis. Providing glutamine by the enteral route may be required because glutamine-enriched TPN has been shown to be of no value after whole abdominal radiation.[65]

GLUTAMINE NUTRITION AND TUMOR GROWTH

Because tumors are avid glutamine consumers, several investigators have examined the effects of glutamine-supplemented diets on indices of malignant cell proliferation *in vivo* and *in vitro*. In 1935, it was demonstrated that the proliferation of cultured HeLa (malignant cervical cells) cells is greatest when the glutamine concentrations are at least 1 mmol/L.[7] This *in vitro* requirement may reflect the continuous demand for glutamine in the absence of the normal *in vivo* supply (0.6 to 0.9 mmol/L). Failure to provide glutamine in the growth medium of cultured malignant cells retards cell division and usually results in cell death. By contrast, *in vivo* studies using tumor-bearing rats showed that the administration of glutamine-supplemented enteral nutrition did not affect tumor weight, tumor DNA content, or tumor glutaminase activity.[54] In similar studies using a mammary carcinoma rat model, there was no significant difference in tumor weight or protein and DNA content and no increase in the incidence of metastases between the groups (Fig. 11; D. Bartlett and M. Torosian, Department of Surgery, University of Pennsylvania, personal communication of unpublished data). Likewise, studies using glutamine-enriched TPN demonstrated no stimulation of tumor growth when tumor weight, tumor DNA content, and tumor glutaminase activity were measured.[55] DNA flow cytometric analysis did not demonstrate any difference in the percentage of aneuploid tumor cells within the G1, S, or G2M cell cycles. However, the ratio of aneuploid to diploid cells in the tumor mass increased by 20% in animals receiving glutamine. Glutamine supplementation had no effect on tumor glutathione levels (Fig. 11).

In summary, glutamine availability to cultured malignant cells will greatly influence the cellular proliferation rate. Clinical studies are necessary to determine if tumor growth can be altered in patients with cancer who receive glutamine-supplemented diets. If glutamine does alter the growth cycle of cancer cells, cycle-specific chemotherapy may be more effective. Of interest are recent studies that indicate that providing oral glutamine and

Table 4. BENEFITS OF ORAL GLUTAMINE NUTRITION PROVIDED TO RATS AFTER WHOLE ABDOMINAL RADIATION*

Diet Provided	No. of Animals Surviving for 8 Days	No. of Animals With Culture-Positive MLNs (day 4)	Jejunal Villous Height (mm)	Jejunal Villous Number (no./cm bowel)
Control	5/11	8/9	0.29 ± 0.03	79 ± 11
Glutamine	11/11†	2/10‡	0.54 ± 0.05†	101 ± 4†

MLNs = mesenteric lymph nodes.
* Modified from Klimberg.[63]
† $p < 0.01$ *vs.* control.
‡ $p < 0.05$ *vs.* control.

methotrexate to animals bearing the MCA sarcoma increases the intracellular tumor concentration of methotrexate.[66] The authors suggest that supplemental glutamine may prevent the development of drug resistance by preventing cellular efflux from tumor cells.

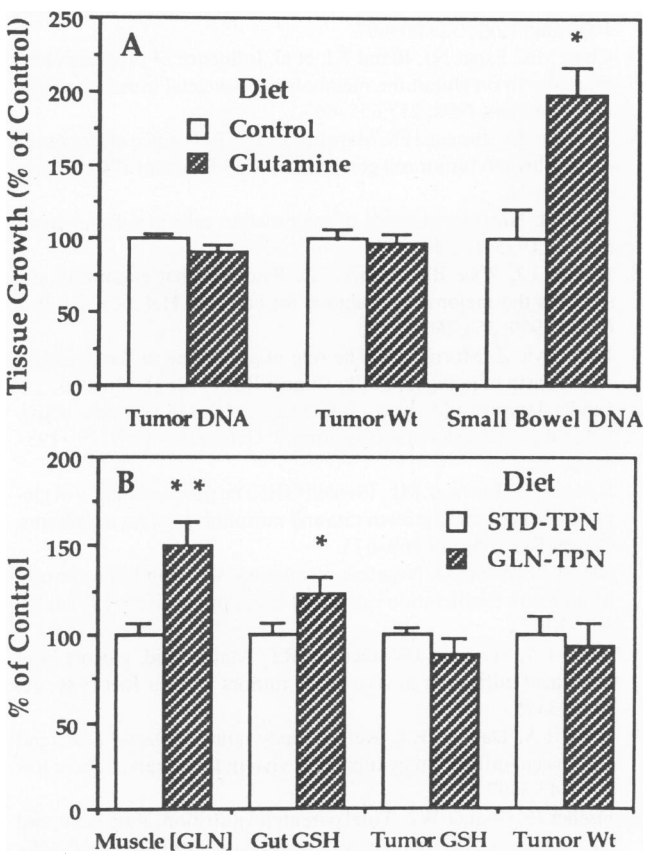

Figure 11. Effects of glutamine nutrition on host tissues and on tumor growth. (A) Effect of a glutamine-enriched oral diet on tumor growth and intestinal growth in rats implanted subcutaneously with a mammary adenocarcinoma (courtesy of Michael Torosian, M.D., Division of Surgical Oncology, Department of Surgery, University of Pennsylvania School of Medicine, unpublished data). (B) Effect of glutamine-enriched total parenteral nutrition on host and tumor (data adapted from Austgen et al.[55]).

GLUTAMINE NUTRITION AND LYMPHOCYTES

Glutamine is essential for lymphocyte proliferation, both as a precursor for nucleotide biosynthesis and as a major energy source.[22] Cell culture studies demonstrate that failure to supplement the culture media with glutamine impairs the ability of lymphocytes to respond to mitogenic stimulation.[67] In macrophages, glutamine may be required for the synthesis of messenger RNA for producing secretory proteins during immune challenge. The obvious implication of these studies is that the immunodeficiency associated with tumor growth and with antineoplastic therapies may, in part, be a metabolic phenomenon that is amenable to therapy with glutamine-containing nutritional regimens.

CLINICAL TRIALS

Studies in human volunteers[68] and in hospitalized patients[69] have failed to demonstrate any toxicity associated with glutamine-supplemented parenteral nutrition. Glutamine in solution undergoes hydrolysis to produce pyroglutamate within days, but this process can be slowed considerably by adjusting the pH and temperature of the solution. In the United States, the majority of studies evaluating the use of glutamine-supplemented diets have used free L-glutamine. In Europe, glutamine dipeptides have commonly been used, and this experience is reviewed elsewhere.[70–72]

The best study to date evaluating the effects of glutamine-enriched TPN in patients with cancer is a randomized, double-blind controlled trial.[73,74] The investigators studied 45 adults who had undergone allogeneic bone marrow transplants for hematologic malignancies. Patients received a standard, glutamine-free TPN solution or an experimental isonitrogenous, isocaloric solution supplemented with L-glutamine (0.57 g/kg/day). Patients received the diets for approximately 4 weeks after

Table 5. RESULTS OF A RANDOMIZED TRIAL OF GLUTAMINE-ENRICHED TOTAL PARENTERAL NUTRITION (GLN-TPN) VS. STANDARD TPN (STD-TPN) AFTER BONE MARROW TRANSPLANTATION*

Study Group	No. of Patients	Nitrogen Balance (g/day)	No. of Patients With Clinical Infections	No. of Patients Without Positive Cultures	Increase in ECW (L)†	Hospital Stay (days)
STD-TPN	21	−4.2 ± 1.2	9 (43%)	1 (5%)	3.2 ± 0.9	36 ± 2
GLN-TPN	25	−1.4 ± 0.5†	3 (12%)§	10 (42%)§	0.4 ± 0.9§	29 ± 1§

ECW = extracellular water.
* Modified from Scheltinga et al.[73] and Ziegler et al.[74]
† n = 10 patients per group.
‡ $p < 0.01$ *vs.* STD-TPN (unpaired t test or Fisher exact test).
§ $p < 0.05$ *vs.* STD-TPN (unpaired t test or Fisher exact test).

transplantation. The patients receiving glutamine-supplemented parenteral nutrition after this procedure had improved nitrogen balance, a diminished incidence of clinical infections, less fluid accumulation, and a shortened hospital stay (Table 5). These clinical improvements were consistent with a role for glutamine in stimulating protein synthesis in skeletal muscle,[75] supporting endothelial function and integrity,[76] and augmenting immune function.[21,22]

SUMMARY

Glutamine is the most versatile of all amino acids,[77] and it is essential for cellular proliferation, tumor growth, and tumor cell survival. If there are differences in the way that normal and malignant cells transport and metabolize glutamine, therapeutic strategies that selectively block glutamine use by malignant cells or enhance the effectiveness of antineoplastic therapies may become possible. Although animal models used to study host–tumor interactions do not extrapolate ideally to the clinical setting, they have provided important and useful information about glutamine metabolism and its regulation in malignant disease. These studies have also served as the basis for initiating the few studies in humans that have been published. There are few clinical trials evaluating the potential benefits of glutamine-enriched nutrition in patients with cancer, but they indicate that additional studies should be initiated. These reports also indicate that pharmacologic doses of glutamine are necessary to benefit the host, and thus, glutamine may be considered a drug and a nutrient (nutritional pharmacology).

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