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Total Parenteral Nutrition in Sick Preterm Infants: Effects of Cysteine Supplementation with Nitrogen Intakes of 240 and 400 mg/kg/day

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Summary: The effects of supplementing total parenteral nutrition (TPN) solutions with cysteine were assessed at two different levels of nitrogen intake by determining nitrogen retention, sulfate excretion, and sulfur-containing amino acid concentrations. Ten infants received 72 mg/kg/day of cysteine-HCl in a TPN solution for a period of 6 days. Five of these infants received 251 ± 48 ($\bar{x}$ ± SD) mg/kg/day of nitrogen, and five received 403 ± 45 mg/kg/day of nitrogen. Two other groups of five infants each received unsupplemented TPN at nitrogen intakes of 235 ± 48 and 412 ± 54 mg/kg/day, respectively. Fluid and nonprotein caloric intakes were similar for all four groups. Cysteine supplementation increased plasma and urine free cyst(e)ine concentrations and enhanced total sulfur retention, but did not enhance nitrogen retention. [Cyst(e)ine refers to the mixture in any proportion of the sulfhydryl (cysteine) and the disulfide (cystine) forms of this compound.] Nitrogen retention, sulfate excretion, cyst(e)ine excretion, and plasma taurine concentrations increased as the result of the increase in nitrogen intake.
Key Words: Cysteine—Total parenteral nutrition—Infants.

Total parenteral nutrition (TPN) is an effective means of nourishing infants who are unable to be fed enterally. It is used to sustain infants until enteral nutrition is fully established or as a means of providing adequate calories and nitrogen to promote growth (1–3). The developing metabolic capabilities of infants, however, make the requirements for individual components of parenteral nutrition regimens uncertain.

Gaull et al. (4) observed that hepatic cystathionase, the rate-limiting enzyme in the production of cysteine from methionine, was virtually absent in second-trimester human fetuses, but that enzyme activity increased with gestational age. Thus, cysteine may be essential for the preterm and newly born term infant. As preterm and term infants increase in postnatal age, however, hepatic cystathionase activity increases rapidly (5). From a developmental standpoint the enzymatic mechanism for the synthesis of cysteine from methionine appears to be in place, but in an evolving state at birth, for both preterm and term infants. Whether or not an exogenous source of cysteine is required during TPN in infancy has not been resolved on the basis of developmental biochemical data.

Several studies have attempted to document clinically the need for an exogenous source of cysteine during infancy. The data most frequently cited in support of a cysteine requirement come from a report by Snyderman (6) that documents impaired nitrogen retention and weight gain in infants in the absence of an enteral source of cysteine. Pohlandt (7) attempted to demonstrate the need for an exogenous source of cysteine during TPN in an indirect manner by documenting that plasma concentrations of half-cystine did not increase in infants receiving TPN when an adequate source of methionine was provided. Recently, Zlotkin et al. (8) cast doubt on the essential nature of cysteine when they observed that infants receiving cysteine-supplemented TPN failed to retain nitrogen and gain weight better than infants who received unsupplemented TPN.

In the sick preterm infant during the first weeks

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of life it is difficult to provide caloric intakes much greater than 60 kcal/kg/day by peripheral intravenous administration. At this relatively low caloric intake, however, nitrogen retention has been demonstrated with a nitrogen intake of 400 mg/kg/day (3). This caloric intake has also been shown to meet the total energy expenditure of infants in a thermoneutral environment (9,10). The effects of supplementing TPN solutions with cysteine at 60 kcal/kg/day and 400 mg/kg/day of nitrogen have not been evaluated. Nor has the effect of supplementing TPN solutions with cysteine at a lower level of nitrogen intake been determined. We report here the effects of cysteine supplementation at nitrogen intakes of 240 and 400 mg/kg/day with a nonprotein caloric intake of 60–70 kcal/kg/day.

PATIENTS AND METHODS

Infants were enrolled in the study protocol if they were at least 2 days old, unable to take enteral feeds, and unlikely to begin enteral feeding within 5–7 days; or if they had been without any enteral intake for 48 h and would be unable to tolerate enteral feeds for at least 5–7 days. Of the 20 infants enrolled in the study, 16 had a primary diagnosis of respiratory distress syndrome, 3 had a primary diagnosis of necrotizing enterocolitis, and 1 had gastroschisis. Clinical information concerning the characteristics of the infants enrolled in the study is given in Table 1.

After obtaining parental permission to enroll an infant in the study protocol, the infant was begun on the supplemented or unsupplemented TPN regimen that had been selected randomly. Infants in the low nitrogen intake groups (Group 1, unsupplemented; Group 2, cysteine-supplemented) received 150 ml/kg/day of fluid, 60 kcal/kg/day of glucose, and 240 mg/kg/day of nitrogen (Aminosyn, Abbott Laboratories, Abbott Park, Chicago, IL). The only sulfur-containing amino acid in Aminosyn is methionine. Infants in the high nitrogen intake groups (Group 3, unsupplemented; Group 4, cysteine-supplemented) received the same nonprotein caloric and fluid intakes as the low nitrogen groups but were given 400 mg/kg/day of nitrogen. The infants in the two groups receiving cysteine supplementation were given 72 mg/kg/day of cysteine-HCl (Abbott) added to the TPN solution. The other components of the TPN solution are listed in Table 2. During the course of the 6-day period of TPN none of the infants received any enteral nutrition. No in-

TABLE 1. *Characteristics of infants studied*[a]

Characteristic	Low nitrogen intake		High nitrogen intake	
	Group 1 TPN n = 5	Group 2 TPN + CYSE n = 5	Group 3 TPN n = 5	Group 4 TPN + CYSE n = 5
Birthweight (kg)	1.46 (900–2300)	1.37 (910–2500)	1.25 (970–1510)	1.01 (890–1150)
Age enrolled (days)	4 (3–5)	7 (4–11)	20 (3–53)	5 (4–5)
Fluid intake (ml/kg/day)	158 ± 16	133 ± 30	158 ± 16	157 ± 43
Nonprotein calories (kcal/kg/day)	63 ± 13	63 ± 5	73 ± 11	67 ± 8
Nitrogen intake (mg/kg/day)	235 ± 48	251 ± 48	412 ± 54	403 ± 45
Cysteine intake (mg/kg/day)	0	72	0	72
Weight change per 6-day study period (g)	−59 (−160 to 40)	+6 (−40 to 60)	−12 (−90 to 60)	−14 (−80 to 20)

Abbreviations used: TPN, total parenteral nutrition; CYSE, cysteine.
[a] Values are means ± SD, or range.

TABLE 2. *Parenteral nutrition solution components*

Component	Quantity per day
Amino acids[a]	240 or 400 mg/kg nitrogen
Glucose	15 g/kg
Sodium chloride	2–6 mEq/kg
Potassium chloride	2–3 mEq/kg
Calcium gluconate	0.9 mEq/kg
Magnesium sulfate	0.25 mEq/kg
Multivitamin concentrate[b]	1.0 ml/kg
Zinc	300 μg/kg
Copper	20 μg/kg

[a] Amino acids provided by Aminosyn (Abbott Laboratories, Abbott Park, Chicago, IL). In grams per 100 g amino acids: isoleucine, 7.3; leucine, 9.5; lysine, 7.3; methionine, 4.0; phenylalanine, 4.4; threonine, 5.3; tryptophan, 1.7; valine, 8.0; tyrosine, 0.6; alanine, 12.9; arginine, 10.0; histidine, 3.0; proline, 8.8; serine, 4.3; glycine, 12.9.

[b] Multivitamin concentrate (USV Pharmaceutical, New York, NY). Each 5-cc vial contained ascorbic acid, 500 mg; vitamin A, 10,000 USP units; vitamin D, 1,000 USP units; thiamine, 50 mg; riboflavin, 10 mg; niacinamide, 100 mg; pyridoxine-HCl, 15 mg; despanthenol, 25 mg; *dl*-α-tocopheryl acetate, 5 IU.

travenous fat emulsions were used during the study period, because the majority of infants were still in the acute phase of respiratory disease and were on ventilators and many had bilirubin concentrations in the 6–10 mg/dl range.

Throughout the 6-day period all infants were maintained on radiant warmers. The infants were weighed daily, and fluid intake and urinary output were accurately recorded hourly. Total parenteral nutrition solutions were administered through peripheral intravenous lines by continuous infusion delivered by IVAC pumps (IVAC Corporation, San Diego, CA). Laboratory measurements of serum electrolytes, blood urea nitrogen, plasma ammonia, serum alanine aminotransferase, serum aspartate aminotransferase, and hemoglobin were made during the 6-day period at the discretion of the primary physician and not as part of the study. On day 6 of the study a 24-h urine collection was begun. Urine was collected in adherent plastic bags attached to the infants' perineum. Urine that leaked from the bag was recorded by weighing the diaper. Urine was collected from the bag hourly and frozen in a plastic container. No stool or significant gastric drainage was recorded during any of the collection periods. Blood was collected in a heparinized syringe through an umbilical artery catheter or by peripheral venipuncture at least 12 h into the 24-h urine collection. The blood was taken immediately to the laboratory on ice, and the red blood cells separated from the plasma by centrifugation at 1,000 $\times g$ in a Beckman microcentrifuge (Beckman Instruments, Palo Alto, CA). The plasma was decanted from the red blood cells, and the plasma proteins were precipitated with a 3% sulfosalicylic acid solution (1:3, volume/volume). After centrifugation at 17,000 $\times g$ for 10 min the supernatant solution was decanted and frozen at −20°C until time of analysis.

Plasma and urine amino acid concentrations were measured with a Beckman 119 CL amino acid analyzer; free cyst(e)ine was measured by the method of Gaitonde (11) as modified by Malloy et al. (12). [Cyst(e)ine refers to the mixture in any proportion of the sulfhydryl (cysteine) and the disulfide (cystine) forms of this compound.] Urine sulfate was measured by the method of Jackson and McCandless (13). Total nitrogen in the urine was determined by Kjeldahl analysis, and nitrogen retention was calculated as nitrogen intake minus 24-h urine nitrogen output. Nitrogen intake was calculated on the basis of each 100 g of Aminosyn being composed of 16% nitrogen.

Analysis of the nitrogen retention, sulfate excretion, plasma sulfur-containing amino acid, and urine sulfur-containing amino acid data was performed by two-way analysis of variance. For each variable measured the effect of cysteine supplementation or lack of supplementation, the effect of high or low nitrogen intake, and the interaction of cysteine and nitrogen intake were determined. The level of a significant effect was set at a p value of <0.05. Significant differences between plasma amino acid concentrations at the low and high nitrogen intakes were determined by the Mann-Whitney test.

RESULTS

The addition of cysteine to TPN solutions did not cause the infants to gain weight more rapidly. The caloric intake of all the groups was low, and gains in weight were the exception (Table 1). Of the sulfur-containing amino acid concentrations measured in the plasma, cysteine supplementation had a significant effect only on free cyst(e)ine ($p < 0.0001$) (Table 3). The concentration of half-cystine bound to plasma proteins was not affected by cysteine supplementation. Neither sulfate excretion nor nitrogen retention was affected by the addition of cysteine. Infants receiving cysteine supplementation did have significant increases in urine cyst(e)ine concentrations ($p < 0.0001$). Cysteine intake had no significant effect on the remainder of

TABLE 3. *Effect of cysteine and nitrogen intake on sulfur-containing amino acid levels, nitrogen retention, and sulfate excretion*[a]

Amino acid, sulfate, or nitrogen	Group 1	Group 2	Group 3	Group 4	Cysteine effect p value	Nitrogen effect p value	Cysteine nitrogen interaction p value
Plasma concentration (μmol/dl)							
Methionine	2.38 ± 0.66	2.84 ± 2.31	3.94 ± 2.19	3.0 ± 3.4	0.8221	0.4249	0.5146
Cystathionine	0.12 ± 0.27	0.32 ± 0.44	0.44 ± 0.87	0.0	0.6054	1.0000	0.1790
Free cyst(e)ine	4.46 ± 1.13	11.34 ± 3.38	4.20 ± 1.34	14.48 ± 3.83	0.0001	0.2509	0.1787
Bound half-cystine	7.40 ± 3.20	7.90 ± 2.80	4.20 ± 2.10	5.40 ± 2.40	0.2500	0.0251	0.1376
Total cyst(e)ine	11.9 ± 3.7	19.2 ± 5.9	8.4 ± 3.1	19.9 ± 6.0	0.0005	0.2250	0.1115
Taurine	4.12 ± 1.99	2.36 ± 1.34	5.96 ± 3.89	11.22 ± 7.07	0.3664	0.0118	0.0807
Urine concentration (μmol/dl)							
Methionine	8.14 ± 2.29	0.10 ± 0.22	3.54 ± 3.5	0.0	0.0001	0.0233	0.0288
Cystathionine	0.74 ± 0.70	1.40 ± 2.55	6.40 ± 5.99	2.08 ± 1.06	0.2349	0.0483	0.1125
Free cyst(e)ine	8.64 ± 2.66	47.16 ± 23.75	22.02 ± 17.9	83.70 ± 21.83	0.0001	0.0082	0.1808
Taurine	24.00 ± 15.85	23.82 ± 27.04	42.46 ± 47.92	43.7 ± 40.58	0.9715	0.2386	0.9625
Sulfate excretion (mg/kg/day)	15.84 ± 9.63	21.22 ± 10.49	52.48 ± 13.52	59.08 ± 10.61	0.2260	0.0001	0.8996
Nitrogen retention (mg/kg/day)	114.00 ± 20.05	150.20 ± 61.98	229.80 ± 34.24	291.60 ± 35.74	0.4552	0.001	0.2425

[a] Values are means ± SD.

the plasma or urine amino acid concentrations measured.

Nitrogen intake had a significant effect on plasma-bound half-cystine and taurine concentrations and on urine methionine, cystathionine, and free cyst(e)ine levels. Nitrogen intake also had a significant effect on a number of other plasma amino acid levels (Table 4).

The urine methionine concentrations in the infants receiving cysteine supplementation reported here are low. We are concerned that this is an artifact produced by a lack of peak separation by the amino acid analyzer. Half-cystine is eluted from the analyzer column just prior to the elution of methionine. In the infants receiving supplementation the large half-cystine peaks measured in the urine may have incorporated the methionine peaks.

We calculated the total sulfur intake, the total sulfur retained, and the percentage of the total sulfur intake retained (Table 5). Aminosyn contains sulfur in the form of methionine (21.5% sulfur) and the antioxidant perservative potassium metabisulfite which provides approximately 18 mg/dl of sulfur per deciliter of Aminosyn. In the infants receiving supplementation cysteine was the only other major source of sulfur (26.5% sulfur). Although sulfur-containing medications were given to all the infants,

TABLE 4. *Effect of nitrogen intake on plasma amino acid levels*

	Nitrogen intake 240 mg/kg/day[a] (μmol/dl) n = 10	Nitrogen intake 400 mg/kg/day[b] (μmol/dl) n = 10
Essential		
Threonine[c]	16.5 ± 7.8	32.0 ± 13.9[d]
Valine[c]	10.9 ± 8.1	17.2 ± 5.3[d]
Isoleucine[c]	3.2 ± 2.0	5.1 ± 1.8[e]
Leucine[c]	5.5 ± 3.6	8.8 ± 2.8[e]
Phenylalanine[c]	5.7 ± 1.6	5.3 ± 2.0
Lysine[c]	12.4 ± 7.7	17.1 ± 9.9
Nonessential		
Alanine[c]	11.9 ± 8.0	20.4 ± 7.4[e]
Glycine[c]	29.4 ± 12.3	65.3 ± 22.0[d]
Serine[c]	10.5 ± 3.0	24.9 ± 10.3[d]
Proline[c]	8.6 ± 4.0	17.6 ± 5.6[d]
Arginine[c]	3.6 ± 2.9	8.1 ± 4.9[d]
Ornithine	5.0 ± 2.5	9.1 ± 5.0
Glutamate	25.1 ± 12.3	38.6 ± 16.7
Aspartate	0.8 ± 0.6	0.6 ± 0.6
Tyrosine[c]	2.9 ± 1.9	2.3 ± 2.4
Histidine[c]	4.9 ± 1.3	6.6 ± 1.9

[a] Mean ± SD of Group 1 and Group 2 concentrations. There were no differences between these two groups.
[b] Mean ± SD of Group 3 and Group 4 concentrations. There were no differences between these two groups.
[c] Present in amino acid solution.
[d] $p < 0.01$, [e] $p < 0.05$. The amino acid concentration at an intake of 400 mg/kg/day nitrogen was significantly different from the amino acid concentration at 240 mg/kg/day nitrogen.

TABLE 5. *Comparison of total sulfur intake and total sulfur retained*[a]

Group no.	Total sulfur intake (mg/kg/day)[b]	Total sulfur retained (mg/kg/day)[c]	Retention (%)
1	18.1 ± 3.2	12.0 ± 5.2	65 ± 20
2 (+ cysteine)	38.2 ± 3.4[d]	29.5 ± 2.1[f]	77 ± 6[e]
3	31.6 ± 4.3	12.4 ± 6.7	38 ± 20
4 (+ cysteine)	50.1 ± 3.6	27.2 ± 5.5	54 ± 9

[a] Values are means ± SD.
[b] Total sulfur intake was calculated as the sum of the sulfur contained in the amino acid solution (Aminosyn) in the form of methionine and potassium metabisulfite. The quantity of sulfur in cysteine has been added for those groups receiving cysteine.
[c] Total sulfur retained was calculated as the total sulfur intake minus the total sulfur excreted (total sulfur excreted = urine sulfur in the form of methionine + cysteine + cystathionine + taurine + sulfate).
[d] $p < 0.05$, [e] $p < 0.005$, [f] $p < 0.0005$. Group 2 was significantly different from Group 3.

the quantity of sulfur intake from this source has been estimated to be low (14). Our calculations show little absolute increase in sulfur retention with an increase in sulfur intake when Group 1 is compared to Group 3 (12.0 ± 5.2 vs. 12.4 ± 6.7 mg/kg/day) or when Group 2 is compared to Group 4 (29.5 ± 2.1 vs. 27.2 ± 5.5 mg/kg/day). The addition of cysteine, however, appears to enhance sulfur retention. Although the total sulfur intakes of Group 2 (38.2 ± 3.4 mg/kg/day, cysteine supplementation) and Group 3 (31.6 ± 4.3 mg/kg/day, no supplementation) are not exactly comparable, the absolute quantity of sulfur retained by Group 2 is more than twofold greater than that retained by Group 3 (29.5 ± 2.1 vs. 12.4 ± 6.7 mg/kg/day, $p < 0.0005$).

DISCUSSION

Our data show no significant enhancement of nitrogen retention when TPN solutions are supplemented with cysteine at nitrogen intakes of approximately 240 and 400 mg/kg/day. These results concur with the findings of Zlotkin and Anderson (8). Our data differ from their data in that we observed a more obvious effect of cysteine supplementation on plasma cyst(e)ine concentrations. We attribute this observation to the method we used for measuring cyst(e)ine that measures both the sulfhydryl and disulfide forms of this compound. We demonstrated previously that a significant quantity of cysteine infused parenterally remained in the sulfhydryl form in the plasma and urine and that automated amino acid analysis could not measure accurately free cyst(e)ine in the plasma or urine (15).

Nitrogen intake had a significant effect on plasma-bound half-cystine and taurine and urine methionine, cystathionine, and free cyst(e)ine concentrations. The effect on these sulfur-containing amino acid levels observed after an increase in nitrogen intake is likely to be the effect of methionine intake which increases concomitantly with nitrogen intake. The decrease in the concentration of plasma-bound half-cystine with an increase in nitrogen intake is an observation for which we have no ready explanation. The fact that plasma taurine and urine cyst(e)ine concentrations increased at the higher nitrogen intake suggests that the metabolic pathway from methionine to taurine is active. This observation supports the reports of Gaull et al. (4) and Zlotkin and Anderson (5) that the activity of hepatic cystathionase, the rate-limiting enzyme in the synthesis of cysteine from cystathionine, matures rapidly with postnatal age.

Sulfate excretion in the urine was not affected by cysteine intake but was significantly affected by an increase in nitrogen intake ($p < 0.0001$).

We observed a greater retention of sulfur in infants receiving cysteine supplementation, as did Zlotkin and Anderson (14). As the requirements for sulfur-containing amino acids or inorganic sulfur are met, however, the percentage of additional sulfur retained decreases. The surplus sulfur is excreted, primarily in the form of sulfate. Previous investigators have correlated sulfur intake with sulfate excretion (16,17).

Cysteine supplementation had no significant effect on the other plasma amino acid levels that we measured. The increase in nitrogen intake from 240 mg/kg/day to 400 mg/kg/day, however, resulted in concentration increases in a number of plasma amino acids. Based on weight gain there appears to be no particular advantage in infusing more nitrogen when the caloric intake is low. Based on nitrogen retention data the infusion of more nitrogen results in enhanced nitrogen retention even at low caloric intakes. The advantages of the higher nitrogen retention and the fate of the retained nitrogen are uncertain.

Whether or not cysteine can be considered an essential amino acid for the newborn, in particular the preterm infant, is from our perspective still uncertain. Data from our studies and from Zlotkin's observations indicate that cysteine does not en-

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