

**Baxter**

June 10, 1998

Dockets Management Branch (HFA-305)  
Food and Drug Administration  
12420 Parklawn Drive, Room 1-23  
Rockville, MD 20857

483 '98 JUN 11 AM 10:28

**RE: Federal Register Notice April 7,1998 (FR Vol 63, Nbr 66, Pages 17011-17012)**

**Docket No. 98N-0182**

Dear Colleague:

Baxter Healthcare Corporation is nominating L-Glutamine as a bulk drug substance for inclusion on the list of bulk drug substances that may be used in pharmacy compounding that do not have a USP or NF monograph and are not components of approved drugs. Attachment 1 - Bulk Drug Substance Checklist includes the information requested in the notice.

We appreciate the opportunity to nominate this bulk drug substance for inclusion on the list for use in pharmacy compounding. If you have any questions regarding this nomination, please contact me.

Sincerely,

*Marcia Marconi*  
DEA

Marcia Marconi  
Vice President  
Regulatory Affairs  
(847) 270-4637  
(847) 270-4668 (FAX)

Enclosure

98N-0182

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**ATTACHMENT 1**

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ATTACHMENT 1

JUN 10 1998

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## BULK DRUG SUBSTANCE CHECKLIST

1. **Ingredient name:** L-Glutamine
2. **Chemical name:** 2-aminoglutaramic acid; glutamic acid 5-amide.
3. **Common name:** Glutamine
4. **Chemical grade or description of the strength, quality, and purity of the ingredient:** See Product Specification Sheets at the Tab entitled PRODUCT SPECS.
5. **Information about how the ingredient is supplied:** White crystals or crystalline powder.
6. **Information about recognition of the substance in foreign pharmacopoeias and the status of its registration in other countries, including whether information has been submitted to USP for consideration of monograph development:** This bulk drug substance has not been submitted to USP for consideration of monograph development. To the best of our knowledge, this bulk drug substance is not listed in the BP, EP or JP. This bulk drug substance is listed in the Food Chemicals Codex, Fourth Edition, effective July 1, 1996, page 174.
7. **A bibliography of available safety and efficacy data, including any relevant peer reviewed medical literature:**
  - a. McCauley R, Kong S, and Hall, J: Glutamine and Nucleotide Metabolism Within Enterocytes. JPEN 22:105-111, 1998
  - b. Fish, J, et al: A Prospective Randomized Study of Glutamine-Enriched Parenteral Compared with Enteral Feeding in Postoperative Patients. Am J Clin Nutr 65:977-983.1997
  - c. Palmer, T et al: Effect of Parenteral L-Glutamine on Muscle in the Very Severely Ill. Nutrition 12:316-320, 1996
  - d. Lacey, Jet al: The Effects of Glutamine-Supplemented Parenteral Nutrition in Premature Infants. JPEN 20:74-80, 1996
  - e. Hornsby-Lewis, Let al: L-Glutamine Supplementation in Home Total Parenteral Nutrition Patients: Stability, Safety, and Effects on Intestinal Absorption. JPEN 18:268-273, 1994
  - f. Khan, K et al: The Stability of L-Glutamine in Total Parenteral Nutrition Solutions. Clinical Nutrition 10:193-198, 1991

Copies of these articles are found after the Tab labeled ARTICLES.

8. **Information about the dosage form into which the drug substance will be compounded, including formulations:** See the Hornsby-Lewis article listed as 7.e above for information about the dosage forms.
9. **Information about the strength of the compounded product:** Diluted in a total parenteral nutritional (TPN) formula to about 0.2 to 0.3 grams per kilogram of patient weight and a final concentration of about 1 to 1.5%.
10. **Information about the anticipated route of administration of the compounded product:** Parenteral administration mixed in a total parenteral nutrition (TPN) formulation.
11. **Information about the past and proposed use of the compounded product, including the rationale for its use or why the compounded product, as opposed to a commercially available product, is necessary:** When the GI tract is not fed, as in TPN, the cells lining the gut begin to die off allowing bacterial translocation and subsequent septicemia. Glutamine is used to maintain the integrity of the gut barrier properties. No commercial y available product exists in the US.
12. **Available stability data for the compounded product:** Hornsby-Lewis et al reported 22 days stability at refrigerated temperatures (See article 7.e. above). Kahn reported 0.6 to 0.9% degradation per day at room temperature (See article 7.f. above).
13. **Additional relevant information:** The stable form glutamine dipeptide is available in Europe in commercial pharmaceutical products.

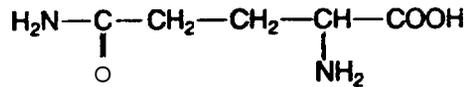
**PRODUCT SPECS**



# L-GLUTAMINE

CAS NO. 56-85-9

## MOLECULAR STRUCTURE AND FORMULA



$\text{C}_5\text{H}_{10}\text{N}_2\text{O}_3$  :146.15  
N : 19.17%

## DESCRIPTION

White crystals or crystalline powder  
odorless  
Slight taste

## SPECIFICATION AND PROCEDURE

State of solution (Transmittance)	More Than 98.0%	sec.-1 c = 10, 2N HCl
Specific rotation $[\alpha]_D^{20}$	+34.2 - +36.2°	Sat.-Z dry c = 2, 6N HCl
Chloride (Cl)	Not More Than 0.021 %	Sec.-4, 0.5 g 0.30 ml of 0.01N HCl
Sulfate (SO <sub>4</sub> )	Not More Than 0.028%	Sec.-5, 0.6 g 0.35 ml of 0.01NH <sub>2</sub> SO <sub>4</sub>
Iron (Fe)	Not More Than 10 ppm	Sec.-6(1), 2.0 g Incineration 2.0 ml of Standard solution
Heavy metals (as Pb)	Not More Than 10 ppm	Sec.-7-(1), 1.0 g 1.0 ml of Standard solution
Arsenic (As <sub>2</sub> O <sub>3</sub> )	Not More Than 1 ppm	SeC.*(1)-B, 1.0 g 1.0 ml of Standard solution
Loss on drying	Not More Than 0.20%	sec.-9-(1) 105Z, 3M
Residue on ignition	Not More Than 0.10%	sec.-10, 2 g
Purity (dry basis)	More-ha 98.5%	Sec.-n-(2), dry → 0.2 g + H <sub>2</sub> O 100 ml → Take 10 ml 0.01 N H <sub>2</sub> SO <sub>4</sub> 1 ml = 1.4615 mg C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub>
Foreign amino acids	Not More Than 1.0% (TLC, 30 μg)	Sec.-13, Solvent A Nin A, Standard (0.12 μg)
Pyrogen*	Free	Sec.-22, 1.0 g/100 ml

\* The pyrogen-free grade will be supplied on request

## IDENTIFICATION

- (1) To 5 ml of sample solution (1 → 50) add 5 drops of dilute hydrochloric acid and 1 ml of sodium nitrite solution; the colorless gas is evolved.  
(2) To 5 ml of sample solution (1 → 1000) add 1 ml of 2% ninhydrin solution and heat for 3 minutes; a purple color is produced.

JUN 10 1998

Spec From: Kyowa Hakko Kogyo Co., Ltd, Tokyo, Japan

5



# Glutamine, L-

## RTECS - Registry of Toxic Effects of Chemical Substances

### Document Outline

- 1.0 SUBSTANCE IDENTIFICATION
- 2.0 SYNONYM(S)/TRADENAME(S)
- 3.0 HEALTH HAZARD DATA
- 5.0 NIOSH DOCUMENTS
  - 0 STATUS IN U.S.

### 1 0 SUBSTANCE IDENTIFICATION

RTECS Number: MA2275100

Chemical Name: Glutamine, L-

CAS Number: 56-85-9

Beilstein Reference Number:

1. BRN 1723797
2. 4-04-00-03038 (Beilstein Handbook Reference)

Molecular Formula: C5-H10-N2-O3

Molecular Weight: 146.17

Wiswesser Notation: ZV2YZVQ

Substance Investigated as: Drug, Mutagen, Human Data

Last Revision Date: 1997

### 2.0 SYNONYM(S)/TRADENAME(S)

1. 2-Aminoglutaramic acid
2. Cebrogen
3. gamma-Glutamine
4. Glumin
5. Glutamic acid 5-amide

JUN 10 1998

6

6. Glutamic acid amide
7. Glutamine
8. L-2-Aminoglutarimidic acid
9. L-Glutamine (9CI)
10. Levoglutamid
11. Levoglutamide
12. Stimulina

### 3.0 HEALTH HAZARD DATAA

#### ACUTE TOXICITY

##### **TDLO/TCLO - LOWEST PUBLISHED TOXIC DOSE/CONC**

*Man*

**TDLo** - ROUTE: Oral; DOSE: 27 mg/kg/1W intermittent ☹

**TOXIC EFFECTS:**

*Behavioral* - Euphoria

##### **LD50/LC50 - LETHAL DOSE/CONC 50% KILL**

*Rat*

**LD50** - ROUTE: Oral; DOSE: 7500 mg/kg ☹

*Mouse*

**LD50** - ROUTE: Oral; DOSE: 21700 mg/kg ☹

#### GENETIC EFFECTS

##### **SISTER CHROMATID EXCHANGE**

*Human*

CELL TYPE: lymphocyte; DOSE: 10 mg/L ☹

#### OTHER MULTIPLE DOSE TOXICITY DATA

*Rat*

ROUTE: Oral; DOSE: 260 mg/kg/30D intermittent ☹

**TOXIC EFFECTS:**

*Behavioral* - Food intake (animal)

*Blood* - Changes in spleen

*Others* - Death

### 5.0 NIOSH DOCUMENTSA

National Occupational **Exposure** Survey 1983: Hazard Code **X4814**; Number of Industries 4;  
Total Number of Facilities 841; Number of Occupations **14**; Total Number of Employees 8491;  
Total Number of Female Employees 5700

JUN 10 1998

7

## 7.0 STATUS IN U.S.▲

EPA TSCA Section 8(b) CHEMICAL INVENTORY

ARTICLES A-F

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ARTICLE A

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## Review

# Glutamine and Nucleotide Metabolism Within Enterocytes

ROSALIE MCCAULEY, PHD; SUNG-EUN KONG, BSC; AND JOHN HALL, FRACS

From the University Department of Surgery, Royal Perth Hospital, Perth, Australia

**ABSTRACT.** Glutamine has an important role as a source of energy for enterocytes. However, it may also have a key role as a source of nitrogen for the synthesis of nucleotides. The relative contribution of *de novo* synthesis and salvage pathways seems to be affected by the position of enterocytes within the crypt-villus axis as well as the dietary intake of nucleic acids and glutamine. Nucleotides are especially important to enterocytes during intestinal development, maturation, and repair. Hence an understanding of nucleotide metabolism within enterocytes has important implications regarding both the composition and route of administration of nutrient solutions. Many

important questions remain unanswered, in particular: Does glutamine stimulate intestinal *de novo* pyrimidine synthesis via the action of carbamoyl phosphate synthetase I? Can *de novo* purine synthesis maintain intestinal purine pools in the absence of dietary nucleic acids? And, what are the specific effects of parenterally administered nucleotides on the metabolism and well-being of enterocytes? A greater understanding of these issues will lead to a more rational approach toward the nutritional modulation of gut dysfunction. (*Journal of Parenteral and Enteral Nutrition* 22:105-111, 1998)

The effects of glutamine (GLN) on the gut certainly are profound and have led to the suggestion that GLN may act as a "biological modifier" and a "pharmacconutrient." However, the mechanisms of action of GLN are poorly defined. It is of interest that, in the context of tumor growth, GLN may act via GLN-dependent proliferation genes.<sup>1</sup> It also should be noted that GLN and epidermal growth factor have a positive additive effect on enterocyte proliferation *in vivo*.<sup>2</sup>

The role of GLN as an important respiratory fuel for the rapidly dividing cells of the gut has been well documented. In contrast, less attention has been directed at the role of GLN as a source of nitrogen for the synthesis of nucleotides (NT). The catabolism of GLN within enterocytes yields nitrogen as well as energy, and the nitrogen derived from GLN can enter various biosynthetic pathways. The term "glutamine oxysynthesis" has been proposed to embrace such duality of action.<sup>3</sup>

In this review we explore one possible explanation for the trophic effect of GLN on enterocytes. It is postulated that GLN might provide enterocytes with both energy and nitrogen for the synthesis of NT. The initial sections detail the trophic effects of GLN and NT on the gut. This is followed by a review of the biochemical pathways leading to the incorporation of nitrogen derived from GLN into NT. We close with a discussion about the ability of GLN to promote NT synthesis within enterocytes.

## GLUTAMINE

GLN, a 5-carbon amino acid with 2 amino moieties, accounts for 30% to 35% of the amino acid nitrogen that is transported in the plasma. This role of GLN as a "nitrogen shuttle" helps to protect the body against the toxic effects of high circulating levels of ammonia. GLN also is important as a respiratory fuel. In autoperfused rat small intestine 57% of GLN carbon is oxidized to CO<sub>2</sub> and 32% of the total CO<sub>2</sub> released by the bowel is derived from GLN.<sup>4</sup> Because GLN is degraded extensively in the gut, this means that both GLN carbon and nitrogen are readily available for the synthesis of other amino acids, lactate, and NT. Complex interorgan exchange mechanisms usually result in a constant level of circulating GLN. However, during periods of catabolic stress the intracellular concentration of GLN declines by >50% and the plasma concentration falls by up to 30%.<sup>5</sup>

GLN is a major respiratory fuel for enterocytes. In 1987, Hwang et al<sup>6</sup> reported that the infusion of GLN-enriched solutions of parenteral nutrients resulted in an increase in the mucosal weight and DNA content of the small bowel. Later it was demonstrated that there is a dose-response relationship between the concentration of Mined GLN and the extent of jejunal atrophy.<sup>7</sup> An intake of at least 4 g/kg/d of GLN was required to reduce the gut atrophy associated with parenteral nutrition in rodents. This dosage is approximately 80% of the total daily protein requirement of the parenterally nourished rat.<sup>8</sup> A large number of laboratory studies have shown that there are functional advantages associated with GLN-enriched parenteral nutrition solutions, which include optimum maturation of the intestine during growth, increased adaptive hyperplasia after intestinal resection, increased disaccharidase activity within the unstirred mucous layer of the brush border, the healing of enterocolitis, and maintenance of the barrier function

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of the gut.<sup>3</sup> These findings have led to the development of stable solutions of GLN dipeptides such as alanyl-GLN.

Phosphate-dependent glutaminase catalyzes the rate-limiting step of GLN degradation in enterocytes. This reaction generates glutamate and ammonia (Fig. 1). Glutamate then can be converted into energy via the tricarboxylic acid cycle or be diverted into various biosynthetic pathways. The latter include the liberation of alanine (for gluconeogenesis) and the production of ornithine, citrulline, and proline. Glutaminase is a mitochondrial enzyme with a  $K_m$  for GLN of 2.2  $\mu\text{mol/L}$  and in the rat jejunum has a specific activity of 4.8  $\mu\text{mol/h/mg}$  protein.<sup>7</sup> These figures indicate that it is a very active enzyme that works at a maximal capacity even in the presence of low concentrations of GLN.

The extent to which supplements of GLN may be beneficial to patients is unclear. A number of clinical studies have suggested that GLN enhances nutritional status by promoting a positive nitrogen balance and conserving skeletal muscle.<sup>8</sup> For example, in patients undergoing elective cholecystectomy, alanyl-GLN-enriched total parenteral nutrition maintained the postoperative concentration of both free GLN and polyribosomes in skeletal muscle.<sup>10</sup> However, there is a lack of controlled clinical trials evaluating the potential benefits of providing GLN to catabolic patients.<sup>7</sup> It is of interest that, in an uncontrolled study, Wilmore's group<sup>12</sup> found that combined treatment with GLN, growth hormone, and a fiber-containing diet significantly increased protein absorption of patients with short-bowel syndrome.

We will now consider NT metabolism within enterocytes. This will provide a foundation for the later discussion that links the ammonia generated by the action of glutaminase with the synthesis of NT.

#### NT AND PROTEIN Metabolism

The nomenclature of NT is complex. NT consist of a purine or pyrimidine base, a pentose sugar, and one or more phosphate groups. Nucleosides (NS) can be viewed as subsets of NT inasmuch as they consist of a purine or pyrimidine base and a pentose sugar. It is important to appreciate that NS and NT can contain either ribose or deoxyribose pentose groups. NT with ribose groups are

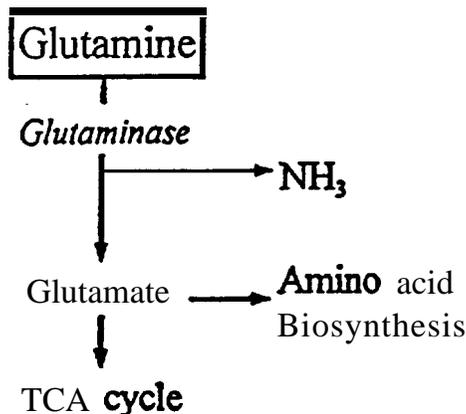


FIG. 1. Pathways of glutamine degradation within enterocytes.

building blocks for RNA, whereas NT with deoxyribose groups are building blocks for DNA. Their presence is especially important during intestinal development, maturation, and repair.

NT promote the growth and maturation of the developing gut. Uauy et al.<sup>3</sup> evaluated the effect of added NT (0.5% for 2 weeks) on gut growth and maturation in weanling rats. These supplements increased mucosal protein and DNA concentration in the jejunum by at least 50%, and this increase was accompanied by an enhancement of disaccharidase activity. NT also promote the healing of damaged gut in weanling rats.<sup>14</sup> The gut has the biochemical pathways to degrade dietary nucleic acids to either NS or purine and pyrimidine bases, and most dietary nucleic acids are absorbed as NS.<sup>15</sup> As cow's milk is lacking in NT content, whereas human milk is rich in cytosine and adenine derivatives, it has been suggested that infant formulas should be enriched with NT.<sup>15</sup>

Dietary deprivation of NT has an adverse effect on the gut. He et al.<sup>16</sup> reported that the addition of NT to enterocyte cultures promoted growth and proliferation. Furthermore, rats fed a NT-free diet have a reduced fractional rate of protein synthesis and a reduced concentration of DNA in the intestine.<sup>7</sup> Leleiko et al.<sup>18</sup> found a dramatic decrease in small intestinal total RNA after the removal of dietary purines and pyrimidines or the administration of 6-mercaptopurine (a purine antimetabolite). It was suggested that all messenger RNA (mRNA) species were not equally affected, and this suggests a potential mechanism by which dietary components may control the synthesis of specific proteins. Leleiko and Walsh<sup>19</sup> have discussed the evidence that the effects of purines may be mediated through regulatory proteins that bind to specific promoter regions genes involved in regulating intestinal growth.

Supplements of NT may help to preserve the structure and function of the gut during parenteral nutrition. Iijima et al.<sup>20</sup> found that rats who received parenteral nutrition enriched with the NS/NT mixture OG-VI (Otsuka Pharmaceutical Factory, Inc, Tokushima, Japan) had increased jejunal weight, DNA content, and crypt cell production rate. Although OG-VI contains precursors for the synthesis of both the purines and pyrimidines, adenosine monophosphate was not included because its administration has been associated with hypotension and bradycardia.<sup>21</sup> The OG-VI-enriched solution had a trophic effect on the intestine greater than the effect of 3.1 g GLN/kg body wt/d, but this dose of GLN is less than the 4.0 g GLN/kg body wt/d that is known to produce optimal changes in the lumen.<sup>7</sup>

Damaged gut requires NT. It has been reported that 4 days of NT/NS-enriched parenteral nutrition significantly improved healing of ulcers induced by indomethacin in rats.<sup>22</sup> Because the NT/NS increased crypt length, crypt-villus tie, and mitotic index, the authors suggested that the effect of NT/NS was related to an increase in the rate of proliferation.<sup>22</sup> A NT-enriched diet also promoted healing of intestine after lactose-induced diarrhea in rats.<sup>14</sup> The animals treated with NT had a greater villous height/crypt depth ratio and less intraepithelial lymphocytes than the rats derived of NT.

The need for the gut to have ready access to NT is relevant to the optimal formulation of enteral and

parenteral nutrients. Nutrient solutions should have the capacity to provide a source of NT to sustain adequate levels of nucleic acid synthesis. In the next section we describe how the gut can derive NT from either existing nucleic acids via salvage pathways or by *de novo* synthesis.

NT SYNTHESIS

Under normal circumstances, the gut metabolizes dietary NT via salvage pathways. However, in times of deprivation both pyrimidine and purines may be synthesized by *de novo* pathways. Figure 2 provides an overview of the biochemical pathways involved in the synthesis of NT. It is important to review this information because it provides a background for discussions about the interrelationships between the metabolism of NT and GLN.

Pyrimidines

Uracil, cytosine, and thymine are the pyrimidine bases commonly found in nucleic acids. The pyrimidine NS (uridine and cytidine) are formed by linkage of the pyrimidine bases to a ribose pentose sugar. The pyrimidine mono-, di- and triphosphates (UMP, UDP, UTP, CMP, CDP, and CTP) are phosphoric esters of the pyrimidine NS. Derivatives of thymine are only found in DNA, whereas derivatives of uracil are found only in RNA.

Carbamoyl phosphate is the substrate for the *de novo* synthesis of the pyrimidine NT (Fig. 3). It can be derived from GLN in the reaction catalyzed by carbamoyl phosphate synthetase II (CPS II) or from ammonia, CO<sub>2</sub>, and Mg adenosine triphosphate (ATP) in the reaction catalyzed by carbamoyl phosphate synthetase I (CPS I). Because ammonia can be generated from GLN in the reaction cata-

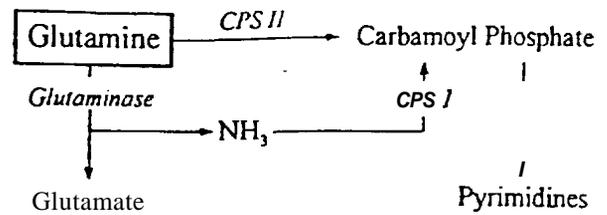


Fig. 3. Metabolism of glutamine nitrogen to pyrimidine nucleotide via carbamoyl phosphate synthetase I (CPS I) and carbamoyl phosphate synthetase II (CPS II).

lyzed by glutaminase, GLN-nitrogen can participate in both of the CPS reactions. CPS I is located in the mitochondria with other enzymes of urea synthesis, whereas CPS II is located within the cytosol as part of a multienzyme complex that contains two other enzymes from the *de novo* pathway. Tatibana and Shigesada<sup>23</sup> have suggested that the distribution of CPS I and CPS II within the cell is to separate the pathways for urea and pyrimidine synthesis.

Analyses of the complementary DNA of rat CPS I and CPS II suggest that they have arisen from the fusion of two ancestral genes: a glutaminase subunit and a synthetase subunit. The glutaminase domain is active in CPS II, enabling the use of GLN as a nitrogen donor. In contrast, the substitution of a swine residue for a cysteine residue in the glutaminase domain of CPS I has resulted in a loss of the ability to bind GLN. Instead, CPS I uses ammonia as the nitrogen donor and has acquired an N-acetyl-glutamate-binding region in the C-terminal half of the synthetase domain.

Jones<sup>24</sup> has suggested that the reaction catalyzed by CPS II is the rate-limiting step in the *de novo* synthesis of pyrimidines in the intestine. Although the intestine contains CPS I, its level of activity is low.<sup>25</sup> The activity of CPS II is regulated by the intracellular concentration of the positive effectors (ATP and 5-phosphoribosyl-1-pyrophosphate) and the inhibitors (UDP and UTP).<sup>27</sup> The final steps in *de novo* pyrimidine synthesis culminate in the formation of UMP, which is the building block for the formation of UDP, UTP, and CTP.

Some individuals lack the last two enzymes of *de novo* pyrimidine synthesis and suffer from orotic aciduria, which is associated with retarded growth and severe anemia. Treatment with cytidine or uridine reverses the anemia and reduces the excretion of orotic acid.<sup>28</sup> We are not aware of any studies that have investigated the jejunum of patients with orotic aciduria.

Pyrimidines can be formed from dietary nucleic acids by salvage pathways. These pathways are complex, as indicated by the fact that pyrimidine phosphoribosyltransferase can salvage both uracil and thymine. The intestine can also salvage appreciable uridine from blood via uridine phosphorylase and has a 20-fold greater activity of uridine phosphorylase than liver.<sup>29,30</sup> As a general comment, there is a lack of detailed knowledge about the specific role of other salvage enzymes.

The activity of pyrimidine phosphoribosyltransferase in the intestine is of great interest because it also converts the anticancer proagent 5'-deoxy-5-fluorouridine to its active form 5-fluorouracil. This reaction is inhib-

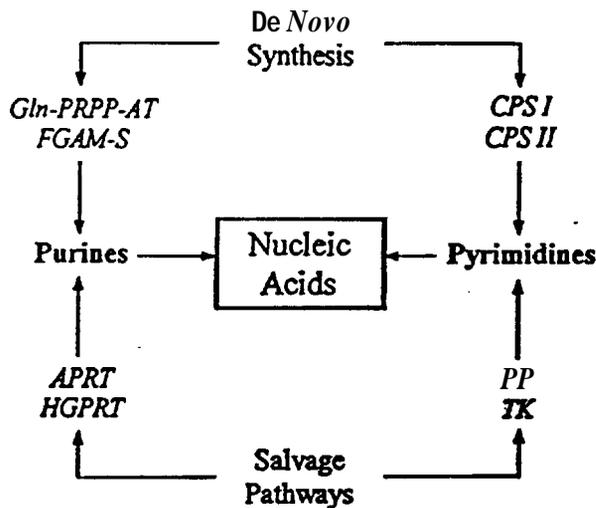


Fig. 2. Overview of nucleotide biosynthesis in enterocytes involving glutamine phosphoribosyl pyrophosphate aminotransferase (Gln-PRPP-AT), phosphoribosylformylglycinamide synthetase (FGAM-S), adenine phosphoribosyl transferase (APRT), hypoxanthine phosphoribosyl transferase (HGPRT), carbamoyl phosphate synthetase I (CPS I), carbamoyl phosphate synthetase II (CPS II), pyrimidine phosphoribosyltransferase (PP), and thymidine kinase (TK).

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ited within the intestinal mucosa by uracil, uridine, and thymine.<sup>31</sup> This observation has implications for the nutrition support of patients receiving anticancer agents. The circadian pattern of thymidine kinase, an enzyme localized to intestinal crypts, and other purine salvage enzymes may explain the observed circadian variation in the toxicity of anticancer drugs.<sup>34</sup>

### Purines

Adenine and guanine are the purine bases commonly found in nucleic acids. The purine NS (adenosine and guanosine) are formed by linkage of the purine bases to a ribose pentose sugar. The purine mono-, di-, and triphosphates (AMP, ADP, ATP, GMP, GDP, and GTP) are phosphoric esters of the purine NS. The derivatives of adenine and guanine are found in both DNA and RNA.

The rate-limiting step of *de novo* purine synthesis is catalyzed by glutamine phosphoribosyl pyrophosphate amidotransferase (Gln-PRPP-AT). The activity of this enzyme is regulated by the intracellular concentration of 5-phosphoribosyl-1-pyrophosphate, AMP, and GMP.<sup>34</sup> A further nine reactions lead to the synthesis of inosine monophosphate (IMP), which can be converted to the other purines (AMP, XMP, and GMP). There are two irreversible reactions in the *de novo* synthesis of purines that involve GLN as a substrate: the rate-limiting step involving Gln-PRPP-AT and the step catalyzed by phosphoribosylformylglycinamide synthetase (FGAM-S).

The capacity of the intestine for *de novo* purine synthesis is equivocal. The early work of Savaiano and Clifford<sup>35</sup> found that rat intestinal cells did not incorporate [<sup>14</sup>C]glycine into adenine and guanine and concluded that intestinal cells lack *de novo* purine synthesis. However, Boza et al<sup>36</sup> recently have challenged the idea that the intestine is not capable of *de novo* purine synthesis. By using [<sup>14</sup>C]-labeled amino acids and gas chromatography-mass spectrometry, it was found that the intestine of pregnant mice derived 92% of RNA-bound purines from *de novo* synthesis. Because there also are contradictory reports about intestinal Gln-PRPP-AT and FGAM-S activity,<sup>37-39</sup> the extent that the gut can generate purines by *de novo* synthesis has yet to be resolved.

The main purine salvage enzymes are hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and adenine phosphoribosyl transferase (APRT). Both of these enzymes are active in intestine,<sup>37</sup> but feeding a purine-free diet caused a 5-fold reduction in the expression of HGPRT and a 10-fold reduction in the expression of APRT mRNA.<sup>38</sup> In accord with these results, Walsh et al<sup>40</sup> found that a purine-free diet caused a 4-fold reduction in the transcription rate of the *HGPRT* gene and were able to identify a regulatory element within the *HGPRT* gene. They postulated that intake of purines would stimulate the synthesis of specific proteins that would bind to and promote the transcription of the *HGPRT* gene.

Purine NS phosphorylase also is a purine salvage enzyme. It catalyzes the formation of inosine and guanosine from hypoxanthine and guanine, respectively. It is of interest that purine NS phosphorylase is an indicator of preservation injury during storage of the donor organ before small bowel transplantation.<sup>41</sup> Purine NS phosphorylase activities in luminal effluents correlated with the duration of preservation time and predicted graft survival.

### NT SYNTHESIS ALONG THE CRYPT-VILLUS AXIS

Cell position within the crypt-villus axis affects NT biosynthesis in enterocytes. Chwalinski and Potten<sup>42</sup> observed that the ability of enterocytes to incorporate [<sup>3</sup>H]thymidine via the salvage pathway decreased as the cells moved from the crypts toward the villus tips. In a more detailed study, Uddin et al<sup>43</sup> used [<sup>3</sup>H]orotic acid and [<sup>3</sup>H]uridine to examine the relative rate and localization of *de novo* and salvage synthesis of pyrimidines in the rat duodenum. Figure 4 has been constructed by using data presented in that study.<sup>43</sup> It demonstrates that in the crypts the cells mainly incorporated uridine via the salvage pathway, whereas the villus cells mainly incorporated orotic acid via the *de novo* pathway. These observations were confirmed by Bissonnette.<sup>44</sup>

### RELATIVE USAGE OF THE *DE NOVO* AND SALVAGE PATHWAYS

The relative contribution of *de novo* synthesis and salvage pathways to intestinal NT pools has significance with regard to the appropriate composition of nutrition support regimens. We are aware of only one study that examined the relative contribution of the two pathways to intestinal pyrimidine pools. Zaharaevitz et al<sup>45</sup> found that *de novo* synthesis made a twofold greater contribution to intestinal NT pools than the salvage pathways. This was in contrast to liver, where salvage pathways made the greater contribution. The relative rates of *de novo* synthesis and salvage pathways in intestine would imply that intestine may be able to maintain pyrimidine pools in the absence of dietary nucleic acids.

Even less is known about the relative rates of *de novo* and salvage purine synthesis in the intestine. However, as previously discussed, rapidly dividing crypt cells are dependent on dietary nucleic acids to maintain NT pools. Thus, feeding a nucleic-acid free diet may impair the proliferative capacity of the crypt cells and thereby reduce villous height and absorptive capacity. This could account, in part, for the atrophic changes observed in the gut of animals fed nucleic acid-free diets.<sup>7</sup>

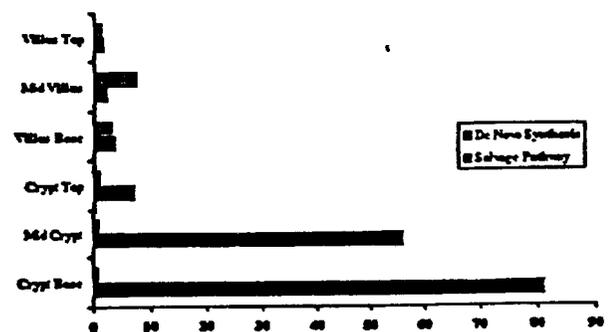


FIG. 4. Relative contribution of *de novo* synthesis and salvage pathways to RNA pools in enterocytes in different positions along the crypt-villus axis. Constructed from data presented by Uddin et al.<sup>43</sup>

## THE RELATIONSHIPS BETWEEN GLUTAMINE AND NT METABOLISM

in this section we build upon the biochemical pathways linking GLN and NT metabolism by presenting evidence in support of the concept that GLN supplements might stimulate the rate of synthesis of NT within enterocytes.

"Radioisotopes cannot be used to study the fate of nitrogen from GLN because  $^{15}\text{N}$  has a half-life of only 10 minutes. In contrast,  $^{14}\text{C}$ , which has an exceedingly long half-life has been used extensively to study the role of carbon as a fuel.<sup>46</sup> Stable isotopes such as  $^{15}\text{N}$  and  $^{13}\text{C}$ , which can be detected by mass spectrometry are an alternative to radioisotopes. For example,  $^{15}\text{N}$ -GLN has been used to trace the fate of enteral GLN in humans. Although this work showed that 54% of enteral GLN was sequestered within the splanchnic bed, the amount of GLN-nitrogen metabolized to NT within enterocytes was not studied."

Windmueller<sup>45</sup> studied the flux of metabolites across isolated segments of rat intestine and concluded that "approximately 80% of the ammonia generated by glutaminase is released into the portal vein, contributing importantly to the large ammonia flux between intestine and liver that also includes ammonia generated by the intestinal microflora. The remaining ammonia apparently is converted to carbamoyl phosphate." This seems to be appropriate because it has been established that carbamoyl phosphate is a substrate for the synthesis of the pyrimidines (Fig. 2). The structure of GLN metabolism within enterocytes means that as GLN is oxidized to release energy, there is a coincident release of nitrogen that can be synthesized into NT. This arrangement is an example of the coupling of a biosynthetic pathway (NT synthesis) to a catabolic pathway (degradation of GLN), and it allows for the precise control of the rate of biosynthesis of important end-products.<sup>49</sup>

The liver is adapted to cope with large amounts of ammonia. It has a highest level of CPS I activity of any tissue and also has a high content of *N*-acetylglutamate,<sup>23</sup> a positive effector of CPS I.<sup>40,51</sup> Thus liver is able to synthesize excess ammonia rapidly into carbamoyl phosphate, which can then be metabolized to urea. One consequence of this adaptation is that carbamoyl phosphate generated by CPS I can contribute to *de novo* pyrimidine synthesis. This arises because the mitochondrial membrane is permeable to carbamoyl phosphate,<sup>52</sup> and carbamoyl phosphate generated in the mitochondria by CPS I can leak into the cytosol and stimulate pyrimidine synthesis (Fig. 6). Hence mitochondrial carbamoyl phosphate can stimulate hepatic pyrimidine synthesis when there is excess ammonia<sup>53,54</sup> or GLN.<sup>55</sup> The effect of an increase in pyrimidine synthesis within hepatocytes on the rate of hepatocyte proliferation has yet to be defined clearly.

The intestine also has CPS I activity and also is exposed to large amounts of ammonia,<sup>56</sup> but because the activity of CPS I in liver is about 28 times that of the intestine, it seems unlikely that excess ammonia or GLN would stimulate intestinal pyrimidine synthesis. However, like the liver, intestine has a high content of *N*-acetylglutamate,<sup>23</sup> the allosteric activator of CPS I, and, although CPS I is less active in intestine than liver, the intestine has a relatively high content of CPS I mRNA. This high CPS I mRNA content might enable the gut to

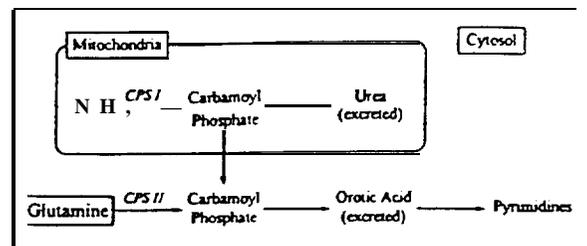


Fig. 5. Carbamoyl Phosphate generated in the mitochondria by carbamoyl phosphate synthetase I (CPS I) may leak into the cytosol and stimulate pyrimidine synthesis. The activity of cytosolic carbamoyl phosphate synthetase II (CPS II) may not always be the rate-limiting step of pyrimidine synthesis.

respond rapidly to any change in concentration of ammonia.<sup>56</sup> In support of this observation, in mice, a 1-hour infusion of  $^{15}\text{NH}_4\text{Cl}$  induced an increase in *de novo* pyrimidine synthesis in the liver (fourfold) and intestine (twofold).<sup>57</sup> Further research is necessary to determine whether GLN can stimulate pyrimidine synthesis within enterocytes. A positive result might help to explain why GLN can have atrophic effect on the jejunum.

There also are unanswered questions about *de novo* purine synthesis within the intestine. On one hand there are reports that intestine has very low activity of Gln-PRPP-AT and FGAM-S<sup>57,58</sup> and is not capable of *de novo* purine synthesis<sup>57</sup>; on the other hand, there are reports that the intestine has appreciable Gln-PRPP-AT<sup>59</sup> activity and that the *de novo* pathway makes a larger contribution than the salvage pathways to the intestinal purine pools.<sup>56</sup> Thus it has yet to be demonstrated that *de novo* synthesis alone can maintain purine pools within the intestine.

After small bowel resection there is an increase in nucleic acid synthesis and cell proliferation in the remaining small intestine. The activity of both glutaminase<sup>60</sup> and enzymes involved in *de novo* and salvage pyrimidine synthesis are increased after small bowel resection.<sup>60</sup> Studies evaluating the effect of GLN on gut adaptation in experimental animals have produced contradictory results.<sup>60-62</sup> It is impossible that the effect of GLN supplements on gut adaptation may be dependent on the route of supply. A key area for further research will be the effect of parenteral and enteral NT supplements on gut adaptation after massive small bowel resection.

In conclusion, our review has stressed the linkage between GLN and NT metabolism within enterocytes. A number of questions remain unanswered. Does GLN stimulate intestinal *de novo* pyrimidine synthesis via the action of carbamoyl phosphate synthetase I? Can *de novo* purine synthesis maintain intestinal purine pools in the absence of dietary nucleic acids? And, what are the specific effects of parenterally administered NT on the metabolism and well-being of enterocytes? A greater understanding of these issues will lead to a more rational approach toward the nutritional modulation of gut dysfunction.

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**ARTICLE B**

# A prospective randomized study of glutamine-enriched parenteral compared with enteral feeding in postoperative patients<sup>1-4</sup>

Judith Fish, George Sporay, Karen Beyer, John Jones, Todd Kihara, Alfred Kennedy, Caroline Apovian, and Gordon L Jensen

**ABSTRACT** Plasma amino acids were measured in 17 postoperative subjects randomly assigned to receive for  $\geq 5$  d tube feeding or total parenteral nutrition (TPN) that had identical energy, nitrogen, and glutamine contents. Subjects required gastric or pancreatic surgery for malignancy and were well-matched for age and body mass index. Tube feeding or TPN began on postoperative day 1 and advanced in daily 25% increments to meet goals of  $105 \text{ kJ} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$ ,  $1.5 \text{ g protein} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$ , and  $0.3 \text{ g glutamine} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$ . Delivered energy, nitrogen, and glutamine were closely matched on day 4. Nitrogen balance and plasma proteins did not differ significantly between feeding groups. Total indispensable amino acids, branched-chain amino acids, and glutamine declined 25% on postoperative day 1 compared with preoperative values. Indispensable and branched-chain amino acid concentrations were restored with 5 d of either tube feeding or TPN. Glutamine concentrations did not differ significantly by feeding group, though a trend suggested that glutamine recovered more slowly in the tube-fed than in the TPN-fed subjects. Plasma amino acids otherwise reflected formula composition with ratios of valine to leucine of 1.24 and  $3.69 \mu\text{mol/L}$  in subjects receiving 5 d of tube feeding or TPN, respectively. These findings suggest that glutamine-enriched tube feeding and TPN can result in similar profiles for most plasma amino acids at carefully matched doses. *Am J Clin Nutr* 1997;55:977-83.

**KEY WORDS** Glutamine, tube feeding, total parenteral nutrition, TPN, enteral nutrition, postoperative patients, amino acids, humans

## INTRODUCTION

Glutamine is the most abundant amino acid in plasma and skeletal muscle (1-3) and was recently recognized as a conditionally essential amino acid. Although glutamine is usually adequately synthesized in the body, plasma and cellular concentrations fall rapidly after injury or surgery (4). Glutamine has many functions, which may increase the demand for glutamine in catabolic states. It serves as the preferred oxidative substrate for enterocytes and may have a vital role in the maintenance of intestinal integrity and function (5, 6). It is also a nucleotide precursor (7) and may regulate protein turnover by shuttling nitrogen between skeletal muscle and visceral organs (8). The gastrointestinal tract has markedly increased glu-

tamine consumption in the stressed state and may, therefore, play a sole role in modulating the protein catabolic response to injury (8, 9). Traditional nutrition support therapies contain very little glutamine because of its unrecognized requirement and its poor stability in solution. Glutamine can be successfully supplemented in parenteral solutions (10-12) or tube-feeding formulas (13). Both modes of nutrition support appear to be well tolerated, but it is unclear whether enteral glutamine is metabolized differently than parenteral glutamine. We hypothesized that there would be an appreciable "first-pass" clearance, with enteral glutamine first metabolized by the gastrointestinal tract and liver before reaching systemic general circulation. This study was designed to compare plasma amino acid profiles in postoperative patients randomly assigned to receive parenteral or enteral nutrition supplemented with glutamine.

## SUBJECTS AND METHODS

### Subjects

Patients between 18 and 75 y of age scheduled for elective upper gastrointestinal surgery were eligible for study. Patients were candidates for postoperative nutrition support and were expected to require nutrition support for  $\geq 5$  d. Exclusion criteria were as follows: insulin-dependent diabetes, renal disease (creatinine concentration  $> 221 \mu\text{mol/L}$ , or  $2.5 \text{ mg/dL}$ ), hepatic disease (total bilirubin concentration  $> 51 \mu\text{mol/L}$ , or  $3 \text{ mg/dL}$ ), immunodeficiency, conditions precluding use of enteral feeding (eg, bowel obstruction or pancreatitis), chronic steroid use, cardiac disease (class III or IV, New York State Heart Association), Glasgow Coma Scale  $< 5$  (14), chronic obstructive pulmonary disease [partial pressure of carbon dioxide ( $\text{PCO}_2$ )  $> 375 \text{ kPa}$ , or  $50 \text{ mm Hg}$ ], metastatic carcinoma, and pregnancy.

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This investigation was approved by the Geisinger Institutional Research Review Board with informed consent by standard protocol. Twenty consenting subjects were randomly assigned preoperatively by a computer-generated random sequence to receive glutamine-enriched enteral or parenteral feeding postoperatively.

#### Nutrition support

Nasogastric postpyloric feeding tubes (8 Fr Frederick-Miller; Cook Company, Bloomington, IN) were placed during surgery and positioned in the jejunum to the ligament of Treitz (15). Central venous access was also obtained.

Tube-feeding and total parenteral nutrition (TPN) formulas were closely matched for energy, protein, nitrogen, and glutamine (Table 1). The measured glutamine content was 113.64  $\mu\text{mol/L}$  in the TPN formula and 109.10  $\mu\text{mol/L}$  in the tube-feeding formula (Table 2). The tube-feeding formula was elemental (Vivonex Plus; Sandoz Nutrition, Minneapolis). The TPN formula was composed of an amino acid solution formulated by combining a commercially available formulation (Ren Amin; Baxter Health Care Corp, Glendale, CA) with free L-glutamine (Ajinomoto USA Inc, Teaneck, NJ). The procedure for glutamine-enriched TPN formulation was described previously (11). TPN solutions were compounded daily as a 3-in-1 admixture. Standard electrolyte, multivitamin, mineral, and trace element solutions were used (Astra, Westboro, MA).

Tube feeding or TPN was initiated at full strength. Either mode of nutrition support was begun at 1800 on day 1 post-surgery and advanced in identical increments on the basis of goal energy requirements of 105  $\text{kJ} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$  (Table 3). Goal nutrition also provided 1.5 g protein  $\cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$  and 0.3 g glutamine  $\cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$ . Nutrition support was continued for 10 d or until the subject was able to consume an oral diet other than clear liquids. Tube-feeding tolerance was monitored with daily recording of nausea, distention, or diarrhea. Subjects were also monitored for complications associated with central venous access (catheter infection, site infection, and venous occlusion). A minimum feeding threshold of 4184 kJ/d by the fourth postoperative day was

established for final data review so that included study subjects would have received adequate enteral feeding for analyses.

#### Laboratory analyses

Venous plasma amino acid concentrations were measured at baseline on days 0, 1, 5, and 10 (Table 3). Blood samples were collected into heparin-containing collection tubes from indwelling catheters at 1000 each sample day. Tube feeding and TPN were stopped 30 min before sampling. Plasma samples were stored at  $-70^{\circ}\text{C}$ . Reference values were determined from analyses of venous plasma samples from four healthy fasting adults.

Plasma was deproteinized by using membrane filtration (Centrifree; Amicon, Beverly, MA) and supplemented with the internal standard S-2-aminoethyl-L-cysteine. The amino acids were separated by ion exchange with a three-boffer lithium citrate system on a Beckman 7300 amino acid analyzer (Somerset, NJ). Postcolumn derivatization with ninhydrin was followed by spectrophotometric detection at 440 and 570 nm. Systems Gold Software was used for peak identification by retention time and for peak integration (16, 17).

Other standard laboratory procedures included the measurement of serum sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, and glucose and complete blood counts on days 0, 1, 3, 5, 7, and 10. Concentrations of magnesium, phosphorus, ionized calcium, albumin, transferrin, and prealbumin were determined and liver function tests were conducted on days 0.5 and 10.

Nitrogen balance was measured on days 1, 4, and 10. Total urine urea nitrogen was determined with a nitrogen analyzer (Antek, Houston) (18) from 24-h urine samples. Nitrogen balance was calculated as follows: [tube-feeding protein intake (g)/6.28] - [total urea nitrogen (g) + 2], or SS [TPN protein intake (gY6.13)] - (total urine urea nitrogen (g) + 2).

Indirect calorimetry was performed on days 1, 5, and 10 postoperatively with the Metascope metabolic cart (Cyber-med, Louisville, Co). Steady state measurements were made over  $\geq 10$  min with ongoing feeding. Measures were not attempted in uncooperative or medically unstable patients, nor in those who maintained  $> 60\%$  inspired oxygen or who were using a continuous positive airway pressure mask.

#### Statistical methods

Continuous data are summarized as means  $\pm$  SEs. Two-sample *t* tests were used to compare patients randomly assigned to the two feeding groups with respect to body mass index (BMI), age at study entry, and nutrients received on day 4. The balance of sex between the two feeding groups was tested with Fisher's exact test. A multivariate analysis of variance (MANOVA) model was fitted to the day-0 and day-5 data for albumin, transferrin, cholesterol, and total lymphocyte count. Within each model, tests for feeding group-by-time interaction, feeding group differences at baseline, and time differences within feeding group were performed. MANOVA was also used to evaluate feeding group, time, and feeding group-by-time interaction effects on each set of acid concentrations measured on days 0, 1, and 5. Significant interaction effects were interpreted to take precedence over corresponding main effects of either time or feeding group. All tests were two sided and a *P* value  $< 0.5$  was considered

TABLE 1  
Composition of tube feeding and total parenteral nutrition (TPN) formulas

Composition	Tube feeding <sup>1</sup>	TPN <sup>2</sup>
Energy (kJ/L)	4300	4300
Energy distribution		
(% amino acids)	18	18
(% fat)	6	6
(% carbohydrate, dextrose)	76	76
Nonprotein energy/nitrogen	115:1	112:1
Total glutamine (g/L)	10.00	10.58
Total nitrogen (g/L)	7.13	7.33

<sup>1</sup> Vivonex Plus (Sandoz, Minneapolis), an elemental formula with amino acids, soybean oil, maltodextrin, modified cornstarch, and standard multivitamins and minerals.

<sup>2</sup> 6.5% Ren Amin (Baxter, Glendale, CA), L-glutamine (Ajinomoto, Teaneck, NJ), 20% Liposyn III (Abbott, Abbott Park, IL), 70% dextrose (Abbott), and standard multivitamins and minerals (Astra, Westboro, MA).

TABLE 2  
Plasma amino acid concentrations on days 0, 1, and 5\*

Amino acid ( $\mu\text{mol/L}$ ) and type Of feeding <sup>a</sup>	Plasma			Formula $\mu\text{mol/L}$
	Day 0	Day 1	Day 5	
<b>Methionine<sup>1</sup> (34 <math>\pm</math> 3)</b>				
TF	33 $\pm$ 11 <sup>b</sup>	28 $\pm$ 6	40 $\pm$ 6	28500
TPN	21 $\pm$ 10	25 $\pm$ 8	56 $\pm$ 15	31 409
<b>Glutamine<sup>3</sup> (490 <math>\pm</math> S9)</b>				
TF	525 $\pm$ 206	387 $\pm$ 156	389 $\pm$ 160	109 010
TPN	5622135	413 $\pm$ 125	474 $\pm$ 179	113 641
<b>Leucine<sup>1</sup> (129 <math>\pm</math> 12)</b>				
TF	136 $\pm$ 52	98 $\pm$ 17	171 $\pm$ 18	144409
TPN	111 $\pm$ 38	78 $\pm$ 17	108 $\pm$ 40	43566
<b>Valine<sup>1</sup> (216 <math>\pm</math> 14)</b>				
TF	195 $\pm$ 51	164 $\pm$ 26	212 $\pm$ 28	47 004
TPN	186 $\pm$ 54	151 $\pm$ 32	3% $\pm$ 141	61379
<b>Isoleucine<sup>5,6</sup> (68 <math>\pm</math> 9)</b>				
TF	79 $\pm$ 39	48 $\pm$ 16	84213	51 682
TPN	57 $\pm$ 20	32 $\pm$ 14	73 $\pm$ 25	345s1
<b>Arginine<sup>1</sup> (75 <math>\pm</math> 9)</b>				
TF	78 $\pm$ 33	45 $\pm$ 12	83 $\pm$ 28	so 939
TPN	77 $\pm$ 18	43213	74 $\pm$ 34	34822
<b>Taurine<sup>5,6</sup> (59 <math>\pm</math> 6)</b>				
TF	57 $\pm$ 7	49 $\pm$ 17	53 $\pm$ 23	3642
TPN	55 $\pm$ 16	37 $\pm$ 12	32 $\pm$ 10	ND
<b>Tyrosine<sup>5</sup> (58 <math>\pm</math> 5)</b>				
TF	69 $\pm$ 20	6629	61 $\pm$ 12	11 021
TPN	60 $\pm$ 20	56 $\pm$ 10	53 $\pm$ 15	1 894
<b>Asparagine<sup>1</sup> (62 <math>\pm</math> 11)</b>				
TF	41 $\pm$ 17	34 $\pm$ 7	24 $\pm$ 6	ND
TPN	44 $\pm$ 17	34 $\pm$ 12	21 $\pm$ 15	ND
<b>Aspartate<sup>1</sup> (7.0 <math>\pm</math> 0.7)</b>				
TF	11.1.272	6.9 $\pm$ 2.1	7.7 $\pm$ 2.9	12888
TPN	6.8 $\pm$ 3.4	6.723.7	3.4 $\pm$ 5.5	49
<b>Phenylalanine<sup>1</sup> (52 <math>\pm</math> 4)</b>				
TF	73 $\pm$ 34	67 $\pm$ 15	99 $\pm$ 34	39293
TPN	58220	56 $\pm$ 10	93 $\pm$ 34	29 221
<b>Lysine<sup>1</sup> (150 <math>\pm</math> 16)</b>				
TF	180 $\pm$ 52	139 $\pm$ 27	141238	33 607
TPN	204 $\pm$ 57	140 $\pm$ 27	155259	29436
<b>Citrulline<sup>1</sup> (28 <math>\pm</math> 1)</b>				
TF	22 $\pm$ 10	15 $\pm$ 6	19 $\pm$ 7	40
TPN	23 $\pm$ 11	13 $\pm$ 6	17 $\pm$ 9	ND
<b>Ornithine<sup>1</sup> (50 <math>\pm</math> 9)</b>				
TF	57 $\pm$ 26	43 $\pm$ 9	73 $\pm$ 16	105
TPN	62 $\pm$ 29	37 $\pm$ 12	63 $\pm$ 24	112
<b>Cystine (54 <math>\pm</math> 4)</b>				
TF	87 $\pm$ 45	54 $\pm$ 32	75 $\pm$ 34	ND
TPN	53 $\pm$ 27	47 $\pm$ 20	57 $\pm$ 52	ND
<b>Proline<sup>1</sup> (185 <math>\pm</math> 47)</b>				
TF	170 $\pm$ 73	115 $\pm$ 36	117 $\pm$ 26	16225
TPN	196 $\pm$ 68	136 $\pm$ 29	153 $\pm$ 75	w 501
<b>Alanine<sup>1</sup> (394 <math>\pm</math> 55)</b>				
TF	421 $\pm$ 258	321 $\pm$ 106	253 $\pm$ 53	9 047
TPN	433 $\pm$ 164	343 $\pm$ 109	294 $\pm$ 149	57 963
<b>Threonine<sup>1</sup> (12S <math>\pm</math> 21)</b>				
TF	114 $\pm$ 35	77 $\pm$ 19	1242 51	28 258
TPN	137 $\pm$ 43	85 $\pm$ 25	162 $\pm$ 51	29722
<b>Glycine<sup>1</sup> (271 <math>\pm</math> S3)</b>				
TF	247 $\pm$ 96	172 $\pm$ 42	139 $\pm$ 34	10372
TPN	266 $\pm$ 67	184 $\pm$ 51	170 $\pm$ 46	35 328
<b>Histidine (76 <math>\pm</math> 8)</b>				
TF	67*20	55 $\pm$ 13	48 $\pm$ 9	10 122
TPN	58 $\pm$ 15	46*35	55 $\pm$ 20	25716

TABLE 2  
Continued

Amino acid ( $\mu\text{mol/L}$ ) and type of feeding <sup>2</sup>	Plasma			Formula
	Day 0	Day 1	Day 5	
Serine <sup>3</sup> (126 $\pm$ 14)				
TF	105 $\pm$ 27	75 $\pm$ 18	69 $\pm$ 17	9147
TPN	109 $\pm$ 36	64 $\pm$ 9	70 $\pm$ 16	14774
Glutamate <sup>3</sup> (24 $\pm$ 6)				
TF	82 $\pm$ 33	82 $\pm$ so	81 $\pm$ 56	290
TPN	86 $\pm$ 45	53:28	94 $\pm$ 87	149
$\Sigma\text{BCAA}$ <sup>3</sup> (413 $\pm$ 34)				
TF	411 $\pm$ 134	309 $\pm$ 52	468 $\pm$ 5s	243095
TPN	354 $\pm$ 107	261255	577 $\pm$ 200	139856
$\Sigma\text{IAA}$ <sup>3</sup> (776 $\pm$ 57)				
TF	81 02248	621:94	872 $\pm$ 126	372753
TPN	774 $\pm$ 218	567:107	1043 $\pm$ 313	259650
Valine:leucine <sup>4</sup> (1.68 $\pm$ 0.08)				
TF	1.48 $\pm$ 0.22	1.68 $\pm$ 0.20	1.24:0.32	0.32
TPN	1.70 $\pm$ 0.19	1.98 $\pm$ 0.32	3.69 $\pm$ 0.60	1.42
Aspartate:asparagine <sup>5</sup> (0.12 $\pm$ 0.02)				
TF	034 $\pm$ 0.32	0.21 $\pm$ 0.07	0.37:0.26	ND
TPN	0.18 $\pm$ 0.12	0.21 $\pm$ 0.14	0.47:0.33	ND
Phenylalanine:tyrosine <sup>3</sup> (0.90 $\pm$ 0.05)				
TF	1.03 $\pm$ 0.33	1.01 $\pm$ 0.19	151:0.43	3.57
TPN	1.00 $\pm$ 0.32	1.0120.13	1.78 $\pm$ 0.46	1s.43
Glutamate:glutamine (0.05 $\pm$ 0.02)				
TF	0.18 $\pm$ 0.11	0.332032	0.29 $\pm$ 0.35	0.033
TPN	0.1720.13	0.13 $\pm$ 0.07	0.20 $\pm$ 0.16	0.001

<sup>1</sup> TF, tube feeding ( $n = 7$ ) on days 0-5; TPN, total parenteral nutrition ( $n = 10$ ) on days 0-5; BCAA, branched-chain amino acids; IAA, indispensable amino acids; ND, not detectable.

<sup>2</sup> Reference control values in parentheses ( $\bar{x} \pm \text{SEM}$ ).

<sup>3</sup> Significant feeding group-by-time interaction,  $P < 0.05$  (MANOVA).

<sup>4</sup>  $\bar{x} \pm \text{SEM}$ .

<sup>5</sup> Significant differences across time,  $P < 0.05$  (MANOVA).

● Significant differences between feeding groups,  $P < 0.05$  (MANOVA).

significant. SAS software (SAS Institute Inc. Cary, NC) was used for the analyses.

## RESULTS

Twenty patients were enrolled into the study; three subjects did not meet goal feeding by day 4: one subject lost tube-feeding access, one had tube feeding held because of high nasogastric drainage, and one subject had tube feeding stopped because of diarrhea. Of the remaining subjects for final data review, 7 received tube feeding and 10 received TPN. There were no significant differences between the two groups for age, sex, and BMI (Table 4). The two groups had similar diagnoses and surgical procedures. Both groups received energy, nitrogen, and total glutamine that did not differ significantly on day 4 (Table 5). Total urinary nitrogen, measured resting energy expenditure, and nitrogen balance on day 4 were also not significantly different between groups (Table 5). An inadequate number of subjects continued to require nutrition support at day 10 and, therefore, a comparison between groups was done only at the earlier time points.

Baseline concentrations of plasma proteins and cholesterol and total lymphocyte counts were not significantly different preoperatively between groups. Both groups had a significant drop in plasma proteins postoperatively (Table 6).

Baseline plasma amino acid profiles did not differ significantly between groups for any measured amino acid. All subjects had hypoaminoacidemia on postoperative day 1. Total indispensable amino acids, branched-chain amino acids, and glutamine declined 25% on postoperative day 1 compared with preoperative day 0 (Table 2). By day 5, plasma concentrations of most amino acids approached baseline values. There were significant differences for feeding group-by-time interactions for methionine, leucine, and valine and significant differences between feeding groups for isoleucine and taurine (Table 2). In each case, the plasma amino acid profile was consistent with the respective tube-feeding or TPN formula composition. Plasma from tube-fed and TPN-fed subjects was readily distinguished on the basis of ratios of valine to leucine on day 5 of 1.24 and 3.69  $\mu\text{mol/L}$ , respectively (Table 2). This ratio served to maximize the inherent differences in formula composition. Plasma glutamine did not differ significantly between groups. Both groups had a drop in plasma glutamine postoperatively and a rise by day 5. There was, however, a trend favoring a return to baseline glutamine concentrations by day 5 in only the TPN subjects, whereas concentrations in the tube-fed subjects on day 5 remained significantly different from those on day 0 (Figure 1). Glutamate, citrulline, arginine, aspartate, and alanine concentrations did not differ significantly by feeding group.

TABLE 3  
Experimental design<sup>1</sup>

Study day	Procedures	Laboratory analyses <sup>2</sup>
0	Enrollment	Venous plasma amino acids, proteins, electrolytes
1	Initiate TPN or tube feeding at 25% of estimated energy needs	Venous plasma amino acids, indirect calorimetry, TUN, electrolytes
2	Increase TPN or tube feeding to 50% of estimated energy needs	—
3	Increase TPN or tube feeding to 75% of estimated energy needs	Electrolytes
4	Increase TPN or tube feeding to 100% of estimated energy needs	TUN
5	100% TPN or tube feeding	Venous plasma amino acids, proteins, electrolytes, indirect calorimetry
7	100% TPN or tube feeding	Electrolytes
9	100% TPN or tube feeding	—
10	Completion	Venous plasma amino acids, indirect calorimetry, proteins, electrolytes, TUN

<sup>1</sup> TPN, total parenteral nutrition; TUN, total urea nitrogen.

<sup>2</sup> Proteins measured were albumin, transferrin, and prealbumin; electrolytes measured were sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, and glucose.

DISCUSSION

Many researchers have suggested possible benefits of either enteral or parenteral glutamine supplementation in stressed subjects. These benefits may differ because the enteral mode of supplementation may be associated with appreciable first-pass clearance of glutamine by the intestine and liver (5, 6, 9, 19-21). The objective of this research design was not to show superiority of enteral or parenteral nutrition, but rather to identify differences in the plasma amino acid profiles that might result from the two modes of glutamine supplementation.

Dechelotte et al (22) evaluated the absorption and metabolic effects of enterally administered glutamine using stable-isotope methods in healthy subjects. Plasma glutamine showed a dose-dependent increase. Byproducts of glutamine metabolism (plasma alanine, glutamate, citrulline, aspartate, and urea) also increased. On the basis of these results, they concluded that glutamine is effectively absorbed by the jejunum.

Dominique et al (23) evaluated glutamine metabolism in healthy adult men through use of stable-isotope methods with isonitrogenous and isoenergetic enteral and parenteral nutrition. They reported a decrease in protein breakdown, with enteral nutrition and a rise in the appearance rate of glutamine in both groups, which was significant only in the enterally fed group. In contrast with the findings of the present investigation,

the parenteral regimen provided no glutamine whereas enteral feeding contained glutamine in small peptides.

Peterson et al (24) described attenuation of the fall in albumin in abdominal trauma patients randomly assigned to enteral compared with parenteral nutrition that delivered comparable amounts of nitrogen and energy. It is difficult to relate their observation to the present study because the enteral and parenteral feedings were not matched for glutamine, the patient population was substantially different, and the difference in albumin was significant on day 10.

Pearlstone et al (25) compared the effects of enteral and parenteral feeding in malnourished cancer patients. They reported enhanced repletion of essential and total amino acid concentrations in those subjects receiving parenteral nutrition compared with those subjects receiving tube feeding or an oral diet. Although the enteral and parenteral formulas were designed to be matched for energy, they differed in nitrogen content, glutamine, and other amino acids. Because the enteral and parenteral feedings were not matched for these key constituents and were not advanced at the same rate, it was not possible to compare these observations with those from the present study.

TABLE 4  
Patient characteristics<sup>1</sup>

Characteristic	Tube feeding (n = 5 M, 2 F)	TPN (n = 5 M, 5 F)
Age (y)	61 ± 4 <sup>2</sup>	62 ± 3
BMI (kg/m <sup>2</sup> )	25.9 ± 1.6	25.9 ± 1.9
Diagnosis	Gastric or pancreatic malignancy	Gastric or pancreatic malignancy
Surgery	Gastric or pancreatic	Gastric or pancreatic

<sup>1</sup> There were no significant differences between groups. TPN, total parenteral nutrition.

<sup>2</sup>  $\bar{x} \pm$  SEM.

TABLE 5  
Nutrition measures on day 4<sup>1</sup>

Measure	Tube feeding	TPN
Glutamine intake (g/24 h)	16.1 ± 1.5 [7]	18.5 ± 0.8 [10]
Protein intake (g/24 h)	72.6 ± 6.8 [7]	78.7 ± 3.3 [10]
Nitrogen intake (g/24 h)	11.5 ± 1.1 [7]	12.8 ± 0.5 [10]
Total urinary nitrogen (g/24 h) <sup>2</sup>	9.7 ± 1.6 [6]	10.9 ± 1.2 [9]
Nitrogen balance (g/24 h) <sup>2</sup>	0.1 ± 1.3 [6]	-0.6 ± 1.1 [9]
Energy intake (kJ/24 h)	6732 ± 636 [7]	7322 ± 176 [10]
Measured REE (kJ/24 h)	8506 ± 498 [5]	7297 ± 586 [7]

<sup>1</sup>  $\bar{x} \pm$  SEM; n in brackets; there were no significant differences between groups. REE, resting energy expenditure; TPN, total parenteral nutrition.

<sup>2</sup> Day-1 values for the tube-feeding and TPN groups, respectively, were as follows: total urinary nitrogen, 8.8 ± 3.9 [5] and 9.4 ± 3.5 [10]; nitrogen balance, -7.5 ± 3.3 [5] and -8.1 ± 2.9 [10].

TABLE 6  
Laboratory assessment<sup>1</sup>

	Tube feeding	TPN
Albumin ( $\mu\text{mol/L}$ )		
Day 0	521.6 $\pm$ 43.5 [7]	463.7 $\pm$ 29.0 [10]
Day 5	391.2 $\pm$ 243.5 <sup>2</sup> [7]	391.2 $\pm$ 29.0 [10]
Transferrin ( $\mu\text{mol/L}$ )		
Day 0	29.2 $\pm$ 3 [6]	22.4 $\pm$ 2.4 [10]
Day 5	18.6 $\pm$ 2.4 <sup>2</sup> [7]	17.5 $\pm$ 2.0 <sup>2</sup> [10]
Prealbumin ( $\mu\text{mol/L}$ )		
Day 0	3.9 $\pm$ 0.6 [6]	3.5 $\pm$ 0.5 [10]
Day 5	1.7 $\pm$ 0.5 <sup>2</sup> [7]	2.2 $\pm$ 0.5 <sup>2</sup> [10]
Total lymphocyte count ( $10^9/\text{L}$ )		
Day 0	1.35 $\pm$ 0.15 [6]	13420.23 [9]
Day 5	0.897 $\pm$ 0.23 [6]	1.14 $\pm$ 0.18 [10]
Cholesterol (mmol/L)		
Day 0	3.8 $\pm$ 0.4 [7]	5.3 $\pm$ 0.8 [10]
Day 5	3.2 $\pm$ 0.4 [7]	4.0 $\pm$ 0.5 <sup>2</sup> [10]

<sup>1</sup>  $\bar{x} \pm \text{SEM}$ ; n in brackets. There were no significant differences between treatment groups.

<sup>2</sup> Significantly different from day-0 value within treatment group.  $P < 0.05$ .

Alverdy (26) fed a glutamine-enriched diet to animals and compared them with animals receiving an identical solution parenterally. They reported less mortality from methotrexate in the oral diet group than in the intravenously fed group. There was also less bacterial translocation to the spleen in the orally fed group than in the intravenously fed group. Although this study shows an outcome benefit to intraluminal feeding, it does not separate the effects of glutamine from those of other nutrients.

Our study was designed to carefully match enteral and parenteral feeding for energy, nitrogen, and glutamine. Usual practice is to begin parenteral nutrition at goal rates whereas enteral feedings are advanced to goal rates over several days. We chose to administer TPN at the same advancement schedule as that for enteral feeding so that differences in plasma amino acid profiles would not reflect differences in the amounts of administered nutrition. This design resulted in the administration of enteral and parenteral feedings that were not significantly different in amounts of energy, nitrogen, or glutamine. Both groups achieved zero nitrogen balance by day 4, despite manifesting the expected stress response postsurgery. There was a postoperative decline in plasma proteins, cholesterol, and amino acids but no significant difference between groups. The limitations of measuring nitrogen balance and these laboratory indexes at a single post-term follow-up in postoperative subjects must be emphasized because nitrogen balance and laboratory indexes may also be sensitive to perturbation by nonnutritional factors like fluid status, infection, and inflammation. Generalized hypoaminoacidemia and reduced glutamine concentrations have been described after injury in critically ill patients (13, 27). These changes likely result from the mobilization of body amino acids that are associated with acute injury response (19, 28).

By day 5 of feeding (both enteral and parenteral), most plasma amino acids were restored to baseline values. The differences in plasma amino acid concentrations between groups on day 5 reflected differences in their respective formula compositions. The ratio of valine to leucine differed

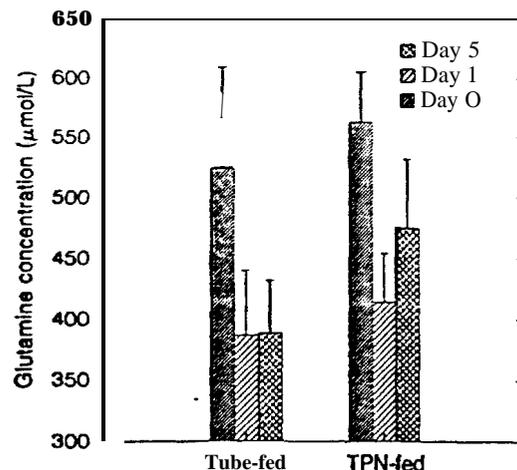


Figure 1. Mean ( $\pm \text{SEM}$ ) plasma glutamine concentrations on days 0, 1, and 5 in tube-fed (n = 7) and TPN (total parenteral nutrition)-fed (n = 10) subjects. Glutamine did not differ significantly between feeding groups, but concentrations on day 5 remained significantly different from day-0 values only in the tube-fed subjects (paired t test,  $P < 0.05$ ).

greatly in the parenteral and enteral formulas; therefore, the plasma ratios of these amino acids served to easily distinguish the formula received.

The small intestine is the primary site of glutamine uptake and metabolism in the body (5). In a stressed state, glutamine uptake by the intestine is accelerated. Studies in dogs have shown that postlaparotomy, glutamine consumption by the intestinal tract is increased by 75% (4). Enterocytes obtain glutamine by absorption across the brush border from the lumen and also from circulating glutamine. Enterocytes have high concentrations of glutaminase, which catalyzes the hydrolysis of glutamine to glutamate and ammonia. It appears that enterocytes metabolize glutamine similarly regardless of whether glutamine enters from the lumen or across the arterial blood (9). The end products of glutamine metabolism are released into portal circulation and extracted or metabolized by the liver before reaching systemic circulation (29).

It seems that enteral glutamine would produce a different circulating amino acid profile than would parenteral glutamine because of this first-pass metabolism. Only the TPN subjects exhibited a trend favoring restoration of plasma glutamine by day 5. We and other investigators have also observed little change in plasma glutamine concentrations with the enteral provision of glutamine at comparable dosing concentrations in either normal volunteers (11) or critically ill patients (13). Ziegler et al (10) reported a significant rise in plasma glutamine with parenteral glutamine administration. Because glutamine and products of glutamine metabolism did not differ significantly by feeding group in the present study, these observations will require further investigation.

Our study suggests that carefully matched parenteral and enteral supplementation will result in comparable profiles for most circulating amino acids. The multiple other sites of glutamine metabolism (skeletal muscle, kidney, and brain) must also have an influence on plasma amino acids (29). The position of the formula fed appears to be a key determinant of

plasma amino acid concentrations. Further studies with long-term feedings or different glutamine doses may give better insight into the potential differences between parenteral and enteral supplementation. It is possible that the provision of parenteral glutamine at 100% of the projected requirement from the first day would have resulted in higher plasma glutamine concentrations by the fifth day. Although plasma amino acid concentrations are useful in evaluating glutamine metabolism, intracellular glutamine metabolism is also an important aspect of evaluating the response to glutamine supplementation. Tissue biopsy, arteriovenous differences across extremities, and turnover studies were beyond the scope of the present study, but might serve to better define how glutamine supplementation affects amino acid metabolism and what dose of glutamine is optimal for patients in stressed states. □

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ARTICLE C

# Effect of Parenteral L-Glutamine on Muscle in the Very Severely Ill

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## ABSTRACT

Glutamine (Gln)-supplemented perioperative total parenteral nutrition (TPN) has been reported to reduce the loss of intramuscular glutamine following routine surgery. This study investigates whether glutamine-supplemented TPN alters muscle biochemistry acutely in the very severely ill patient. Thirty-eight patients (age 19-77 yr; mean 55 yr), critically ill (APACHE II range 8-31; median 17) admitted to the intensive care unit (ICU) were recruited to receive either conventional TPN (CTPN) or an isonitrogenous, isoenergetic feed supplemented with 25 g crystalline L-glutamine per 24 h (GTPN) in a prospective, double-blind, block-randomized study. In a representative sample of these patients, relatives consented to a paired muscle biopsy taken before feeding (10 GTPN/9 CTPN patients; ICU Day 2-4) and repeated 5 days later (16 patients; ICU Day 7-9). Muscle biopsies and matching plasma samples were analyzed using a coupled glutaminase-glutamate dehydrogenase enzymatic assay. A correction was made using sodium to account for the massive changes in extracellular fluid volume. The average muscle Gln content before feeding was very low. Between biopsies no consistent pattern of change was seen with or without exogenous Gln. It also proved difficult in these very sick patients to correct low plasma Gln with L-Gln-TPN during the initial phase of the severe illness. TPN supplementation with 25 g/24 h L-glutamine appears inadequate in the acute period. So counteract the muscle and plasma biochemical changes seen in these patients. It is unknown whether any larger dose could alter this state. Nutrition 1996; 12316-320

Key words: Glutamine, muscle, parenteral nutrition, critically-ill

## INTRODUCTION

L-Glutamine is classified as a nonessential amino acid in humans but at the cellular level there is the capacity for its synthesis. Dietary intake, together with endogenous production, is normally adequate to satisfy requirements. It is, however, a very important amino acid because it is the primary nitrogen donor in DNA synthesis at the cellular level and is also involved in interorgan ammonia transport and bicarbonate generation in the kidney. When metabolic demand for glutamine exceeds available supply, as is thought to occur in critical illness, a relative deficiency results. This may have adverse consequences. Many cell subpopulations require glutamine for both norms and stimulated cell division, e.g., enterocytes of the GI tract, white blood cells, and fibroblasts. Failure of such cellular populations, as might occur during substrate deficiency, might result in breakdown of gastrointestinal integrity, impaired immune responsiveness, and failure of wound healing. These are all clinical features seen in the most critically ill patients who are the subject of this study.

Muscle wasting in the critically ill is a severe clinical problem. Glutamine-enriched total parenteral nutrition (TPN) when given preemptively has been shown to result in an improvement in muscle biochemistry after a medium intensity surgical stress (open cholecystectomy). In contrast, this short report examines the effect of L-glutamine-supplemented TPN on muscle and plasma glutamine biochemistry in patients already very severely ill. This short paper does not report on other outcome measures that will require a larger cohort of patients.

## MATERIALS AND METHODS

Glutamine requirements in the critically ill are not known. At the time of study design, no other work in these very seriously ill patients had been published. Normal dietary intake contains ~4-8 g of L-glutamine, a variable proportion of which may be absorbed by the gastrointestinal tract. Early work by Furst and colleagues<sup>7</sup> suggested that after severe trauma, glutamine demand was markedly increased and suggested to be in

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excess of 20 g/24 h. A target dose of 25 g L-glutamine/24 h was chosen for this study on largely empirical grounds as a compromise between what might be clinically desirable and pharmaceutically practical. L-glutamine is absent from commercially available parenteral amino acid solutions due to perceived manufacturing and storage difficulties. Following the method of Hardy, Grimble, and McElroy,<sup>8</sup> two TPN all-in-one admixtures [glutamine enriched (GTPN) and conventional (CTPN)] were formulated for central venous administration, resulting in the administration of 15.5 g nitrogen and 2000 nonnitrogen calories (1:1 CHO to fat) per 24-h period. In addition, GTPN resulted in the administration of 25 g L-glutamine, with correspondingly less nitrogen from other amino acid sources (Table I). Even in GTPN the quantities of amino acid were adequate to satisfy recommended daily amounts.

This study was formally approved by the St. Helens & Knowsley Research Ethics Committee. After ICU admission and hemodynamic stabilization, a clinical decision as to the need for TPN support was made by the attending consultants. Provided exclusion criteria were absent (hepatic failure, nonresected malignant disease, pregnancy, or age under 16), the patient's relatives were approached for consent for study inclusion in a two-stage process. Consent was requested first for studying TPN administration and, second, in those patients without major disturbance of coagulation or symptomatic thrombocytopenia, for obtaining paired percutaneous muscle biopsies of the tibialis anterior muscle. A two-stage consent was necessary to satisfy ethical considerations in this highly stressful situation.

Patients were prospectively randomised using a double blind, sealed envelope, block-randomization technique. Block sizes were limited to six patients so that changes in general ICU therapeutic techniques would have equal effect on glutamine and control patients. Baseline plasma and muscle samples were taken before TPN administration and repeated 5 days later. Muscle biopsy of tibialis anterior was performed using the conchotome<sup>9</sup> technique and the sample immediately pre-

served in liquid nitrogen pending assay. The muscle sample was powder homogenized and an acid extract made using 2% perchloric acid and assayed for glutamine and glutamic acid using a coupled glutaminase-glutamate dehydrogenase assay.<sup>10</sup> Previous work within our department clearly showed detectable changes in muscle histology and biochemistry in this patient population over this short time period.<sup>11,12</sup> A longer interval was not selected so as to limit the influence of secondary clinical events, not primarily related to TPN administration.

In preparation for this work, the enzymatic glutamine-glutamate assay was optimized for the expected substrate concentrations in human muscle tissue based on results in the literature and our own preliminary work. All assays were performed in duplicate, together with known standard concentrations of both substances being assayed. These standards indicated a mean recovery of L-glutamine of 82.7 ± 9.8% (SD) and of L-glutamic acid of 86.2 ± 10.6% (SD). Statistically, the recovery fraction shows a normal distribution for both assays and does not show any drift over time. These results are in accordance with others who have used this assay technique.<sup>10</sup> Results presented in this paper have been corrected for variability revealed by standard assay.

Sodium content of the acid extract was assayed using a model 5000 Atomic Absorption spectrophotometer with air/acetylene flame (Perkin-Elmer, Beaconsfield, Bucks, UK) and used to correct assay results for changes in extracellular fluid volume using the method of Jackson.<sup>13</sup> Plasma samples were "snap frozen" in liquid nitrogen and deproteinized using perchloric acid before enzymatic assay.

Statistical comparisons were made using *t* test and Mann-Whitney *U* test as appropriate (ARCUS PRO-STAT V.3.0, Iain E. Buchan, University of Liverpool). Serial measurements were analyzed according to Matthews.<sup>14</sup> Measurements for each patient were summarized by a descriptive statistic (e.g., slope of measurement versus time). Summary values were then compared between groups using either parametric or nonparametric inferential statistics as appropriate. Results are indicated as mean or median ± 95% confidence intervals.

## RESULTS

Thirty-eight patients, age range 19-77 yr (mean 55 yr), fulfilled the study entry criteria, and consent was obtained. Diagnoses included perforated abdominal viscus, acute pancreatitis, blunt trauma, burns, etc. Although individual diagnoses may vary (Table U), all patients exhibited some degree of the systemic inflammatory response syndrome (SIRS)<sup>15</sup> and so can be considered to exhibit similarities with regard to their metabolic response. Illness severity according to the APACHE II score was similar between glutamine and control groups, indicating a successful randomization process (overall median APACHE II 17, range 8-31). TPN was commenced typically 3 days after ICU admission (median 3 days, interquartile range 2-4 days). There was no significant difference in duration of pre-TPN ICU stay between control and study groups.

Before TPN administration, glutamine levels were markedly depressed in both plasma (~54% of normal) and muscle (-19% of normal<sup>17</sup>; Table III). Glutamate levels were dramatically elevated in plasma (335%). Extracellular fluid volume expressed as a percentage by weight of the muscle biopsy specimen was 39% (95 ± 5% CI), suggesting a marked increase from normal values of ~20%<sup>18</sup> during the initial few days of critical illness.

No consistent changes in plasma glutamine levels were seen in the interval between biopsies (or equivalent timespan in the nonbiopsied patients). An increase in plasma glutamic acid levels was seen in both GTPN and CTPN patients, the magnitude of which was not significant between groups (Table IV)

TABLE I

CONSTITUENTS OF SUPPLEMENTED (GTPN) AND UNSUPPLEMENTED (CTPN) REGIMES		
	GTPN (d)	CTPN (ml)
2.5% L-glutamine solution (Sterile supply pharmacy, Whiston Hospital)	1000	
Eloamin 15 (Leopold Pharma & Oxford Nutrition UK)	500	
Eloamin 10 (Leopold Pharma & Oxford Nutrition UK)		1000
Sterile water		300
Glucose 50% (Baxter)	500	
Glucose 20% (Galen)	500	
Elo lipid 20% (Leopold Pharma & Oxford Nutrition UK)	500	

Each 24-h regime contains 3.1 L fluid, 15.5 g nitrogen, 2000 Kcal (1:1 CHO:fat). Electrolytes and trace minerals added as required. GTPN and CTPN = L-glutamine-supplemented and conventional total parenteral nutrition, respectively.

TABLE II  
DIAGNOSES PRECIPITATING ICU ADMISSION

Glutamine Patient Diagnosis	Ix	APC	Control Patient Diagnosis	Bx	APC
Smoke Inhalation, pneumonitis		1	Asthma, COAD		2
ARDS, pneumonia	Yes	8	Resp. failure, MOF		5
Septicaemia, ARF	Yes	23	Cardiac arrest, MOF		18
Septicaemia, MOF	Yes	23	Sepsis, ruptured bladder		23
Septic shock, MOF		23	Septicaemia, pyelonephritis	Yes	23
Septicaemia, heart failure		23	Sepsis, peritonitis, ARF	Yes	23
Septicaemia, pancreatic abscess		23	Peritonitis, perforated colon		23
Cholangitis, ARF		23	RTA multiple injury	Yes	25
Septicaemia, perforated colon	Yes	23	RTA, multiple injury		25
Burns 40%		24	Ca oesophagus resection		36
Fall, skull, vertebral & rib fractures		25	Perforated GU, MOF		37
RTA, lung contusion	Yes	25	Bowel obstruction, resection	Yes	37
Head injury, lung contusion	Yes	25	Rectal perforation, MOF	Yes	37
Status epilepsy, alcohol, pneumonia	Yes	32	Perforated GU	Yes	37
Seizure, retained products of conception	Yes	32	Perforated colon		37
Peritonitis		37	Bowel obstruction, resection	Yes	37
Perforated colon	Yes	37	Diaphragmatic hernia, head injury		50
Pancreatitis, ARF	Yes	53	Pancreatitis		53
			Pancreatitis	Yes	53
			MU acidosis, pneumonia ARF	Yes	55

ICU = intensive care unit Bx = patients who consented to have muscle biopsies; APC = Apache II diagnostic code; COAD = chronic obstructive airway disease; ARDS = acute respiratory distress syndrome; MOF = multiple organ failure; ARF = acute renal failure; RTA = road traffic accident; GU = gastric ulcer.

but was significantly different to zero, indicating a generalized increase in plasma glutamic acid levels regardless of the type of nutritional supplementation.

\* Muscle glutamine levels did not show a consistent pattern of change with either nutritional regime, although individual patients did show significant gains and losses (Fig. 1). Similarly, no significant trends for muscle glutamic acid or the proportion of the muscle samples taken up by extracellular fluid were detected over the timespan under study.

#### DISCUSSION

If the supply of glutamine in the diet is reduced, and the demand the same or increased, then the body must look for

alternative sources. The two available options are either an increase in synthesis, or mobilization of stored amino acid. L-Glutamine is synthesized de novo in humans within skeletal muscle, the liver, and the pulmonary tree. The lungs are able to increase synthesis during episodes of surgical stress<sup>18</sup> and subsequent sepsis.<sup>19</sup> However, pulmonary glutamine flux has not been evaluated in septic patients where that is evidence of pneumonia, ARDS, or other dysfunction. Similarly, whereas liver glutamine synthesis is increased during acidosis,<sup>20</sup> this is as a result of increased ammonia production and forms a scavenging pathway, as route to the kidney where the ammonia is excreted. A consistent finding in all studies of interorgan glutamine flux is a marked increase in glutamine release from

TABLE III.

PLASMA AND MUSCLE GLUTAMINE AND GLUTAMATE LEVELS BEFORE TPN ADMINISTRATION		
	Prefeeding Values (mean ± 95% CI)	Comparison With Normal Values (%)
Plasma glutamine mmol · L <sup>-1</sup>	0.343 ± 0.062	54
Plasma glutamate mmol · L <sup>-1</sup>	0.077 ± 0.020	535
Muscle glutamine (mmol · kg <sup>-1</sup> wet weight)	3.076 ± 0.886	19

Normal glutamine: plasma 0.637 mmol L<sup>-1</sup>; and muscle 16.5 mmol kg<sup>-1</sup> wet weight<sup>21</sup>; normal glutamate: plasma 0.023 mmol L<sup>-1</sup>.

TABLE IV.

CHANGES IN PLASMA AND MUSCLE GLUTAMINE AND GLUTAMIC ACID LEVELS DURING THE FIRST 5 DAYS OF TPN		
	GTPN	CTPN
Plasma glutamine changes	+0.038 ± 0.077	-0.005 ± 0.026
Plasma glutamic acid changes	+0.016 ± 0.012†	+0.012 ± 0.008†
Muscle glutamine change	-0.074 ± 0.552	+0.046 ± 0.443
Muscle glutamic acid change	+0.017 ± 0.284	+0.100 ± 0.145
ECF changes	+0.2 ± 2.8	+0.1 ± 1.8

Results expressed as mean ± 95% CI of the trend in level for each patient expressed as mmol · day<sup>-1</sup> (plasma), mmol · kg<sup>-1</sup> · day<sup>-1</sup> (muscle) or %d<sup>-1</sup>.

† Significantly different from zero (p < 0.05).

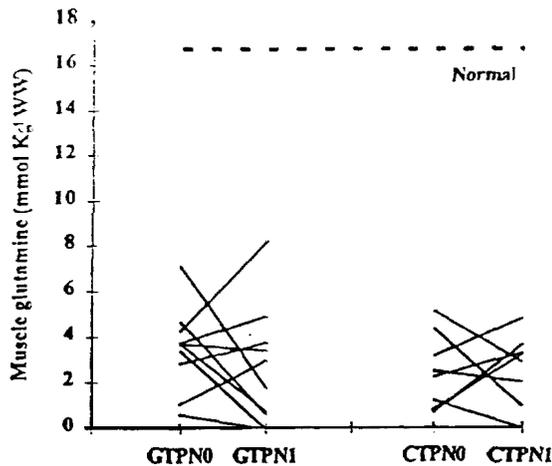


FIG. 1. Muscle glutamine before, after, and five days total parenteral nutrition (TPN) administration.

the skeletal muscle where it is the most abundant free amino acid. Muscle intracellular glutamine levels are known to fall during surgical stress, and this may have a direct influence on muscle protein synthesis.<sup>21</sup>

Plasma and muscle glutamine levels were very low before the instigation of TPN on Day 3 of the patients' illness. This suggests that the balance of glutamine supply and demand at this point has already been exceeded and the maximal rate of production or release of muscle glutamine will already be taking place. In healthy volunteers, infusion of the stress hormones adrenaline, glucagon, and cortisol increases limb amino acid efflux and reduces markers of protein synthesis.<sup>22</sup> The primary trigger for this response may be an increase in circulating glucocorticoid levels, which can produce a similar effect.<sup>23</sup> Exogenous administration of 25 g/day of L-glutamine in this situation appears to be unable to turn around the changes that have already taken place. It is unknown whether commencing treatment earlier or giving larger doses would alter this.

Plasma glutamine levels, although not a reflection of body stores, do give some indication of the state of whole body metabolic flux. Plasma levels, low at the start of nutritional supplementation, remain low, despite a large proportion of all amino acids being supplied as glutamine. This suggests either supply (endoge-

nous production plus exogenous administration) never catches up with demand or that an unknown control process favors a lower plasma glutamine concentration. Muscle glutamine levels show a similar pattern indicating this, although massive quantities of glutamine are released by skeletal muscle each day; body consumption is greater by orders of magnitude. Unlike the normal surgical situation in which there is a metabolic response to a single stress 'trigger' - the 'operation' - in these very severely ill patients, the stress 'trigger' is ongoing with repeated episodes of systemic inflammation, pain, and tissue trauma. Whole body glutamine consumption might increase in a supply-dependent manner as more glutamine is made available so more is used for tissue repair and immune function. This study has shown a marked increase in ECF volume within the muscle studied and is consistent with previous observations. No significant changes were observed during the period of this study. It has recently been argued<sup>24</sup> that changes in cellular hydration state might be the mechanism whereby intracellular muscle glutamine levels are controlled. If this were the case, then it might not prove possible to change muscle intracellular glutamine levels by nutritional manipulation alone during this severely ill period.

The finding of an elevation in plasma glutamate has been noted in septic surgical patients, although not commented on as a specific finding. Plumley et al. (Table II)<sup>25</sup> noted a 174% increase in arterial glutamate levels in patients without underlying lung damage, during early stages of systemic sepsis. They also found a small, statistically nonsignificant 'bump' in levels in patients with uncomplicated surgical stress. Plasma glutamate levels might be an indirect reflection of flux through the glutamine-glutamic acid metabolic pathway, a high level indicating high levels of glutamine utilization. Such a hypothesis would be consistent with the finding of increasing levels post-surgery, sepsis and in the critically ill with systemic inflammatory response syndrome. This may be relevant to the administration of glutamic acid, which forms part of the amino acid mixture in most commercially available formulations.

Changes in muscle may not be the most important effect of glutamine supplementation. Exogenous glutamine administration may have a significant impact in those tissues where it is an essential substrate, e.g., the GI tract and liver, fibroblasts, and in particular the immune system, while the musculoskeletal system relies on the role of a storage organ consumed in times of need and replenished in times of plenty. Although there are a number of encouraging clinical studies,<sup>23,26</sup> there remains a need for studies in the very severely ill to confirm a positive efficacy and influence on outcome.

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ARTICLE D

1



## The Effects of Glutamine-Supplemented Parenteral Nutrition in Premature Infants

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**ABSTRACT.** Background: Glutamine (GLN) is the primary fuel for rapidly dividing cells, yet it is not a constituent of parenteral nutritional formulas administered to newborns. The aims of this prospective, randomized, double-blind trial were (1) to confirm the safety of glutamine supplementation for premature infants and (2) to examine the effects of glutamine-supplemented parenteral nutrition on length of stay, days on total parenteral nutrition (TPN), days on the ventilator, and other clinical outcomes. Methods: Premature infants received either standard or glutamine-supplemented TPN and were monitored throughout length of stay for various health and biochemical indices. The group was examined as a whole (n = 44; birth weight range 530 to 1250 g) and in two weight subgroups, <800 and ≥800 g. Results: Serum ammonia, blood urea nitrogen, and glutamate tended

to be higher in the GLN groups, but the levels were well within normal limits. In the <800-g cohort (n = 24), glutamine-supplemented infants required fewer days on TPN (13 vs 21 days,  $p = .02$ ), had a shorter length of time to full feeds (8 vs 14 days,  $p = .03$ ), and needed less time on the ventilator (38 vs 47 days,  $p = .04$ ). There was a tendency toward a shorter length of stay in the NICU (73 vs 90 days, NS). These findings were not observed in the infants ≥800 g (n = 20). Conclusions: Glutamine appears to be safe for use in premature infants and seems to be conditionally essential in premature infants with extremely low birth weights. Larger multicenter trials are needed to confirm these observations and further evaluate the efficacy of GLN in these high-risk premature infants (*Journal of Parenteral and Enteral Nutrition* 20:74-80, 1990)

The premature infant is extremely vulnerable to insufficient nutrient intake and specific nutrient deficiencies. In utero, under optimal conditions, its nutrient supply is ideal and constant. After delivery, the infant is faced with the more hostile ex utero surroundings where the food supply may be intermittent inadequate, or imperfect. Unlike the adult, the premature infant has limited nutrient stores to meet metabolic requirements during short-term fasts. Even when nutrients are provided enterally or intravenously, the immature intestinal tract and liver may not always optimize absorption and metabolism of such feedings. Unfortunately, other priorities of medical care often preclude optimal nutrient delivery, contributing to additional nutritional risk.

The amino acid glutamine (GLN) is the most abundant amino acid in muscle and plasma of adult humans;<sup>1</sup> it is important for cell growth and synthesis and is an essential nutrient for the replication of cells in tissue culture.<sup>2,3</sup> In addition, GLN is an important fuel source for lymphocytes,<sup>4,5</sup> macrophages,<sup>6</sup> and enterocytes;<sup>7-9</sup> this amino acid also plays a key role in acid-base homeostasis<sup>10,11</sup> and serves as a necessary precursor for the major intracellular antioxidant glutathione.<sup>12-14</sup>

Traditionally, GLN was thought to be "nonessential" and was not included in IV amino acid mixtures because of its

relative instability in solution. However, after usual guidelines for preparation of IV nutrient solutions, GLN has been shown to be stable,<sup>15-18</sup> and a variety of human<sup>19-22</sup> and animal studies<sup>13,14,23-25</sup> have demonstrated an improved outcome when GLN was added to parenteral nutrition.

GLN supplementation may be of particular benefit to preterm infants receiving IV feedings. First, infants are growing, and GLN is one of the primary nutrients that provides purine and pyridine precursors for cell replication. Second, GLN is needed for maturation of the intestinal tract and may aid in prevention of enterocolitis.<sup>26,27,28</sup> Lastly, GLN enhances growth, development and function of the immunologic system<sup>29</sup> and thus should be of benefit to the premature infant who is vulnerable to infections. This study reports our preliminary safety and efficacy findings of L-GLN-supplemented parenteral nutrient solutions used in a neonatal intensive care unit (NICU).

### MATERIALS AND METHODS

The study was approved by the Committee for the Protection of Human Subjects from Research Risks, Brigham and Women's Hospital (BWH). An eligible infant's attending physician gave permission for the investigators to discuss the study with the parents. Informed consent was obtained for each infant.

#### Entry Criteria

Study subjects were selected from among premature infants in the NICU who were less than 4 days old and who had been receiving total parenteral nutrition (TPN) for less than 3 days. Included in the study were infants who were determined to be at high risk of necrotizing enterocolitis (NEC) according to previously established criteria.<sup>30</sup> In-

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Infants were included if they met at least six of the following criteria: birth weight < 1500 g; gestational age < 32 weeks; 5-minute Apgar score < 6, need for > 21% oxygen; need for ventilatory assistance; low blood pressure for age; suspected intraventricular hemorrhage; the presence of seizures; the presence of patent ductus arteriosus; the presence of umbilical, arterial and venous catheters; and birth weight < 1000 g (giving special consideration to the extremely low-birth-weight infant). Infants were excluded from the study for severe central nervous system damage incompatible with a prolonged life, renal failure limiting protein intake, and inborn errors of metabolism or liver disease preventing the delivery of appropriate nutrient requirements.

### Study Design

The major aims of this study were (1) to test whether GLN was well tolerated in this population of premature infants and (2) to investigate the potential benefits of GLN-supplemented TPN for these infants using a variety of clinical indicators, including length of stay (LOS), days on TPN, ventilator use, and incidence of infectious episodes.

Because of safety concern, this study was initiated as an open-label trial. After the initial four patients received glutamine, infants were then randomized by balanced assignment into control and treatment groups, and the study was blinded so that none of the investigators, nursing staff, or dietitians knew which solution the infants were receiving. The only person aware of the assignments was the research pharmacist, who kept the code sealed until the time of data analysis. Infants who met the entry criteria were randomized by gestational age (< or  $\geq$  32 weeks), high-risk NEC score, and multiple births (in the event of twins, siblings were assigned to opposite groups). The treatment consisted of addition of GLN to the TPN solution. GLN was initially added at 15% weight per volume of the amino acid mix (n = 4), increased to 20% (n = 15) and later increased to 25% (n = 3). Both the control and GLN diets were isocaloric and isonitrogenous (Table I).

### General Procedures

The clinical care, including nutrition support, of the infants was managed by the staff of the NICU, which followed generally accepted protocols. After initial blood cultures, all of the infants received antibiotic therapy for at least the first 3 days of life. Follow-up blood cultures were obtained when clinically indicated to evaluate bacteremia. Infants were weighed daily on a standard infant gram scale unless precluded by the patient's condition (eg, if the infant were on a high-frequency ventilator). Routine blood was drawn daily during the initial course and subsequently twice a week or more often if medically indicated to monitor electrolytes, blood gases, complete blood count, and indices of renal function. For the present study, 1 mL of blood was drawn before the initiation of the study infusion and once a week thereafter to monitor amino acid and ammonia concentrations; this was conditional on the other requirements for obtaining blood samples. Therefore, not all infants were measured for all variables. In the majority of infants, blood for the reported GLN assay was drawn at the end of the first week of TPN (approximately day 7).

Plasma levels of glutamine and glutamate were determined on a protein-free filtrate by an enzymatic spectrofluorometric method.<sup>24,25</sup> Plasma ammonia was measured by an enzymatic method (Sigma Kit 170-B, St Louis, MO).<sup>26</sup> Standard clinical laboratory methods were used for the assessment of hematocrit, blood urea nitrogen (BUN), white blood cell count (WBC), and bicarbonate (derived from blood CO<sub>2</sub> measurement);<sup>27</sup> standard microbiological techniques were employed to determine presence of positive blood cultures.

### Standard Therapy

Blood transfusions were generally administered to maintain hematocrit > 0.30 (30%) volume %, and > 0.40 (40%) if an infant required oxygen or was symptomatic. Parenteral nutrition was advanced over 3 to 4 days as tolerated. Protein and lipids were increased by 0.5 to 1.0 g/kg per day and dextrose was advanced to maintain normal blood glucose using up to 10% dextrose via peripheral infusions or concentrated dextrose solution was infused by a central catheter. Nasogastric feedings were initiated once patients no longer required umbilical arterial access and demonstrated metabolic, respiratory, and gastrointestinal stability.

Enteral feedings were advanced gradually in strength (from 1/4 strength to full strength) and then by increasing volume in the absence of gastric residuals, heme-positive stools, and significant respiratory

TABLE I  
Composition of solutions

	Standard solution (Aminosyn PF; 7% solution) (mg/100 mL)	Study solution (Aminosyn PF + glutamine; 7% solution) (mg/100 mL)
Lysine	475	3s0
Leucine	631	665
Phenylalanine	300	240
Valine	452	262
Isoleucine	634	427
Methionine	125	100
Threonine	360	288
Tryptophan	125	100
Alanine	490	a92
Arginine	861	669
Glycine	270	216
Histidine	220	176
Proline	570	456
Glutamate	576	4s1
Serine	347	278
Aspartate	370	296
Tyrosine	44	a s
Taurine	50	34
Glutamine	0	1400**

\* 20% glutamine solution.

or metabolic decompensation. Advancement to full enteral nutrition generally required 7 to 14 days. Breast milk or infant formulas (including Similac Special Care [Ross Laboratories, Columbus, OH] and Enfamil Premature Formula [Mead Johnson, Evansville, IN]) were used. Parenteral nutrition was discontinued when fluid needs were met by the enteral route or when parenteral fluids ran below 1.5 mL/h.

This therapy was continued on the basis of the clinical status of the infant or the presence of positive blood cultures. Infants were discharged from the NICU when they were approximately 35 weeks gestational age, had achieved cardiovascular stability and thermoregulation, were able to take all focal orally, and had consistent weight gain. Infants were either transferred home or to level I or II nurseries before discharge and were followed to discharge there.

### Endpoints

Primary endpoints included time to full enteral feeds, days on TPN, ventilator days throughout BWH length of stay (LOS), and weight gain per day (averaged across days at BWH). Secondary endpoints included plasma GLN levels ( $\mu\text{mol/L}$ ), BUN and incidence of elevated BUN (> 8.9  $\mu\text{mol/L}$  [ $> 25 \text{ mg/dL}$ ]), the frequency of a low WBC (<  $5 \times 10^9$  cells/L) throughout LOS at BWH, frequency of positive blood cultures, LOS at BWH (equivalent to LOS in the NICU at BWH), and total LOS, (which included LOS at BWH plus the number of days [if any] at the transfer hospital).

### Statistical Analyses

Infants entered into the study were excluded from analysis for the following reasons: (1) the study solution was not given for at least 7 continuous days; (2) less than 1.5 g amino acids/kg per day was given during the period of IV feedings (averaged over the total days of TPN); (3) the infant developed sepsis or other serious illness that precluded IV feedings or altered normal transition to enteral feeding; (4) the infant developed conditions (including NEC) necessitating surgery; or (5) the infant died during the course of the study. Exclusion criteria were developed a priori. Dietary intakes were analyzed using Neonova Nutrition Optimizer, version 3 (Ross Laboratories, 1990). Data were analyzed using standard statistical packages (STATA version 2.05 [Santa Monica, CA, 1990] and Systat version 5.2 [Evanston, IL, 1992]).

No clear dose-response effects in mean plasma glutamine concentration at the three different GLN doses were observed, except that the 20% dose was significantly different from zero (Figure 1). Mean group differences were examined by parametric (Student's *t* test) and nonparametric (Kruskal-Wallis) tests, depending upon the normality of their distribution (Lilliefors' test for normality). Data were analyzed using Cox regression analysis to assess any interaction between treatment group and potential confounding variables, in particular birth weight. Because of a significant interaction between treatment and birth weight (see Results and Discussion section), outcomes for two birth

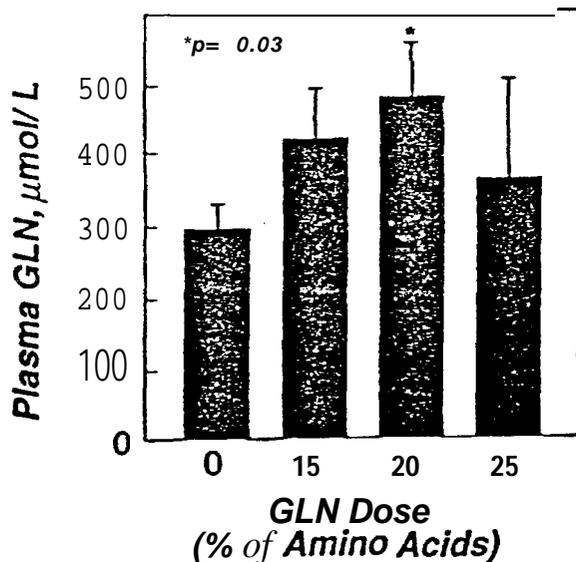


FIG. 1. Plasma GLN concentration generally increased as the percentage of amino acids given as glutamine increased. The 20% dose is significantly different from the 0 dose, by ANOVA (LSD). The actual quantity of glutamine administered to each infant depended on infusion rate of the nutrient solution and the individual body weight of the infant.

weight subgroups (< 800 g and  $\geq$  800 g) were examined within each group. Analysis of covariance was employed to control for birth weight class. Fisher's exact test was used to test between-group differences in frequencies of abnormal laboratory values. Potential outliers in the data set were examined by analyzing residuals (differences between observed and predicted values of dependent variable). Cook's distance, a measure of the degree to which the estimated coefficient would change if the sample were deleted, was employed to detect any observations with an unusually large measure of influence. Leverage was also used to examine the "extremeness" of observations. Data are reported as means  $\pm$  SD.

## RESULTS

### Group as a Whole

Between May 1990 and August 1992, 78 infants were enrolled; 33 of these were subsequently excluded for the following reasons: 16 (8 controls and 8 treatment group) had insufficient time on TPN; 4 (3 controls and 1 treatment group) developed serious conditions that prevented study participation because of surgery or transfer; 4 (2 controls and 2 treatment group) developed NBC, which precluded enteral feedings; 9 (4 controls and 5 treatment group) died from complications relating to the underlying condition. One, a statistical outlier (for two dependent variables LOS at BWH and TPN days), was excluded. This infant had a birth weight of 1470 g, APGAR of 4 and 113-day length of stay. (Tests were done with and without this individual and exclusion of this observation had no effect on the significance of the data or conclusions.) The final sample size of the study population was 44 (19 males, 25 females). There were no baseline differences between the control and treatment groups (Table II) nor between the males and the females, nor in the number of infants with central vs peripheral Knee (data not shown).

Since no differences in mean plasma glutamine concentration were seen among the three GLN doses (Figure 1),

the results were pooled. As shown in Table III, the GLN group had higher mean plasma GLN levels (439 vs 295  $\mu\text{mol/L}$ ,  $p = .04$  [normal range of plasma GLN in 1-month-old breast-fed neonates: 142 to 550  $\mu\text{mol/L}$ ]). In addition, plasma glutamate levels tended to be higher in the GLN-supplemented group (188 vs 152  $\mu\text{mol/L}$ ,  $p = .07$  [normal plasma levels of glutamate in 1-month-old breast-fed neonates: 24 to 243  $\mu\text{mol/L}$ ]), but there were no significant differences between serum bicarbonate or glutamate-glutamine ratios. There was a trend toward higher plasma ammonia levels in the GLN group, but this difference was not statistically significant (5 vs 4  $\mu\text{mol/L}$ , {0.9 vs 0.7  $\mu\text{g/mL}$ }  $p = .16$ ; the normal range of ammonia in 0- to 2-week-old infants is 5.6 to 9.2  $\mu\text{mol/L}$  [0.96 to 1.57  $\mu\text{g/mL}$ ]).

The GLN-supplemented group had a higher proportion of infants with at least one case of high BUN (12/17 vs 5/17,  $p = .04$ ) during the study when compared with controls. Mean BUN during TPN (averaged over days of TPN) also tended to be higher in the GLN group (7.8 [range: 2.9 to 11.4  $\text{mmol/L}$ ] vs 6.4  $\text{mmol/L}$  [range: 1.4 to 18.6  $\text{mmol/L}$ ], NS), though the means of both groups were within the normal range, which for premature infants is 1.1 to 8.9  $\text{mmol/L}$  (3 to 25  $\text{mg/dL}$ ). Total calorie and protein intakes were significantly higher only for week 1 in the control group (323 vs 294  $\text{kJ/kg}$  per day [77 vs 70  $\text{kcal/kg}$  per day],  $p = .05$ ; 2.1 vs 1.9  $\text{g protein/kg}$  per day,  $p = .03$ ). Low white cell counts were seen in fewer infants who received GLN-supplemented TPN compared with controls (2/22 vs 7/22,  $p = .13$ ) (Table III) (mean values: GLN vs control: 20.1 vs 21.7  $\times 10^9$  cells/L, NS). The number of red blood cell transfusions and average lymphocyte counts did not differ between groups. There were no other statistically significant differences between the groups.

### Two Weight Classes

Life-table analysis (Kaplan-Meier) revealed that at varying lengths of stay (BWH), the treatment had varying effects (Fig. 2). Cox regression demonstrated a significant interaction between birth weight and treatment in determining LOS. Because of this, we examined the effect of GLN, controlling for birth weight groups, < 800 g and  $\geq$  800 g, as this was the general midpoint of the group (Table IV).

The four weight subgroups were similar at baseline, except that in the  $\geq$  800-g treatment group the majority of subjects tended to be male (7 vs 2,  $p = .09$ ), two of whom were triplets. Each of the other three subgroups had nearly twice as many females as males. However, there were no outcome differences between sexes in the group as a whole, except for a trend toward greater weight gain per day in males (16.4 vs 13.9  $\text{g/d}$ ,  $p = .09$ ).

### The $\geq$ 800-g Cohort

In the  $\geq$  800-g cohort, there were no statistically significant differences in plasma GLN, glutamate, or BUN (Table IV). During TPN (but not for LOS at BWH), the GLN-supplemented group had more infants with positive blood cultures (5 vs 0,  $p = .01$ ) and a higher percentage of positive cultures after weighting by the number of cultures (100% vs 0%,  $p = .01$ ), although this trend remained for LOS at BWH, the differences were no longer statistically significant (Table IV). Among infants  $\geq$  800 g

with positive cultures, there was no difference in the number with central vs peripheral lines. Finally, males in this cohort had a significantly greater average weight gain than females (17.3 vs 12.3 g/d,  $p = .05$ ). They also tended to gain more weight than males in the < 800-g group (17.3 vs 15.2 g/d, NS).

#### The < 800-g Cohort

Serum GLN was higher in the GLN-supplemented group (400 vs 225  $\mu\text{mol/L}$ ,  $p = .05$ ), but there were no differences between the treatment and control groups in serum glutamate or ammonia (Table IV). The frequency of elevated BUN levels was higher during TPN, but the difference was not statistically significant. The GLN-supplemented group had fewer days on TPN (13 vs 21 days,  $P = .02$ ) and correspondingly shorter time to full feeds from the start of enteral feeding (8 vs 14 days,  $p = .03$ ), and spent less time on ventilator (38 vs 47 days,  $p = .04$ ). During the

third week of TPN, the GLN-supplemented group had a significantly higher lymphocyte count vs controls (25.9 vs 15.8,  $p = .04$ ). Length of stay was also shorter in the supplemented group (73 vs 90 days), although this difference did not reach statistical significance.

#### DISCUSSION

*In utero*, the fetus derives a large amount of GLN from the placenta and from amniotic fluid, the latter of which is thought to contribute up to one fifth of the total protein load. After birth, the amino acids in greatest concentrations in mother's milk are glutamine, glutamate, and taurine.<sup>44</sup> Studies in fetal sheep have revealed that glutamine is 'quantitatively the major transported gluconeogenic amino acid'<sup>45</sup> and our rates of delivery are below the rates of transfer measured in these studies. Therefore, it is only in the artificial setting of a hospital when the baby takes nothing by mouth that GLN is withdrawn from the infant because it is not provided in standard TPN or in usual infant formulas. We therefore started our dose-response study providing a solution that contained 15% of amino acids as glutamine, and with no evidence of short-term toxicity we increased the dose to 20%. A small number of infants were studied at the 25% level, but no clear advantages were observed.

#### Safety of GLN Administration

GLN appeared to be well tolerated in this study populations, as indicated by the within-normal levels of plasma ammonia and glutamate and improved GLN levels in both weight classes. Among infants  $\geq 800$  g, the plasma glutamate levels were higher in the GLN group, but these differences were also seen in this group at baseline (Table IV). In the < 800-g group, plasma glutamate was slightly higher (177 vs 142  $\mu\text{mol/L}$ , NS), but not significantly. Moreover, the infants showed no developmental disorders to indicate any neurotoxic effects of GLN or glutamate. These findings are similar to those of a GLN dose-response study

TABLE II  
Characteristics of study groups

	Control	GLN*
n	22	22
Birth weight (g)	6002 ± 165	8112 ± 175
Gestational age (week)	26 ± 1	26 ± 2
Apgar score (5-minute)	6.2 ± 1.5	6.4 ± 2.0
Sex (M/F)	7/15	12/10
Entry age (days)	4.1 ± 0.9	3.7 ± 0.8
IVH (+/-)	3/19	1/21
Birth number	1.2 ± 0.4	1.4 ± 0.7
# single births	15/22	16/22
# twins	5/22	4/22
# triplets	0/22	2/22
Delivery (V vs C)	9/13	5/17
Baseline		
Plasma glutamine ( $\mu\text{mol/L}$ )	363 ± 139	334 ± 169
Plasma glutamate ( $\mu\text{mol/L}$ )	159 ± 132	187 ± 110
Plasma ammonia ( $\mu\text{mol/L}$ )	7 ± 5	6 ± 2
BUN (mmol/L)	6.7 ± 2.2	7.0 ± 2.4

\*No differences between groups.

TABLE III  
Clinical outcomes of two groups

	Control	Glutamine	p
n	22	22	NS
Ventilator days (LOS)	35 ± 23	31 ± 17	NS
Length of stay: BWH	74 ± 30	71 ± 29	NS
Length of stay: total	92 ± 25	88 ± 21	NS
TPN days	16 ± 10	14 ± 6	NS
Day of 1st enteral feeds	6.5 ± 6.6	5.6 ± 3.1	NS
Time to full feeds (from 1st day of enteral feeding)	10.7 ± 7.4	8.3 ± 5.0	NS
Frequency of low WBC count (no. of patients, TPN)	6/22	2/22	NS
Frequency of low WBC counts, (no. of patients, LOS)	7/22	2/22	NS
WBC level, (TPN) ( $10^6$ cells/L)	20.7 ± 11.2	20.1 ± 12.1	NS
Frequency of high WBC count (no. of patients, TPN)	14/22	11/22	NS
Plasma glutamine ( $\mu\text{mol/L}$ )	295 ± 125	439 ± 221	.04
Plasma glutamate ( $\mu\text{mol/L}$ )	152 ± 44	188 ± 50	.07
Plasma ammonia ( $\mu\text{mol/L}$ )	4 ± 1	5 ± 2	NS
BUN (mmol/L) (during TPN)	6.4 ± 4.2	7.8 ± 2.4	NS
High BUN (no. of patients, TPN)	5/17	12/17	.04
Weight gain per day (kg)	15.0 ± 5	15.0 ± 5	NS
Frequency of positive cultures (no. of patients, TPN)	5/21*	7/22	NS
Frequency of positive cultures (no. of patients, LOS)	7/21*	9/22	NS

The only difference between males and females was that on average, males had higher weight gain per day (16.4 vs 13.9,  $p = .09$  than females). TPN refers to the time period (in days) during which TPN was administered in the BWH NICU; LOS refers to the total length of stay at BWH (except for total, which includes time spent in a transfer hospital whenever applicable).

\*n = 21 as 1 infant had no cultures tested.

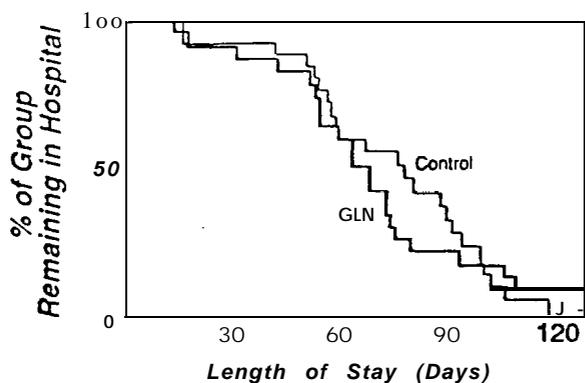


FIG. 2 The percentage of infants hospitalized over time for infants in the control ( $n = 22$ ) vs GLN-treated ( $n = 22$ ) groups. At earlier weeks of hospital stay, the percentage of infants in the NICU was approximately the same for both groups. However, at later weeks, more control infants remained in the NICU; at 80 days, eg, <25% of the infants in the GLN-supplemented group remained hospitalized, compared with >40% of the control infants. Cox proportional hazard regression revealed that length of stay was determined by a significant interaction between birth weight and the treatment received ( $p < .03$ ). Therefore, subgroups of two birth weight cohorts (<800 and  $\geq 800$  g) were created to control for the effects of birth weight on this response.

in healthy adults<sup>20</sup> where increasing loads of GLN did not result in clinical toxicity or abnormal glutamate levels, but there was a nonsignificant increase in glutamate in proportion to the GLN dose.

There was, however, a greater number of GLN-supplemented infants with at least one episode of high (>8.9 mmol/L) BUN during TPN in both weight classes (Table IV), although this difference was not significant. Because of the slight elevation in BUN of the GLN-supplemented group that was observed as the GLN dose was increased, the 20% GLN supplementation appears to be appropriate. We further analyzed the total nitrogen intake and N-kilocalorie ratios of the two groups in each weight class. There were no significant differences in these indices either by week (week 1 through 4 of TPN) or averaged over the days of TPN (data not shown). In the  $\geq 800$ -g infants, the only differences seen were in week 1 for total nitrogen (controls: 0.372 #kg per day vs GLN-supplemented 0.804 g/kg per day,  $p < .02$ ), and this reflected a higher overall intake of total protein for week 1. There were no significant differences in N-kcal ratios in either weight class.

#### Efficacy of GLN Administration

Among subjects  $\geq 800$  g, a greater number of GLN-

TABLE IV  
Two weight classes

	< 800 g			$\geq 800$ g		
	C	GLN	p	C	GLN	p
n	11	13		11	7	
Birth weight (g)	684 $\pm$ 52	691 $\pm$ 76	NS	917 $\pm$ 134	985 $\pm$ 119	NS
Gestational age (weeks)	25.1 $\pm$ 1.9	25.5 $\pm$ 1.4	NS	26.6 $\pm$ 2.1	27.0 $\pm$ 1.0	NS
Apgar score (5-minute)	5.8 $\pm$ 1.4	5.7 $\pm$ 2.1	NS	6.6 $\pm$ 1.6	7.3 $\pm$ 1.2	NS
Sex (M/F)	3/8	5/8	NS	4/7	7/2	.09
Entry age (days)	4.2 $\pm$ 4.2	3.8 $\pm$ 0.9		3.720.7	4.02 LO	NS
IVH (+/-)	3/8	1/12	NS	0/11	0/10	NS
Birth number	12% 0.4	1.2 $\pm$ 0.4	NS	1.3 $\pm$ 0.5	1.6 $\pm$ 0.9	NS
# single births	9/11	10/13	NS	6/11	6/9	NS
# twins	2/11	3/13	NS	3/11	1/10	NS
# triplets	0/11	0/13	NS	0/11	2/10	NS
Delivery mode (vaginal or c-section)	6/5	4/9		3/8	1/8	NS
Baseline						
plasma glutamine ( $\mu$ mol/L)	325 $\pm$ 143	355 $\pm$ 198	NS	401 $\pm$ 133	288 $\pm$ 112	NS
Plasma glutamate ( $\mu$ mol/L)	212 $\pm$ 123	186 $\pm$ 122	NS	95 $\pm$ 110	168 $\pm$ 116	NS
Plasma ammonia ( $\mu$ mol/L)	8 $\pm$ 6	6 $\pm$ 2	NS	5 $\pm$ 1	5 $\pm$ 1	NS
BUN (mmol/L)	7.6 $\pm$ 1.4	8.2 $\pm$ 1.8	NS	5.7 $\pm$ 2.9	5.4 $\pm$ 2.1	NS
Outcome						
Ventilator days (total)	47 $\pm$ 23	38 $\pm$ 14	.04	23 $\pm$ 17	20 $\pm$ 17	NS
Length of stay, BWB (days)	90 $\pm$ 21	73 $\pm$ 23	NS	58 $\pm$ 31	64 $\pm$ 36	NS
Length of stay, total (days)	104 $\pm$ 19	93 $\pm$ 18	NS	79 $\pm$ 26	78 $\pm$ 21	NS
TPN days	21 $\pm$ 11	13 $\pm$ 5	.02	11 $\pm$ 4	14 $\pm$ 7	NS
Day of first enteral feeds	9 $\pm$ 9	6 $\pm$ 3	NS	4 $\pm$ 1	5 $\pm$ 3	NS
Time to full feeds	14 $\pm$ 9	8 $\pm$ 4	.03	8 $\pm$ 4	9 $\pm$ 6	NS
Plasma glutamine ( $\mu$ mol/L)	225 $\pm$ 82	400 $\pm$ 237	.05	375 $\pm$ 121	499 $\pm$ 198	NS
Plasma glutamate ( $\mu$ mol/L)	142 $\pm$ 48	177 $\pm$ 56	NS	166 $\pm$ 36	210 $\pm$ 31	NS
Plasma ammonia ( $\mu$ mol/L)	4 $\pm$ 2	5 $\pm$ 2	NS	5 $\pm$ 2	5 $\pm$ 2	NS
BUN (mmol/L) (during TPN)	6.8 $\pm$ 4.7	7.5 $\pm$ 2.8	NS	5.7 $\pm$ 3.7	8.2 $\pm$ 2.0	NS
Frequency of high BUN (no. of patients, TPN)	3/10	7/10	NS	2/7	5/7	NS
Frequency of positive cultures (no. of patients, TPN)	4/11	3/13	NS	0/10†	5/9	.01
Frequency of positive cultures (no. of patients, LOS)	5/11	5/13	NS	3/10†	5/9	NS
% Positive cultures (TPN)	17 $\pm$ 23	18 $\pm$ 30	NS	0 $\pm$ 0	39 $\pm$ 49	.02
% Positive cultures (LOS)	14 $\pm$ 20	15 $\pm$ 30	NS	6 $\pm$ 9	26 $\pm$ 36	NS

Values are means  $\pm$  SD.

\*Between-group differences controlling for birth weight class, ANOVA for means (LSD); Fisher's exact test for frequencies.

†One infant had no cultures tested, therefore  $n = 10$ .

supplemented infants had positive Mood cultures during TPN (5 vs 0,  $p = .01$ ), but not for BW/length of stay (5 vs 3, NS); this was the only difference of note in these infants. His finding is inconsistent with other studies of GLN supplementation in humans.<sup>21,22</sup> This difference may have been an artifact of the small sample size; it underscores the need for larger clinical trials in this area.

In the very-low-birth-weight (< 600 g) cohort, GLN-supplemented TPN was associated with shorter time to full feeds, fewer days on TPN, reduced LOS in the neonatal intensive care unit, fewer infants with episodes of low WBC, earlier initiation of enteral feeding, and fewer days on the ventilator. Reduced length of stay with GLN supplementation has been reported in two other recent clinical trials<sup>21,22</sup> and may have been attributable to improved nutritional status,<sup>27</sup> enhanced bowel mucosal development,<sup>22</sup> and normalization of body water.<sup>21,22,46</sup> The decrease in days on TPN along with earlier enteral feeding may have contributed to improved intestinal cell maturation and nutrient absorption in the treated infants. This might have led to the observed earlier discharge from the NICU. Berseth and Nordyke<sup>47</sup> reported that in this high-risk population, early provision of enteral nutrients was associated with earlier maturation of intestinal motor activity. In addition, higher lymphocyte counts were noted during week 3 in the GLN-supplemented, < 800-g infants. This is consistent with the recent report of Ziegler et al<sup>48</sup> who found a significant increase in lymphocytes in recovered bone marrow transplant patients who received GLN.

#### Conditional Essentiality of GLN

It is not clear whether the  $\geq 800$ -g group may have had a larger treatment effect if the gender distribution of infants had been more similar. The fact that the < 800-g infants showed a discernible treatment effect may be an illustration of the "conditional essentiality" of GLN,<sup>49</sup> whereby the physiologic stressors on the extremely-low-birth-weight infants may have generated an even greater need for exogenous GLN.

#### Study Limitations

Because of the relatively small sample size of this pilot study, differences may have been undetectable statistically, although some of these differences may be clinically significant. The size of these infants also made it difficult to obtain blood samples in sufficient quantities for a more detailed metabolism study. In addition, the decision to initiate enteral feedings is made subjectively by the staff rather than on the basis of definitive criteria. Other possible confounding variables include varying discharge criteria for transfer hospitals and multiple unknown maternal risk factors. Despite these limitations, this study provides evidence that GLN supplementation appears to be safe in this population of premature infants throughout their stay in the NICU. Nevertheless, the full impact of a neonatal intervention cannot be fully assumed until a much longer period of time has\* *ie*, until the children have reached school age (as in studies of iron deficiency anemia, etc). Therefore, a larger study with long-term follow-up would be required to unequivocally determine the safety of this treatment.

#### Conclusions

GLN supplementation may reduce the length of stay in the hospital and promote enteral nutrition in these neonates. This preliminary study should serve as the basis for a larger multi-institutional, randomized, prospective, clinical trial of GLN supplementation to facilitate appropriate evaluation of GLN in neonates as well as of the multiple variables present in this patient population.

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**ARTICLE E**



## L-Glutamine Supplementation in Home Total Parenteral Nutrition Patients: Stability, Safety, and Effects on Intestinal Absorption

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**ABSTRACT.** A study was conducted to determine safety and efficacy of L-glutamine when added to total parenteral nutrition (TPN) solutions of patients receiving TPN in the home. Stability studies were performed on various concentrations of L-glutamine in TPN solutions mixed by the Pharmix method. These showed that glutamine was stable in home TPN solutions for at least 22 days. The daily home TPN solutions of seven stable patients were then supplemented with glutamine at a dose of 0.285 g/kg of body weight for 4 weeks. The glutamine-TPN solutions were prepared weekly. Five patients received the full 4 weeks of glutamine-TPN. In two patients, administration of glutamine-TPN mixtures was stopped at the end of week 2 and week 3 because of elevations in liver enzymes. A third patient's liver enzymes rose at the end of week 4. These abnormalities subsided after discontinuation

of the glutamine-TPN solution. Plasma levels of glutamine increased during the first 3 weeks of supplementation but these increases were not statistically significant. D-Xylose absorption studies performed before and after the administration of glutamine-TPN did not reveal any improvement in small-bowel absorptive capacity. In conclusion, stable glutamine-TPN solutions for use by home TPN patients can be formulated. However, supplementation of home TPN solutions at this dose was associated with apparent hepatic toxicity and did not demonstrate a beneficial effect on intestinal absorptive capacity as measured by D-xylose absorption. Therefore, on the basis of this study, routine supplementation of home TPN solution with glutamine cannot be recommended (*Journal of Parenteral and Enteral Nutrition* 18268-273, 1994)

Patients with various forms of gastrointestinal failure of adenosine triphosphate, purines, and pyrimidines.<sup>2</sup> require long-term home parenteral nutrition. Over the past 3 decades, extensive research resulted in a nutritionally and metabolically adequate total parenteral nutrition (TPN) formula that includes glucose, lipids, amino acids, minerals, vitamins, and trace elements.

Commercial amino acid solutions for use in TPN include the essential amino acids tryptophan, leucine, isoleucine, threonine, histidine, lysine, valine, methionine, and phenylalanine and the nonessential amino acids serine, alanine, tyrosine, arginine, glycine, and proline. Glutamine is a nonessential amino acid that has not been a component of TPN amino acid solutions, mainly because of problems arising from the difficulty of getting glutamine into solution, its lack of stability in TPN solutions, and the fact that it is nonessential. Glutamine is, however, the most abundant amino acid in the body. It makes up approximately 60% of the free amino acids found intracellularly in skeletal muscle.<sup>1</sup> It has several important functions: It carries amino nitrogen from peripheral tissues to the splanchnic area; it is a major factor in renal acid-base regulation; and it donates nitrogen for the formation

There is a positive correlation between the concentration of glutamine and the rate of muscle protein synthesis. Glutamine is a major fuel source for rapidly dividing cells such as enterocytes, fibroblasts, colonocytes, and reticulocytes. It is therefore the preferred fuel for the small-bowel mucosa.

It has been suggested that, under stress, glutamine may act more as an essential amino acid. Studies have documented decrease in glutamine pools during catabolic illness or stress? Patients receiving glutamine after an operation have been shown to have a decreased catabolic effect.<sup>4</sup> Those patients had improved nitrogen balance, muscle protein synthesis, and intracellular muscle glutamine levels compared with a group receiving TPN without glutamine.

There has been interest in adding glutamine to TPN because it may be conditionally essential during periods of stress. To date, reported studies in humans have described mixing glutamine-TPN solutions on a frequent basis to keep the mixtures stable.<sup>2-4</sup> These studies in humans were performed on hospitalized patients. We have undertaken a study to determine whether glutamine can remain stable in a TPN solution for prolonged periods to allow at-home use by patients receiving TPN. We also evaluated the clinical safety of these solutions and their potential effects on intestinal absorption.

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MATERIALS AND METHODS

An Investigational New Drug application was filed with the Food and Drug Administration for the use of glutamine in TPN solutions. The study protocol was approved by the Investigational Review Board at Memorial Sloan-Kettering Cancer Center. Patients were recruited from our home TPN population. All patients gave informed consent before joining the study.

Patients

Patients were eligible if they received TPN in the home for at least 5 nights a week and fulfilled the following criteria: age >18 years, no requirements for insulin, serum bilirubin level <1.5 mg/dL, serum creatinine level <1.8 mg/dL, and no chemotherapy or radiation therapy in the preceding 6 months. Seven home TPN patients (three women, four men) were studied. Their ages ranged from 38 to 81 years, and their weights ranged from 45 to 76 kg (Table I). All patients had been receiving home TPN for at least 1 year (average 8.4 years), and all were clinically stable. Patients were removed from the study if they had an elevation in LFT results of 2 times the baseline; an elevation in ammonia, blood urea nitrogen, creatinine, or glucose of 1.5 times the baseline; or any new physical or mental status change.

Glutamine-TPN Mixture

Stability studies of glutamine-TPN solutions were performed before the solutions were administered to patients. Glutamine was mixed in TPN solutions in concentrations of 1 and 1.5% wt/vol, and solutions were kept at 4°C for 22 days (Table II). The composition of the mixtures was calculated to give a wide range of concentrations of amino acids, glucose, electrolytes, and trace elements in an effort to duplicate the various formulas prescribed to home TPN patients. Glutamine,

ammonia, glutamic acid, pH, amino acids, sterility, particulate matter, electrolyte concentrations, color, dextrose concentration, visual appearance, and bag weight were quantitated in triplicate for each formula on days 0, 8, 15, and 22.

The solubility of glutamine in solute-laden solutions has been problematic in the Past-The Pharmix<sup>6</sup> compounding process was used because it allows complete solubilization and solute uniformity in the preparation of the enriched parenteral solutions. This compounding process allows the glutamine to be solubilized first, before it is combined with any other components. In this method, the sterile water for injection is added to a stainless steel mixing tank in sufficient quantity to fulfill the total prescription volume. The glutamine is solubilized in the sterile water for injection before adding any additional nutrient components. Once the glutamine is in solution, all other components are added in a systematic way to insure complete dissolution. The pH of the compounded solution is adjusted with either glacial acetic acid or phosphoric acid in quantities sufficient to maintain a pH range between 5 and 7. The refractive index of the compounded solution is also tested to ensure complete solubilization.

This process also involves a cold sterilization technique of two membrane filters. This ensures that the glutamine is not exposed to a heated process that can catalyze product degradation and promote the generation of potential toxic by-products.

Glutamine-TPN Administration

Glutamine was added to TPN solutions at a dose of 0.285 g/kg of body weight. Therefore, a typical 70-kg patient received 20 g of glutamine per day over a 10-hour to 12-hour infusion (Table III). Our regular home TPN formula typically includes amino acids in the amount

TABLE I  
Patient characteristics

Patient	Sex	Age (y)	Weight (kg)	Time on TPN (y)	Diagnosis
1	M	81	64	5	Inflammatory enteropathy
2	M	45	7a	1	Testicular cancer; s/p small-bowel resection and radiation
3	M	64	76	13	Intestinal pseudo-obstruction
4	F	71	49	4	Colon cancer s/p small-bowel and right-colon resection
6	F	81	62	13	Uterine cancer s/p small-bowel resection and radiation
6	M	42	72	16	Crohn's disease s/p small-bowel resection
7	F	38	46	6	Malrotation s/p small-bowel resection

TPN, total parenteral nutrition; s/p, status post.

TABLE II  
Composition of glutamine-supplemented TPN solutions used in the stability study

Formula	A	B	C	D
Glutamine (%)	1.0	1.5	1.0	1.5
Amino acids (%) <sup>*</sup>	1.0	1.0	6.0	6.0
Dextrose (%)	5.0	5.0	30.0	30.0
Electrolytes (mEq/L)	0	0	†	†
Trace elements	0	0	‡	‡

\* Exclusive of glutamine.

† Electrolytes (mEq/L): potassium, 114; sodium, 143; magnesium, 17; calcium, 7; chlorine, 144; phosphate, 21 mmol/L.

‡ Trace elements: zinc, 13 mg/L; copper, 0.4 mg/L; chromium, 15 µg/L; manganese, 1 mg/L; selenium, 20 µg/L.

of 1 g/kg of body weight. The glutamine was considered part of the total amino acid dose. The remainder of the glutamine-TPN solution was based on the regular TPN formula of each individual patient. These formulas contain amino acids, dextrose, electrolytes, trace elements, and water. Vitamins are added on a daily basis, and the lipid is given separately. No three-in-one solutions were used. These formulas had been used for at least 6 months before the study. On the first day of the study, the glutamine-TPN formula was administered in the Day Hospital to each patient so that unexpected clinical responses could be monitored. Subsequently, patients used self-administered glutamine-TPN solutions at home for the duration of the study.

#### D-Xylose Absorption Test

A D-xylose absorption test was performed at baseline and at the end of the study. Patients were given 25 g of D-xylose orally. They had serum D-xylose levels determined at 0, 1, 2, 3, and 5 hours after drinking the D-xylose solution. They also were asked to empty their bladders at baseline, and a 5-hour urine collection for measurement of D-xylose was obtained.

#### Monitoring

Blood tests including complete cell count, venous ammonia concentration, serum glutamic oxaloacetic transaminase concentration, serum glutamic pyruvate transaminase concentration, alkaline phosphatase concentration, lactic acid dehydrogenase concentration, bilirubin level, blood urea nitrogen level, creatinine level, and glucose level were performed. Plasma amino acid profiles were also obtained.

Analyses of plasma amino acids were performed as follows: Blood samples were collected in tubes containing 15% ethylenediaminetetraacetate and kept on ice. Plasma was separated by centrifugation and stored at  $-70^{\circ}\text{C}$ . Amino acids were separated as follows: 400  $\mu\text{L}$  of plasma was mixed with 400  $\mu\text{L}$  of Seraprep (Pickering Laboratories, Mountain View, CA) containing 250  $\mu\text{mol}$  of L-norleucine (Sigma, St. Louis, MO) per liter as an internal standard. The resulting deproteinized plasma was then centrifuged, and the supernatant amino acids were separated by high-performance liquid chromatography (Perkin Elmer, Plainfield, NJ) with a lithium cation exchange column. Individual amino acids were identified and quantified by postcolumn derivitization with ninhydrin (Pickering Laboratories). The response factor for calculation of glutamine concentration was determined from an external glutamine standard formulated from a 1:1 mixture of 250  $\mu\text{mol}$  of pure crystalline L-glutamine (Gibco, Grand Island, NY) per liter in water and Seraprep. Response factors for other amino acids were determined from commercially available amino acid standard solution (Amino Acid Calibration Standard, Pickering Laboratories).

The blood tests, including amino acid profiles, were done at baseline and at weekly intervals for the 4 weeks of administration of glutamine-TPN and for 2 weeks thereafter, when patients were receiving their regular TPN without glutamine. Patients were called every other day and were asked about any new physical or mental symptoms.

## RESULTS

#### Stability of Glutamine-TPN Solutions

The stability studies on the four glutamine-TPN formulas indicated that the glutamine concentration

TABLE III  
Glutamine, amino acids, and calories in individual daily TPN solutions

Patient	Glutamine (g)	Amino acids (g) excluding glutamine	Total calories (protein, fat, and carbohydrates in TPN solution)
1	18.3	38.3	2340
2	20.6	51.3	2888
3	21.5	64.0	2674
4	14.4	26.1	2261
5	14.0	66.6	1779
6	21.4	54.0	2314
7	13.0	32.0	1846

TPN, total parenteral nutrition.

TABLE IV  
Stability data for formula D at day 0, 8, 15, and 22

	Day 0	Day 8	Day 15	Day 22	Percent change at day 22
pH	6.30	6.31	6.30	6.32	0.32
Dextrose (%)	100.00			92.71	-7.29
color (Klett units)	26.00	25.66	24.66	25.66	-1.31
Particulate matter	PASS	PASS	PASS	PASS	
Ammonium (ppm)	<16	<16	<15	22.47	
Glutamic acid	Neg	Neg	Neg	Neg	
Bag weight (% loss)	0.00			-0.09	-0.09
Pyrogen	PASS				
Sterility	PASS			PASS	

remained within United States Pharmacopeia guidelines when solutions were kept at 4°C for up to 22 days (Tables IV and V). Dextrose and electrolyte levels remained within United States Pharmacopeia limits. No significant increases in glutamic acid or ammonia concentration were detected. The other amino acids in the solution also remained stable. Sterility and pyrogen assays were negative for all samples. Color, pH, visual appearance, and bag weight all remained within acceptable limits.

**Outpatient Outcome With Glutamine-TPN**

Five patients received glutamine-TPN for the entire 4-week study. In the two other patients, glutamine-TPN administration was discontinued because of elevation of liver function test (LFT) results at the end of weeks 2 and 3. One of the five patients who completed the 4 weeks of supplementation was noted to have elevated LFT results at the end of week 4. All patients in the study had stable LFT results for the year before the study. The specific changes in liver enzymes in the three patients are listed in Table VI. Patients who developed an increase in LFT results of 2 times baseline were removed from the study. Ammonia levels did not increase significantly in any patients. The liver enzyme abnormalities had returned to baseline by 2 weeks after discontinuation of the glutamine-TPN formula. None of the patients who developed increased LFT results had any complaints referable to the liver. Because all values returned to baseline rapidly, no ultrasound, computed tomography scan, or liver biopsy was performed. Patients developed no physical or mental status complaints. Of note, anecdotally, three patients described a sense of increased well-being while receiving the glutamine-supplemented TPN solution.

**Plasma Amino Acid Levels**

The plasma glutamine levels before glutamine supplementation and at weekly intervals after beginning supplementation are presented in Figure 1. At baseline, the mean level was  $560 \pm 60 \mu\text{mol/L}$ . This was statistically similar to levels of  $634 \pm 31 \mu\text{mol/L}$  and  $627 \pm 28 \mu\text{mol/L}$  reported previously in healthy controls.<sup>7</sup> The mean level rose to  $655 \pm 46 \mu\text{mol/L}$  after 1 week

of glutamine supplementation, but then a gradual decline began. After 4 weeks of glutamine supplementation, the mean plasma glutamine level declined to the presupplementation level. Two weeks after discontinuation of glutamine, the level had decreased to  $453 \pm 82 \mu\text{mol/L}$ . A Wilcoxon matched pairs test was performed, comparing each mean plasma glutamine level obtained with the levels for every week (eg, baseline compared with values at week 1, week 2, week 3, week 4, and posttreatment weeks 1 and 2). There was no statistically significant change noted despite the trend toward an early increase followed by a subsequent decrease in plasma levels. Mean plasma levels of taurine, tyrosine, methionine, asparagine, aspartate, histidine, alanine, glycine, glutamate, and serine did not change significantly compared with baseline values. The mean plasma levels of tryptophan and phenylalanine increased significantly from baseline to week 1. Valine and isoleucine levels increased significantly from week 2 to week 3 and from baseline to week 1 postsupplementation, respectively. Lysine and leucine values decreased significantly from baseline to week 3 and from week 1 to week 2 postsupplementation, respectively.

**D-Xylose Absorption Tests**

D-Xylose absorption tests were performed in six of the seven patients. Patient 3, with pseudo-obstruction did not undergo this test because he could not tolerate drinking the solution. He was also the patient who received only 3 weeks of glutamine. All patients began with markedly abnormal D-xylose absorption. There was no evidence of any significant improvement in intestinal absorption of D-xylose after 4 weeks of glutamine-TPN in five patients or after 2 weeks of glutamine in one patient (Fig. 2). Blood levels of D-xylose at hours 1 and 5 did not increase after glutamine supplementation, nor was there any increase in 5-hour urinary excretion of D-xylose.

**DISCUSSION**

This study demonstrates the feasibility of administering a TPN solution containing glutamine to home TPN patients. The Pharmix method was used to mix the TPN solutions.<sup>7</sup> When TPN solutions were supplemented with glutamine, they remained stable in solution for at least

TABLE V  
Amino acid stability data for formula D at day 0, 8, 15, and 22

Amino acids (%)	Day 0	Day 8	Day 15	Day 22	Percent expected at 22 days
Glutamine ( $\pm 1.11\%$ )	100.00	105.43	101.14	93.02 (NS)	96.53
Alanine	100.00	106.64	92.98	93.13 (NS)	92.23
Arginine	100.00	102.60	91.21	92.69 (NS)	91.71
Glycine	100.00	105.69	93.19	92.49 (NS)	92.81
Histidine	100.00	105.95	95.69	96.16 (NS)	96.36
Isoleucine	100.00	108.65	90.90	96.11 (NS)	94.72
Leucine	100.00	104.69	93.15	96.31 (NS)	95.28
Lysine	100.00	115.12	90.60	90.87 (NS)	91.76
Phenylalanine	100.00	107.07	90.91	94.14 (NS)	90.73
Proline	100.00	104.61	90.40	92.67 (NS)	91.69
Serine	100.00	108.11	90.84	95.26 (NS)	94.05
Threonine	100.00	105.13	94.38	92.86 (NS)	93.48
Valine	100.00	112.24	90.61	98.84 (NS)	98.98

NS, not significant. There was no statistically significant decrease in any amino acid concentration over the test period.

22 days. Because of this stability, the bags containing the glutamine-TPN solutions could be delivered to the patients' homes on a weekly basis, and daily compounding of the solutions was unnecessary.

Animals receiving long-term TPN may develop intestinal mucosal atrophy, particularly if they have no oral intake. It has been postulated that this atrophy is caused or worsened by the lack of glutamine.<sup>8</sup> Grant and Snyder<sup>9</sup> found that rats given glutamine-TPN would maintain mucosal weight in the stomach and colon but not the small bowel. O'Dwyer et al.<sup>10</sup> reported that rats receiving glutamine had increased intestinal weight, DNA content, and intestinal villous height.

Intestinal villous height was not measured in our study. However, the D-xylose absorption studies in our patients did not indicate any improvement in small-intestinal absorption after 4 weeks of glutamine supplementation. The D-xylose absorption test is the standard clinical test for evaluating the absorptive capacity of the small-bowel mucosa. However, it has some limitations. It measures xylose absorption specifically and does not measure other types of nutrient absorption. It is also not sensitive enough to pick up subtle changes in absorptive capacity.

A possible explanation for lack of improvement in absorption is that our patients may have already achieved

TABLE VI  
Specific liver enzyme changes in three patients

	Baseline	Highest value	2 Weeks after study
Bilirubin NL = 0.6-1.2	0.7	0.7	0.2
Alkaline phosphatase NL = 38-126	175	206	208
AST NL = 5-40	3a	126	36
LDH NL = 313-618	520	946	276
Ammonia NL = 18-54	44	52	Nor done
ALT NL = 5-40	16	41	24
<b>Patient 6</b>			
Bilirubin NL = 0.8-1.2	0.3	0.5	0.3
Alkaline phosphatase NL = 30-100	151	226	152
AST NL = 5-40	4a	175	22
LDH NL = .3-180	189	191	126
Ammonia NL = 18-54	63	41	202
ALT NL = 5-40	58	95	33
<b>Patient 3</b>			
Bilirubin NL = 0-1.0	0.8	1.4	0.7
Alkaline phosphatase NL = 30-88	113	189	131
AST NL = 10-37	24	272	21
ALT NL = 5-37	20	142	37
LDH NL = 60-200	171	377	150
Ammonia NL = 13-64	90	57	32

NL, Normal laboratory value; AST, serum glutamic oxaloacetic transaminase; LDH, lactic acid dehydrogenase; ALT, serum glutamic pyruvate transaminase.

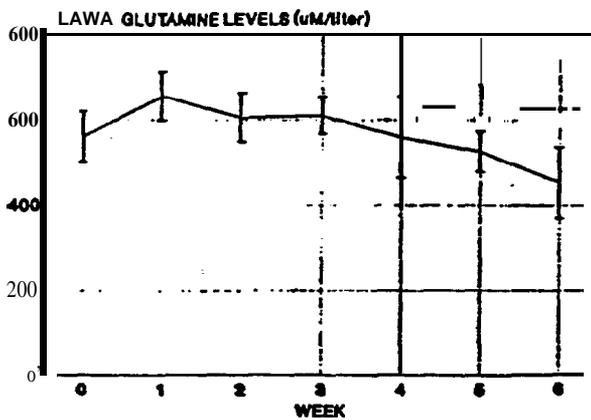


FIG. 1. Mean plasma glutamine levels. There was no significant increase at any time during the study.

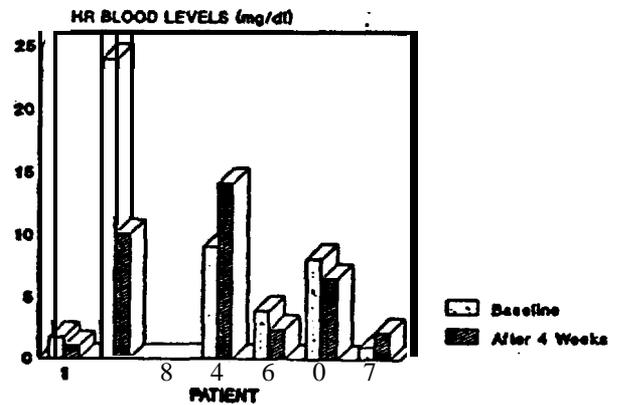


FIG. 2. Results of the D-xylose absorption test. There was no significant improvement in intestinal absorption of D-xylose during the study period.

maximal small-bowel adaptation, inasmuch as they had intestinal failure and had been receiving TPN and oral feedings for long periods. It is also conceivable that it takes a longer period of administration of glutamine to improve small-bowel function. Whether glutamine given during the early stages after resection of the small bowel would result in an accelerated rate of improvement in absorption cannot be determined from this study.

Although our patients had been receiving home TPN for an average of 8 years, their plasma glutamine levels were comparable to those of normal healthy people. Glutamine supplementation did not result in any sustained increase in serum glutamine levels.

It has been suggested that glutamine supplementation can prevent bacterial translocation from the intestinal tract and thus reduce the rate of sepsis. Alverdy<sup>10</sup> found that chemotherapy-treated rats given TPN without glutamine had a 100% mortality rate and upon necropsy had higher percentages of positive cultures from mesenteric lymph nodes, liver, spleen, and blood. Burke et al<sup>11</sup> found a statistically significant difference in the rate of bacterial translocation in both enterally fed and glutamine-TPN-fed rats compared with rats fed TPN alone. These studies suggested that parenteral glutamine supplementation in animals might help protect them from bacterial translocation. Our study did not evaluate rates of infections because it was performed in a stable, small population for a relatively short period.

Recently, Ziegler et al<sup>6</sup> reported a reduction in the infection rate in bone marrow transplant patients whose TPN was supplemented with glutamine at 0.57 g/kg of body weight. They also noted an improvement in nitrogen balance and a decrease in the length of hospital stay. These patients were acutely ill after radiation, chemotherapy, and bone marrow transplantation.

Our patients received only half the above dose. They received 0.285 g of glutamine per kilogram of body weight in their TPN. They received this lower dose because their TPN infusion lasted only 10 to 12 hours instead of the 24-hour period used in inpatient studies. Because glutamine-TPN had not been previously given in an outpatient setting, we chose to use a rate that had previously been shown to be safe. The question arises whether the results would have been different if a larger dose had been used.

The only apparent side effect from glutamine supplementation was an increase in liver enzymes, which resolved upon cessation of the glutamine supplementation in affected patients. It is of concern that three of our patients developed this elevation in liver enzymes

A previous study reported a slight rise in alkaline phosphatase and bilirubin levels during the 1-month administration of glutamine-TPN to bone marrow transplant patients.<sup>6</sup> Transaminase levels were not mentioned in that report. Transaminase level increases were the most impressive laboratory value change in our population.

In summary, this study showed that a stable glutamine-TPN solution can be made for the home setting. However, it does not give evidence that glutamine supplementation causes improvement in small-bowel absorption in stable home TPN patients who are taking food orally. Evaluation of other potential benefits in this patient population such as a decrease in sepsis rate requires a larger number of patients and a much longer study. The potential liver toxicity from glutamine supplementation has to be kept in mind if such studies are undertaken.

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ARTICLE F

1

## The stability of L-glutamine in total parenteral nutrition solutions

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**ABSTRACT**—An assessment was made over a period of 14 days of the rate of glutamine degradation in different intravenous solution: kept at 22–24°C, 4°C, –20°C and –80°C. At room temperature (22–24°C) degradation rates in mixed parenteral nutrition solutions and amino-acid/dextrose solutions ranged from 0.7–0.9%/day, in Perifusin 0.6%/day, and in dextrose alone as low as 0.15%/day. At 4°C, glutamine degradation was <0.1–0.2%/day in all solutions examined, at –20°C it was minimal (<0.04%/day) and at –80°C, it was undetectable.

Glutamine degradation was found to be associated with the formation of equimolar quantities of ammonia. No glutamate formation was detected.

It is concluded that it is possible to store glutamine in parenteral nutrition solutions kept at 4°C, with about 2% loss over a period of 14 days. The degradation is sufficiently slow to consider the use of intravenous glutamine in nutritional therapy.

### In production

All the currently available commercial amino-acid solutions that are used in total parenteral nutrition (TPN) lack free glutamine. This is largely because of fears about the instability of glutamine and possible toxicity of some of its degradation products (pyroglutamic acid and ammonia). However, there is surprisingly little information about the stability of glutamine, and some of the work with parenteral nutrition solutions is confusing. For example, in one preliminary report it was suggested that the rate of degradation of glutamine in the presence of other nutrients is as low as 0.1%/day at room temperature (1). In another preliminary communication the rate of glutamine degradation in a parenteral nutrition solution was reported to be higher, but less than 5%/day (2). However, other workers have reported the rate of glutamine degradation in buffers, kept at room temperature and a pH similar to that of parenteral nutrition solutions, is even higher at about 10–20%/day (3,4, 5).

If free glutamine is to be included in parenteral nutrition solutions as an alternative to the use of glutamine containing dipeptides (6), it is essential to have information about its stability and the type of degradation products that are formed during

storage. In previous studies we have found that the stability of glutamine in solution depends not only on temperature and pH, but also on the composition and molarity of other constituents in the solutions (7, 8). It became obvious that there was a risk in attempting to predict the rate of degradation of glutamine in one solution as a result of observations made on another solution. It also became clear that the stability and products of glutamine degradation should be assessed in a variety of parenteral nutrition solutions. This study aimed to obtain such information at various temperatures, including those that are likely to exist during storage or administration of the intravenous solutions.

### Methods

L-glutamine (Degussa, Frankfurt, Germany) was added to the solutions to form a final concentration of about 1% (w/v) (70 mmol/l). The composition of the various intravenous solutions is indicated in Table 1.

An mixture was prepared in standard sized total parenteral nutrition (TPN) bags under strict aseptic conditions. The solutions contained electrolytes (except valinin/dextrose) and micronu-

**Table 1** Detailed composition of each type Of solution/mixture used to assess the stability of glutamine

Constituents	Aminoplex (A)†/ dextrose	Aminoplex (B)††/ dextrose	Eloamin (A)†††/ dextrose	Eloamin (B)†††/ dextrose	Synthamin††††/ dextrose	Synthamin/ dextrose/ lipid	Vamin*/ dextrose	Perifusin**
Glutamine (mmol/l)	74	66	74	68	74	68	74	74
K (mmol/l)	46.6	43.2	46.2	42.5	32.5	26		30
Na (mmol/l)	49.1	45.3	49.2	45.3	3s	28		40
Nitrogen (g/l)	7.11	8.5	6.8	8.1	6.4	5.12	-9	5.4
Carbohydrate (kcal/l)	577.2	532	557.7	529.1	563	450.4	460	-
Fat (kcal/l)	-	-	-	-	-	400		
Phosphate (mmol/l)	17.3	16.0	17.2	1s.9	15.0	12		
Mg (mmol/l)	4.5	4.4	4.02	3.7	2.5	2		5
Ca (mmol/l)	67.3	62.5	67.6	62.6	35	28	-	9
Acetate (mmol/l)	2.9	2.7	2.9	2.6	-	-		
Trace elements***	1.0	1.3	7.8	10.3	62.5	50		10
Trace elements***	Elotrace B	Elotrace B	Elotrace B	Elotrace B	Additrace	Additrace	-	
Vitamins****	MVC9 + 3	MVC9 + 3	MVC9 + 3	MVC9 + 3	Multibionta	Multibionta	-	

† Geistlich Sons Ltd, Cheater, UK

†† Leopold Pharma, Graz, Austria/Oxford Nutrition, Oxford, UK

††† Baxter, Egham, Surrey, UK

• Kabi Vitamins Ltd., Alton, Middlesex, UK

\*\* Merck Ltd., Alton, Hunts, UK

• \*\*\* 1 ampoule of trace elements, present in ~2.5L solutions (Elotrace B, Leopold Pharma, Graz, Austria/Oxford Nutrition, Oxford, UK; Additrace, Kabi, UK)

• \*\*\*\* 1 ampoule of multivitamins present in 2.5L solutions (MVC Lyphomed, Chicago, USA/Oxford Nutrition Ltd UK; Multibionta, Merck, UK)

trients (Table 1). The L-glutamine was first dissolved in sterile doubly distilled water for injection B.P. (2.5% w/v or 173 mmol/l), passed through a 0.2  $\mu$ m filter to remove particulate matter, and then added to the above intravenous solutions/mixtures (Table 1), and dextrose solutions alone to achieve a final dextrose concentration of 5–25% w/v. As a control the same intravenous solutions/mixtures were prepared without the addition of glutamine. As further controls, ammonia (as ammonium chloride, Analar, British Drug House, Poole, Dorset, UK), L-glutamate and L-pyroglutamate (Sigma, Dorset, UK) were added separately to the same solutions (without glutamine) to obtain final concentrations of 10, 10 and 40 mmol/l respectively. Portions of these solutions were sampled at the same intervals (see below) as the above solutions and analysed to assess the recovery and possible interconversion of glutamate, glutamine, pyroglutamate and ammonia.

Aliquots (20 ml) of each solution were removed from the TPN bags and kept in sterile glass vials at room temperature (22–24°C), 4°C, and –80°C (all except mixed bag containing lipid). The Synthamin containing solutions were also stored at –20°C. The TPN bags (Ultrastab, Oxford Nutrition, Oxford), which are impermeable to gases such as oxygen were also kept at room temperature, and the contents sampled at intervals. Duplicate samples from all the solutions were taken at day 0 (shortly after addition of glutamine), day 7 and day 14. In some cases (dextrose only solutions and all Synthamin containing solutions), additional measurements were made on days 3 and 30. The pH of each solution (see Table

2) was measured at room temperature before, during and at the end of the test period.

Glutamine (9), glutamate (10), and ammonia (Roche, urea UV method, Kit No:0711144) were measured in duplicate using standard enzymatic techniques adapted for use on the Cobas Fara (Roche) centrifugal autoanalyser. The 'all in one' mixture which included the lipid emulsion (stored only at 22–24°C and 4°C) was filtered through a 0.2  $\mu$ m filter prior to analysis in order to remove the lipid.

#### Calculations

Degradation rates (see Table 2) were derived from (a) the sequential decline in glutamine concentrations, and (b) a combination of initial glutamine concentration and appearance of ammonia (the degradation of glutamine was found to be associated with the formation of equimolar quantities of ammonia; see (7) and results of this paper). The second method was used partly because no dilutions were necessary prior to analysis, and partly because it is able to detect small changes in concentrations more accurately than the first method (the initial ammonia concentration was close to zero - cf. glutamine concentration). Both methods of calculation took into consideration the initial concentrations of ammonia and glutamate, and the amounts formed over the test period in control solutions that did not have additions of L-glutamate or ammonia.

The degradation rate K (%/day) was derived by plotting the log of glutamine concentration (as % of original) against time (days). K is the antilog of the gradient thus formed.

Table 2 Degradation rates (%/day) of L-glutamine in various solutions/mixtures at different temperatures using the glutamine method (A) and the ammonia method (B)†

Solution ††	pH	Temperature					
		22–24°C		4°C		–80°C	
		A	B	A	B	A	B
Synthamin/Dextrose	5.8	0.89	0.90	0.10	0.10	un	un
Synthamin/Dextrose/Lipid	5.8	0.70	–	0.20	–	–	–
Aminoplex A/Dextrose	5.9	0.67	0.68	0.20	0.10	un	un
Aminoplex B/Dextrose	6.0	0.67	0.77	0.18	0.10	un	un
Eloamin A/Dextrose	5.8	0.75	0.65	0.25	0.10	un	un
Eloamin B/Dextrose	5.8	0.74	0.71	0.20	0.14	un	un
Vamin/Dextrose	5.6	0.65	0.73	0.18	0.13	un	un
Perifusin	6.7	0.53	0.58	0.10	0.08	un	un
Dextrose 15%	4.2	0.17	0.14	0.10	0.09	un	un

† See text for details of method A and B

†† For detailed composition see Table 1

un = undetectable

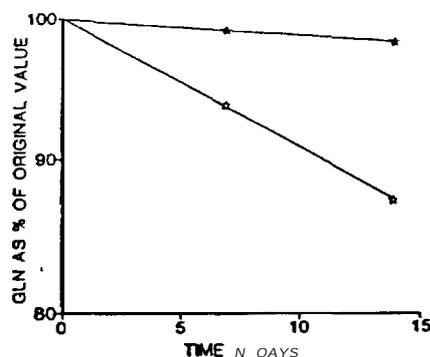


Fig. 1. the degradation of L-glutamine (GLN) ● 22-24°C in a dextrose solution 15% w/v (\*) and in a Synthamin/dextrose mixture (\*). see Table 1 for detailed composition. Results ● e plotted on a semilogarithmic scale (y axis only).

### Results

The intrabatch coefficient of variation for the measurement of glutamine, glutamate and ammonia were 1.1%, 0.6% and 0.5%, respectively. The corresponding values for interbatch variation were 1.7%, 0.8% and 0.7%. Measurement of these metabolites in duplicate samples gave virtually identical results, and therefore, only the mean results are given below.

Rates of glutamine degradation calculated by the two methods agreed closely with each other (Table 2) because, as expected (7), ammonia appeared in the solution at an approximately equimolar rate as the loss of glutamine (Fig. 2).

The degradation rate of glutamine was highest at 22-24 °C (0.6-0.9%/day), five-fold lower at 4°C (0.1-0.2%/day) and undetectable at -20°C. In the TPN samples stored at -20°C (Synthamin/dextrose), degradation was minimal (<0.04%/day) over a period of 14 days (results not shown in Table 2). In dextrose solution (5-25% w/v 188-938 kcal/l) at 22-24°C the degradation rate ranged from 0.10-0.20%/day, the lowest degradation rate corresponding to the highest dextrose concentration. In dextrose 15% w/v (563 kcal/l) the rate of degradation was 0.15%/day (Fig. 1). Therefore, the presence of additional constituents in TPN solutions (Table 1) enhances glutamine degradation (Table 2).

The initial concentrations of ammonia in the amino-add containing solutions were less than 0.25 mmol/l and remained close to this value in the control samples or those to which glutamate or

pyroglutamate was added (14-day period at room temperature). In the control experiments in which ammonia and glutamate were added to the various solutions, both metabolites were completely recovered at the end of the study. Furthermore, in the control experiment in which pyroglutamic acid was added to the intravenous solutions, no ammonia, glutamate or glutamine appeared.

The pH of any solution did not change by more than 0.2 pH units during the study period. Initial ammonia values were close to zero in the amino-acid containing solution/mixture (<0.25 mmol/l and did not rise above 0.5 mmol/l in any of the control samples). Glutamine degradation rates in samples stored in gas impermeable bags, were virtually identical to those stored in glass vials (room temperature).

### Discussion

The recognition of the potential importance of glutamine in the metabolism of various cells and tissues (2, 11-16), has led to attempts to administer it as a component of parenteral nutrition solutions. The use of soluble and stable glutamine containing dipeptides, which are readily hydrolysed within

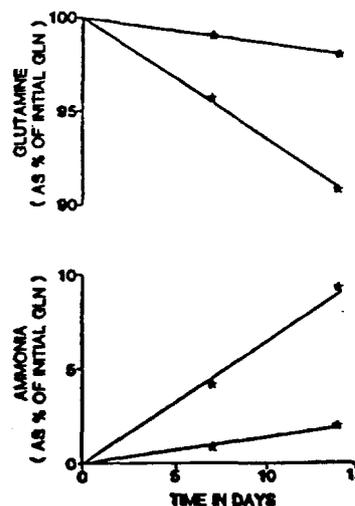


Fig. 2. Graph showing the equimolar formation of ammonia from the degradation of L-glutamine (GLN) in Aminoplex (A)/dextrose at 4°C (●) and at 22-24°C (★) (see Table 1 for detailed composition). Upper graph shows results derived by measuring glutamine alone. Lower graph shows results derived from repeated measurements of ammonia, and initial glutamine concentration.

the body to yield free amino-acids, provides one way of achieving this aim (6). Another, more economic option, is to administer free glutamine, but (his option depends on the stability and possible toxicity of glutamine and its degradation products.

Since heating for even short periods of time may cause substantial degradation of glutamine (3, 8), heat sterilisation was avoided in this study. Instead (the glutamine solution was aseptically prepared and filtered through a 0.2 $\mu$  filter prior to mixing it with the other nutrients.

In this study there was close agreement between the rate of loss of glutamine and the rate of appearance of ammonia, which increases confidence in the results. The formation of ammonia occurs irrespective of whether glutamine degrades to glutamate or pyroglutamate. Since no glutamate was formed it is presumed that glutamine degrades quantitatively to pyroglutamate.

The rate of glutamine degradation in typical parenteral nutrition solutions (0.8%/day at room temperature, or 0.1-0.15%/day at 4°C) was found to be slower than that reported in several other solutions (3, 4, 5) e.g. in buffers maintained at similar pH and temperature as the parenteral solutions. The rate of glutamine degradation in the commonly used intravenous solutions is sufficiently slow to consider the possible clinical use of such solutions as a viable option (although the degradation of glutamine in atypical TPN solutions including those with unusual mineral concentrations requires investigation).

There are at least four ways in which this option may be achieved. *One* is to prepare a fresh glutamine solution and mix it with other nutrients on the day of administration. This reduces to a minimum the time over which glutamine can degrade. *Second*, since the degradation of glutamine at 4°C is several-fold less than at room temperature, storage of such solutions at 4°C is demonstrably associated with the loss of only about 2% glutamine over a period of 14 days. *Third*, it may be possible to prepare, store and/or administer glutamine solutions containing dextrose alone, which slows glutamine degradation even further (Fig. 1, Table 1). *Fourth*, it may be possible to freeze a solution of glutamine in water, dextrose, or TPN solution (e.g. -20°C -WC) and thaw it for infusion or for mixing with other nutrients on the day of requirement. The evidence suggests that over a period of at least 2-4 weeks there is minimal or undetectable degradation of glutamine in intravenous solutions stored

between -20°C and -80°C (this work and (7,8)). In addition glutamine does not appear to affect the stability or size of the lipid particles in 'all in one mixture' (8).

It should be emphasised that the use of glutamine in TPN solutions in clinical practice should take into account not only the stability of glutamine (and formation of degradation products) but also the possible toxicity effects of such infusions, which might vary in different circumstances. In one recent study in which glutamine containing solutions were administered in healthy subjects (17), no toxicity effects were identified and no significant measurements in ammonia concentrations were noted.

The increase in ammonia concentration in TPN solutions kept at room temperature (0.5 mmol/L/day in solutions containing about 70 mmol glutamine/L) is small and not likely to be an important clinical problem. The body handles up to about 1 mole of ammonia per day (14gN), which is used to form urea. Furthermore, administration of an oral bolus dose of about 7g (-131 mmol) ammonium chloride (i.e. 0.1g/kg in a 70kg man), which is used clinically as a diagnostic test for renal tubular acidosis, provides 20-30-fold more ammonia than a TPN solution containing a total of as much as 5mmol ammonia. Although the latter delivers ammonia directly into the systemic circulation, it is administered very slowly (frequently over 24 h - cf. bolus dose of ammonium chloride).

Alleged toxicity of pyroglutamic acid (also known as 5-oxoproline, 2-pyrrolidone-5-carboxylic acid) requires further investigation, but it is unlikely to cause symptoms if given in small amounts. This is partly because tissues are normally exposed to small concentrations of pyroglutamic acid (18), (it is also a normal constituent of mammalian protein/polypeptides, and an intermediate in the gamma-glutamyl cycle (19,20), and partly because of recent studies in normal subjects involving administration of TPN solutions containing glutamine have not been associated with detectable toxic effects (17). Furthermore protein hydrolysates such as 'Aminosol' (a casein hydrolysate), which was at one time used routinely in TPN, contained substantial amounts of pyroglutamic acid (4% of total N, corresponding to 34 mmol pyroglutamic acid per 12g N[21]). Again, toxicity effects due to pyroglutamic acid or ammonia were apparently not identified.

In summary this study suggests that it should be possible to prepare, store and administer glutamine in parenteral nutrition solutions, with little

198 STABILITY OF L-GLUTAMINE IN TPN SOLUTIONS

degradative loss, but administration of such solutions in clinical practice requires further evaluation of their benefits and possible toxicity.

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