To the Graduate Council:

I am submitting herewith a dissertation written by Mitzi Wilkinson Davis entitled "The Response of Premature Infants to Parenteral Amino Acids in the First Week of Life." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Home Economics.

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The Graduate School

THE RESPONSE OF PREMATURE INFANTS TO PARENTERAL AMINO ACIDS IN THE FIRST WEEK OF LIFE

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Mitzi Wilkinson Davis

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In Memory of

Robert Gordon Wilkinson

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Those who mean the most never truly leave us.
They live on in the values they imparted,
the wisdom they shared,
and the love they brought into our lives.

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ABSTRACT

This study investigated the response of premature infants to parenteral amino acids and related that response to gender, amino acid concentration at birth, amino acid status prior to infusion, postnatal age, caloric intake, weight, intake of individual amino acids, and gestational age. Plasma samples were collected from 21 premature infants (gestational age 31.2 ± 2.3 weeks) before the initiation of amino acid infusion and after 24 hours of infusion. Mixed umbilical cord samples were available from 10 of these infants. Eleven (11) infants (gestational age 30.8 ± 2.8 weeks) receiving parenteral amino acids with no enteral supplementation were evaluated at one week. Plasma amino acid concentrations were compared to those reported in breast fed term infants and in preterm umbilical cord samples. Intakes were compared to published recommendations. The amino acid source was Aminosyn (Abbott Laboratories, Chicago, Ill.).

Neither gender, plasma amino acid concentration at birth or weight were influencing factors. Changes in concentration of most amino acids appeared to be more a result of endogenous regulation than simple intake. Alanine infused at a high concentration showed relatively little plasma accumulation while the same concentration of glycine in the infusate lead to excessive plasma concentrations of glycine and possibly serine. Plasma concentration of histidine was high in most infants and varied directly with gestational age. Plasma phenylalanine levels varied inversely with postnatal age and tyrosine concentration varied directly

with gestational age. There were indications of cysteine and taurine synthesis in some infants. Methionine intakes exceeded recommendations in all infants at one week and were associated with excessive plasma concentration. Valine concentration varied directly with caloric intake, gestational age, and valine intake. Leucine concentration also increased with gestational age from 27-33 weeks, but the relationship after that time is unclear.

The amino acid source (Aminosyn, Abbott Laboratories, Chicago, Ill.) does not appear to have ideal composition for preterm infants. For use during the first week of life when intake of both amino acids and calories is low, the infusate should contain less glycine and methionine and possibly less serine, proline, histidine, and valine.

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CHAPTER I

INTRODUCTION

There is little doubt that the quality of nutrition affects the development of the fetus and the infant. There is also little doubt that optimal nutrition of the infant born prematurely is very difficult to achieve. The preterm infant is by definition functionally compromised and cannot ingest, digest, assimilate, metabolize, synthesize, or excrete nutrients with the competence of the mature newborn. An immature digestive system is a major handicap (1). A weak or absent sucking reflex is not coordinated with swallowing before approximately 32 weeks gestation or 1500 grams body weight. An incompetent cardiac sphincter coupled with a relatively strong pyloric sphincter results in frequent regurgitation and danger of aspiration. The premature has small stomach capacity and decreased gastric acidity, which interferes with protein denaturation. Gastrointestinal immune response is low and abdominal musculature is weak. Carbohydrates and proteins appear to be sufficiently digested and absorbed, but fat absorption is poor (2). In addition to these basic problems, complications such as respiratory distress syndrome with assisted ventilation make oral feedings impossible. There has been, therefore, a long recognized need to provide intravenous nutrition for very premature infants while the gastrointestinal tract matures.

Two reports in 1966 mark the beginning of serious attempts at intravenous alimentation in premature infants. Cornblath, Forbes, and

Phildes (3) demonstrated that early intravenous feedings of glucose to very low birth weight infants led to decreased mortality. Auld, Bhanganada, and Mehta (4) subsequently showed that hypoglycemia and weight loss were also decreased by similar intervention. It was Dudrick and associates (5), however, whose techniques made possible the administration of hypertonic dextrose and protein solutions and who demonstrated that slow continuous infusion of these nutrients could lead to normal progression of growth and development in term infants unable to feed enterally. Since that time parenteral nutrition has also been used extensively in the management of premature infants.

Although parenteral nutrition is now routine in most institutions caring for prematures, the ideal composition of the infusate has yet to be determined. Major concerns include (a) optimal growth for the preterm infant and the calories and nitrogen necessary to achieve that growth, (b) renal and hepatic function of the premature, (c) effects of deficiency and/or excess of individual amino acids in plasma, and (d) development of both synthetic and catabolic pathways at various gestational and postnatal ages.

Although clinical impressions suggest that one cannot view all prematures as a homogenous group, there have been relatively few attempts to systematically determine differences in amino acid metabolism in vivo according to degree of prematurity. This study assessed the response of infants of varying gestational ages to parenteral amino

acids and related that response to gender, plasma amino acid concentration at birth and immediately prior to infusion, postnatal age, caloric intake, weight, intake of individual amino acids, and gestational age. Plasma amino acid concentrations were used to evaluate the appropriateness of individual amino acid intake and the composition of parenteral fluids for premature infants in the first week of life.

CHAPTER II

REVIEW OF LITERATURE

This study was concerned with individual amino acids, their intake and their plasma concentrations, in relation to a number of variables. Following is a selected review of (a) amino acid metabolism in the preterm infant, and (b) recommendations for individual amino acid intake and for composition of parenteral fluids for the premature.

Amino Acids in the Premature

Alanine

Alanine is a nonessential amino acid involved in several aspects of human protein metabolism. In addition to participation in protein synthesis, alanine is the major amino acid source of glucose in either the postabsorptive or fasting state. When needed, nitrogen liberated from amino acids in muscle is transported as alanine to the liver where, in a reversible reaction catalyzed by alanine aminotransferase, the alanine is converted to pyruvate for synthesis of glucose (6).

The fetus presumably does not synthesize glucose in utero, and it is unclear if there are significant modifications of alanine metabolism in the human infant. In rats alanine aminotransferase differs from other enzymes closely involved in gluconeogenesis (glutamate dehydrogenase and aspartate aminotransaminase) in that its activity does not increase postnatally until weaning (7). In humans the aspartate aminotransferase is elevated at birth but alanine aminotransferase is not (8).

Despite the absence of gluconeogenesis the fetal net uptake of alanine is greatest of all amino acids, and at term it is the only non-essential amino acid supplied to the fetus in significant quantities (9). Pohlandt (10) found no elevated plasma levels despite high intravenous intakes in infants and suggested alanine as a suitable source of non-essential nitrogen during parenteral nutrition. Recommended intakes for preterm infants vary from 100-700 mg/kg/day (10-13).

Arginine and Ornithine

In addition to their function in protein synthesis, arginine and ornithine are integral intermediates in the urea cycle (14). Both are continually synthesized in this cycle as arginine is hydrolyzed by the enzyme arginase to produce ornithine and urea. The ornithine is reused in a cyclical process in which it reacts with carbamyl phosphate to form citrulline which then reacts with a second nitrogen from aspartate to reform arginine. In addition to being recycled, ornithine can also be synthesized directly from glutamate in a reaction catalyzed by ornithine aminotransferase. Ornithine aminotransferase attains maximum activity in human liver before the 20th week of gestation and still exceeds adult values at birth (15). The high perinatal activity of ornithine aminotransferase is in agreement with Snyderman's finding of no adverse effects of an arginine deficient diet on infant growth and development (16).

Despite its endogenous production, the addition of arginine to parenteral fluids has been found to be clinically advantageous. Gullino et al. (17) observed that arginine decreased the accumulation of

ammonia resulting from the deamination of other amino acids. This finding has been replicated by others (18, 19) and arginine is now routinely used to protect against the toxic effects of ammonia by enhancing the urea cycle.

A second important action of arginine is its stimulation of endogenous insulin secretion (20) which tends to be slow and weak in the premature infant (21). The release of adequate insulin is necessary for proper assimilation of the concomitantly administered glucose and the prevention of hyperglycemia. Arginine infusion has also been shown to induce the release of an immunodetectable growth hormone (22) which, in addition to other actions, is thought to impair insulin inactivation and thus prolong insulin half-life (23).

Although considered a nonessential amino acid, significant amounts of arginine are now almost universally added to total parenteral fluids. As a basic amino acid it serves a useful function in increasing the pH of the parenteral solution, thereby decreasing the chance of metabolic acidosis. Arginine is exceedingly well tolerated at doses as high as 1200 mg/dl (11), but the fetus is thought to extract less than 87 mg/kg/day at term from umbilical plasma (10). Dosage recommendations for arginine vary from 110-250 mg/kg/day (10-13). Administration of ornithine is not considered necessary when adequate arginine is provided.

Glycine and Serine

Glycine and serine are nonessential amino acids which can be interconverted through the glycine cleavage system (8). Serine can

also be synthesized from glucose (14). The enzyme involved in the equilibration of serine and glycine, serine hydroxymethyl transferase, has been found to be as active in human fetal and newborn liver and kidney as in adult tissues (25).

Glycine is utilized in the synthesis of proteins, heme, purines, glycocholate, glutathioine, hippuric acid, and creatine. Serine, in addition to its interconversion with glycine, participates in the synthesis of cephalins, ethanolamine, choline, cysteine and glucose. Both of these amino acids are metabolically active and have rapid turnover rates (14).

Because it is comparatively cheap, glycine has been used as a means of increasing the nitrogen content of parenteral amino acid solutions. This high glycine intake has not always been innocuous, however, as it has been associated on several occasions with hyperammonemia and hyperglycinemia (11). The premature's need for glycine is unclear as Pohlandt (10) found very little daily fetal extraction of glycine from umbilical plasma, whereas Jackson (26) has estimated a fetal requirement of 220 mg/kg/day for anabolic purposes only. Recommendations for intravenous intake of glycine range from 100-400 mg/kg/day and serine from 98-200 mg/kg/day (11-13). Pohlandt (10), however, found a much lower combined dose of only 50 mg/kg/day necessary to prevent undesirably high plasma levels.

Histidine

Histidine is considered an essential amino acid in children and infants but not in adults. Assumption of its endogenous production is

based on Rose's observation that 3 adult males remained in nitrogen balance when fed a synthetic histidine-free diet for 8 days (27). Snyderman et al. (28), however, found that infants fed a diet lacking histidine developed a skin rash and had a reduced growth rate and lower nitrogen retention when compared to controls receiving histidine. The synthetic pathway utilized in humans and the effects of low dietary histidine over longer periods of time have not yet been fully determined.

Histidine is utilized in protein synthesis and formation of histamine (14). The term fetus extracts approximately 120 mg/kg/day from umbilical plasma (11), and recommended parenteral intakes vary from 48-120 mg/kg/day (11-13).

Leucine, Isoleucine, and Valine

The essential amino acids leucine, isoleucine, and valine are called branched chain amino acids because each contains a methyl group which is not part of the longest carbon-to-carbon chain (14). As a group they are unique in that the major site of their metabolism is not liver as it is for other essential amino acids. The branched chain amino acids instead pass through portal circulation and liver virtually unchanged (29) and travel via plasma to target organs where they are incorporated into proteins, metabolized into nonessential amino acids, or catabolized in muscle, kidney and gut wall (11). Catabolism begins with reversible transamination into keto acids (8). The transaminase for branched chain amino acids is most active in muscle (30) which, by virtue of its size and enzymatic activity, becomes important in nitrogen

metabolism. The premature infant's muscle mass is in no way comparable to that of an adult (25% vs. 45% of body weight), suggesting potential alterations in the metabolism of branched chain amino acids in the premature.

A second somewhat unique characteristic of branched chain amino acids is their antagonistic relationship. Harper and associates (31) were first to observe that excess supply of any one of these amino acids increased the requirement for the other two in the rat. Swendseid (32) documented this effect in humans who developed depressed plasma levels of isoleucine and valine following excessive leucine intakes. The exact mechanism for this antagonism is unknown, but its existence should be considered in formulating amino acid solutions for parenteral use.

Leucine is somewhat similar to arginine in its ability to stimulate secretion of insulin (20). Stimulation of insulin is thought to be an advantage in the premature as it is the primary growth promoting hormone in the fetus, newborn, and very young infant (33).

Leucine, isoleucine, and valine are frequently recommended as sources of nonspecific nitrogen in parenteral fluids. A wide range of intakes of branched chain amino acids have been given without excessive elevation in peripheral blood (11). This may be due to diverse metabolic pathways which could be protective in the event of excessive branch chain amino acid intake.

At term, fetal extraction is approximately 59 mg isoleucine, 122 mg leucine and 187 valine per kg/day from the umbilical plasma (10). Recommended intravenous intakes for premature infants vary

from 60-200 mg/kg/day of isoleucine, 125-400 mg/kg/day of leucine, and 100-700 mg/kg/ day of valine (10-13).

Lysine

Lysine has been recognized as an essential amino acid for many years, yet knowledge of its metabolism is fragmentary. In addition to participating in protein synthesis, lysine is a source of butyrobetaine, a precursor of carnitine, the intracellular transport agent for fatty acids, but other functions remain obscure (14). As exemplified by the greater than two-fold variance in recommendations for oral intake, establishment of requirements is difficult (11). Lysine is well conserved and efficiently recycled in times of limited intake, perhaps because of the marked tendency of muscle to store free lysine (6). The small muscle mass of infants is possibly a disadvantage in this regard as they may lack a sufficient reservoir for dealing with excess plasma lysine. As the most potent inhibitor of arginase, the enzyme responsible for cleaving urea from arginine, lysine could interfere with ammonia elimination if its concentration significantly exceeded physiological levels (34). Hyperlysinemia has also been associated with mental retardation even when plasma lysine levels are only moderately elevated (34). From a teleological point of view, a possible argument against attempts to maintain low plasma concentrations is the observation that the fetus has a very high intrauterine lysine concentration and a daily lysine retention of approximately 336 mg/kg/day (10). Recommendations for parenteral intake vary from 120-334 mg/kg/day (10-13).

Methionine and Cysteine

Methionine is an essential sulfur containing amino acid and is the substrate for the synthesis of taurine and cysteine. In addition, methionine participates in protein synthesis and is a source of methyl groups for nucleic acid modification, methylation of amino acid residues and the synthesis of a large number of biological compounds including creatine, epinepherine, and phosphatidyl choline. Cysteine, likewise, is involved in the synthesis of such important compounds as glutathioine, iron sulfide complexes, keratin and various enzymes (14).

It is well accepted that the transsulfuration pathway is the means by which older children and adults convert the sulfur of ingested methionine to cysteine (35). There is considerably less certainty about the formation of cysteine in the newborn however. In 1972 it was reported that the activity of cystathionase, the last enzyme in the pathway of transsulfuration of methionine to cysteine, is virtually absent in extracts of human fetal liver and brain during the second trimester (36). Indications from animal studies are that cystathionase activity is low in liver throughout gestation and low in brain throughout pre- and postnatal development (37). Further, the human placenta has been found to have no transsulfuration enzyme activity, suggesting that the fetus is entirely dependent on the mother for cysteine (36-38).

Feeding studies appeared to support the view of cysteine as an essential amino acid when Snyderman (39) reported at least one preterm infant who still had a need, based on nitrogen balance and growth studies, for dietary cysteine at 5 months of age. This has been, however, the only published case of cysteine deficiency, and the methodological and clinical details of that study have never been reported. Cystathionase activity in other studies appeared to be a postnatal phenomenon with adequate activity in both term and preterm infants appearing about the second postnatal week (40). Full activity was reached more slowly in premature than term infants (25). Significant cystathionase has recently been found in the human fetal kidney.

All enteral formulas contain considerable amounts of cysteine, but only one commercially available intravenous amino acid solution (Vamin, Pharmacia, Montreal, Quebec) contains cysteine in significant amounts (41). The absence of cysteine is primarily due to poor solubility (42). Despite the fact that acceptable nitrogen retention and growth have been reported in infants given cysteine-free intravenous solutions (43), the suspicion remains that cysteine might be a limiting amino acid for protein synthesis and growth in the low birth weight infant in the early days of extrauterine life.

In 1981 Zlotkin, Bryan, and Anderson (41) reported results of the first clinical study done to determine the essentiality of cysteine for the intravenously fed infant. They found no difference in nitrogen retention, weight change, or growth in length or head circumference between control infants and those supplemented with 77 mg of

cysteine per kg/24 hours. The supplemented group did have a 60% increase in plasma cysteine and a 300% increase in urinary cysteine excretion. Possible reasons for lack of differences between groups include a short balance period (3 days following a 3 day adaptation period), errors inherent in nitrogen balance studies per se (44), and/or the nonessentiality of cysteine. A note of caution was introduced when sulfur balances indicated a significant increase in plasma sulfate (45). The authors suggested that the addition of cysteine to an intravenous solution already high in sulfate because of the antioxidant perservative potassium bisulfite (such as contained in Aminosyn), could result in sulfate accumulation with unknown metabolic effects. Increased sulfate has been related to hypercalciuria and may be an etiological factor in the bone disease associated with parenteral nutrition in preterm infants (46).

Malloy et al. (47) compared cysteine supplemented infants with controls receiving total parenteral nutrition for 6 days and essentially replicated Zlotkin's findings. The addition of cysteine was associated with increases in plasma and urine cysteine but no differences in weight gain or nitrogen retention. There was again greater retention of sulfur possibly indicating that cysteine was preferentially retained.

The question of whether cysteine is an essential amino acid for all infants, for those receiving intravenous nutrition, or for infants of certain gestational and/or postnatal ages has yet to be answered. On the other hand, methionine is unquestionably an essential amino acid for all ages. There is concern about the proper intake, however, as

animal experiments indicate that high doses are far from innocuous. Sauberlich (48) and Peng (49), working independently, found that based on growth depression, methionine was the most toxic of the essential amino acids. If the premature infant does have significant alterations in the transsulfuration pathway and less synthesis of sulfur containing amino acids, careful attention must be given to methionine intake if toxicity is to be avoided.

The fetus at term extracts approximately 15 mg methionine and 19 mg cysteine per kg/day from the umbilical plasma (10). Recommended intravenous intakes for methionine vary from 15-80 mg/kg/day and for cysteine from 0-85 mg/kg/day (10-13).

Phenylalanine and Tyrosine

Phenylalanine and tyrosine are known as aromatic amino acids because of the presence of a benzene ring in their carbon structures (14). Both are involved in protein synthesis and tyrosine is, in addition, a substrate for the synthesis of epinepherine, norepinepherine, thyroxin, and melanin (8). In the human adult, tyrosine is a non-essential amino acid derived from the irreversible hydroxylation of phenylalanine by phenylalanine hydroxylase (14). Phenylalanine must be supplied in the diet for individuals of all ages, but the need for an exogenous source of tyrosine in the premature has been a source of debate. Based on nitrogen balance studies in infants fed a defined mixture of amino acids, Snyderman (39) suggested that tyrosine was essential in the premature infant. Raihi (50), however, was able to demonstrate some activity of phenylalanine hydroxylase as early as

the 8th week of gestation and activity similar to that of the adult by the 13th week. It appears, therefore, that the enzymatic mechanism for conversion of phenylalanine to tyrosine is present in the viable premature, but it is possible that the activity of this enzyme complex is inadequate for the rapid growth of the premature, and there may be a requirement for exogenous tyrosine. A second consequence of delayed maturation of the phenylalanine hydroxylase system is transient phenylalanemia (12).

Elevated plasma levels of tyrosine resulting in a disorder known as transient neonatal tyrosinemia are also a concern. Evidence regarding its significance is conflicting, but at least two investigators have associated elevated plasma tyrosine with subsequent learning disabilities (51, 52). Transient neonatal tyrosinemia is thought to occur as a consequence of the relative delay in the maturation of p-hydoxyphenylpyruvic acid oxidase, an enzyme involved in tyrosine catabolism (50). Significant elevations of tyrosine occur more frequently in premature than full-term neonates and in those infants receiving a high protein intake (52).

While the duration of tyrosinemia and phenylalanemia are undoubtedly important in the development of adverse outcomes, the nutritional goal is to satisfy requirements for growth and development but spare the vulnerable brain any potential insult resulting from hyperaminoacidemias. Proper dosage of these amino acids are consequently of great concern. The term fetus extracts approximately 54 mg of tyrosine and 59 mg of phenylalanine per kg/day from umbilical plasma

(10). Recommendations for tyrosine intake vary from 0-120 mg/kg/day and for phenylalanine from 59-230 mg/kg/day. The amino acids should, however, be considered together when determining dosages as the administration of tyrosine will decrease the requirement for phenylalanine (10-13).

Proline

Proline is a gluconeogenic amino acid found in many body proteins. Its major synthesis is from glutamic acid via glutamic semialdehyde and its primary catabolic pathway takes the reverse pathway but through a different enzyme system (53). Proline may also be hydroxylated to form hydroxyproline, a major constituent of collagen, or may be converted to ornithine (54). Although proline is a nonessential amino acid, question has been raised about the adequacy of its endogenous production. Watts, Bradley and Mann (55) suggested that the rate of synthesis or proline was less than that of other nonessential amino acids. Juergens and Dolif (56) found improved nitrogen balance following proline enrichment of parenteral solutions containing essential amino acids as proposed by Rose for adults. Heine (57), however, found no effect of proline supplementation on nitrogen balance in 6 infants receiving proline-free parenteral solutions containing essential amino acids as proposed by Rose with glycine added as a nonessential nitrogen source. High intakes of proline and/or marked imbalances of amino acids have, on the other hand, been associated with hyperprolinemia in infants (10, 56). In adults

undergoing parenteral alimentation, proline was found to be second only to valine in its rise in blood concentration per millimole infused per hour, suggesting that its degradation or use in protein synthesis was limited (58). The fetus extracts approximately 127 mg/kg/day from umbilical plasma (10). Recommended intravenous intakes for infants range from 50-135 mg/kg/day (10-13).

Taurine

Taurine is an abundant and ubiquitous sulfonic amino acid (59). Along with inorganic sulfate, it is the major end product of methionine metabolism in mammals. Considerable biochemical evidence exists, however, that taurine is not merely an end product of sulfur metabolism but rather a functionally important metabolite. While its actual roles have not been fully characterized, multiple functions are suggested. Taurine may act as a neurotransmitter or neuromodulator in the brain where it has been implicated in epilepsies in both human and animal models (60). It is thought to be a regulator of membrane excitability in cardiac and skeletal muscles. It may play a role in the maintenance of the structural integrity of the retina, especially the photoreceptor cells, and may be involved in endocrine and reproductive function (61).

At the present time the only proven biochemical role for taurine in man is its conjugation with bile acids to form bile salts. Unlike many other species, the human can conjugate bile acids with either glycine to form glycocholate or taurine to form taurocholate (62). The

taurine conjugated bile acids are more efficient as they are less likely to be deconjugated and degraded by gut flora. They, therefore, have slower turnover rates and greater conservation in the enterohepatic circulation (63). They also maintain critical micellar concentration further down the gut (64). It has also been shown that bile acids, especially taurocholate, are important in the control of hepatic cholesterol synthesis and, therefore, of total body cholesterol pool size (65, 66).

Taurine is not considered essential for either children or adults, but several lines of evidence suggest that the premature infant may have a need for an exogenous source of taurine. The enzyme involved in what is presumed to be the major synthetic pathway, cysteine sulfinic acid decarboxylase, has very low activity in the fetal liver (64). The finding of a very high concentration of taurine in the brain during development at a time of limited capacity for synthesis implies the need for exogenous taurine (67). This high concentration in fetal and newborn brain decreases slowly postnatally (68) and has lead to the suggestion that taurine may have a role in the development as well as function of the brain (64).

Taurine is present in higher concentration in human milk (25-35 micromoles/dl) than in infant formula (1-3 micromoles/dl) or cow's milk (1 micromole/dl) from which most such formulas are derived. Commercially available soy formulas contain no taurine (69). Infants fed human milk remain primarily taurine conjugators of bile acids whereas those fed taurine deficient formula become predominately glycine conjugators (70). This ability to conjugate with either glycine or taurine

may be an important safety mechanism to prevent depletion of limited supplies. Both preterm and term infants fed artificial formulas have been shown to have significantly lower concentrations of taurine in their plasma and urine than did infants fed human milk, suggesting a dietary requirement in the rapidly growing neonate (40, 71). Jarvenpaa et al. (72), however, found that taurine supplementation of whey predominant formulas had no effect on growth of premature infants either in the immediate postnatal period or for up to 4 months postnatally.

Studies of taurine metabolism during total parenteral nutrition are very limited. Beagle pups, whose sulfur metabolism is similar but not identical to that of humans, had a decrease in taurine conjugated bile acids after being treated with taurine-free but methionine-sufficient parenteral fluids for 14 days (73). The significance of this is uncertain. While not a factor during parenteral nutrition, an adequate bile salt pool may be important in the successful initiation or reinstitution of enteral feedings.

Rigo and Senterre (74) monitored 14 low birth weight infants perfused with a solution described as having no taurine and little cysteine. They found consistently low levels of plasma taurine in both the under 1500 g and under 2000 g babies. The investigators felt these results indicated that preterm infants have a limited capacity for taurine synthesis from cysteine and methionine precursors and may be dependent upon an external taurine supply for adequate growth and development. Commercial amino acid solutions currently contain no taurine.

Threonine

Threonine is an essential amino acid for both adults and infants. In addition to participating in protein synthesis, it is a precursor for glycine, alanine, and asparagine, but these can be readily synthesized from other amino acids (11). Hydroxyl groups of threonine residues are also a frequent site for carbohydrate linkage in the formation of glycoproteins (14).

The fetal plasma concentration of threonine is high, but the significance of this elevation is unknown. The intravenous administration of high doses of threonine has been associated with hyperammonemia and hyperthreonemia. Ghadimi found intakes in infants of more than 100 mg/kg/day frequently resulted in marked elevations of plasma threonine, whereas levels remained within acceptable limits when intakes were limited to 60 mg/kg/day (11). Term fetal extraction from cord blood is approximately 131 mg/kg/day (10), and recommended parenteral intakes for infants vary from 60-150 mg/kg/day (10-13).

Tryptophan

Tryptophan is an essential amino acid required for the synthesis of body protein and the synthesis of at least 3 physiologically active substances: serotonin, nicotinic acid, and melatonin (8). The primary catabolic pathway for tryptophan involves the conversion of tryptophan to formylkynurenine by tryptophan pyrrolase (14). This enzyme is apparently present in most term infants but is reported to be absent in the premature (7). Tryptophan is usually the least

abundant amino acid in the diet and is generally the amino acid supplied in the smallest amounts in parenteral fluids. The fetus extracts approximately 124 mg/kg/day at term from umbilical plasma (10). Recommendations for intravenous intakes are fairly uniform around 30 mg/kg/day except for that of Pohlandt who found 80-120 mg/kg/day necessary to maintain acceptable plasma tryptophan levels (10-13).

Recommended Intakes of Parenteral Amino Acids for the Premature

Table 1 presents the recommendations of several investigators for parenteral intake of amino acids in the preterm infant. These recommendations were determined in a variety of ways. Snyderman (13) based her recommendations on the oral amino acid requirements of full term infants. She multiplied requirements for essential amino acids by a factor of 1.3 to allow for individual variation. This factor was chosen rather than the traditional 2 to compensate for the 10-15% of ingested nitrogen normally lost in the stool of enterally fed infants and because of "concern for harmful excess." Nonessential amino acids were recommended in the proportions found in human milk with the exceptions of glutamic acid and aspartic acid which were recommended in minimal amounts. Snyderman recommended that the nitrogen that would have been supplied in glutamic and aspartic acids be supplied instead in glycine and alanine.

TABLE 1

Recommended parenteral amino acid intakes[1]

	Ghadimi[2]	Stegink[3]	Burger[4]	Pohlandt[5]	Snyderman[6]
Aspartic Acid	12.5	71[7]	135	0	40
Glutamic Acid	12.5	72[8]	490	0	40
Threonine	60	143	150	60-130	120
Serine	100	98	80	50[9]	138
Proline	50	132	135	120	160
Glycine	100	203	255	50[9]	330
Alanine	100	282	290	320-700	370
Valine	200	188	80	180	140
Cysteine	85	84	50	40	60
Methionine	15	54	40	22-45	60
Isoleucine	200	127	70	60-100	150
Leucine	400	216	125	130-210	200
Tyrosine	12.5	79	50	20	120
Phenylalanine	100	104	95	59-76	120
Tryptophan	30	43	30	80-120	30
Lysine	120	334	160	175-330	140
Histidine	50	74	110	50-120	48
Arginine	250	187	110	210	102
Total	1897.5	3069	2500[10]	1576-245	2368

[1]All values are mg/kg/day.

TABLE 1 (continued)

[2]Ghadimi, H. (1975) Newly devised amino acid solutions for intravenous administration. In: Total Parenteral Nutrition: Premises and Promises (Ghadimi, H., ed.), pp. 93-442, John Wiley & Sons, New York.

[3]Stegink, L. D. (1975) Development of a crystalline amino acid mixture for parenteral alimentation of young infants. In: Intravenous Nutrition in the High Risk Infant (Winters, R. W. & Hasselmeyer, E. G., eds.), pp. 411-420, John Wiley & Sons, New York.

[4]Burger, U. & Wolf, H. (1976) Investigations on the utilization of parenterally administered amino acids by premature and hypotropic neonates. Europ. J. Pediatr. 122, 169-175.

[5]Pohlandt, F. (1979) Studies on the requirement of amino acids in newborn infants receiving parenteral nutrition. In: Nutrition and Metabolism of the Fetus and Infant (Visser, H. K. A., ed.), pp. 341-364, Martinus Nijhoff, the Hague.

[6]Snyderman, S. E. (1975) Amino acid requirements. In: Intravenous Nutrition in the High Risk Infant (Winters, R. W. & Hasselmeyer, E. G., eds.), pp. 205-214, John Wiley & Sons, New York.

[7]Plus 71 mg/kg/day asparagine.

[8]Plus 587 mg/kg/day glutamine.

[9]Combined dose of glycine and serine.

[10]Includes 45 mg/kg/day ornithine.

Stegink (12) based his recommendations on three sources: (a) Snyderman's essential amino acid requirements for orally fed term infants, (b) the amino acid concentration of cord blood, and (c) plasma amino acid concentrations of infants infused with different protein hydrolysate solutions. Snyderman's values for all amino acids were taken as the absolute minimum even when they were higher than the values obtained by other criteria. This was the case with methionine, isoleucine, leucine, phenylalanine, histidine, and lysine. For other amino acids the levels calculated from cord blood were selected with the exceptions of glycine, which was increased to provide nonessential nitrogen, and threonine, which was averaged because of its disproportionally high cord concentration.

Ghadimi (11) evaluated the tolerance to increasing doses of amino acid solutions to arrive at his recommendations. Subjects were 8 infants less than 7 months of age and 52 neonates, including 35 prematures of undescribed gestational age. Amino acid solutions were based on "metabolic pathways of each amino acid with emphasis on economy of the substance, the role of different tissues in metabolism, side effects, nitrogen requirement, enzymic immaturities, and minimal and safe oral requirements of essential amino acids." Plasma aminograms determined after increasing amounts of test solutions were often decisive in selecting the upper limit of recommended dosage ranges.

Pohlandt (10) approached the task in two ways. He first of all assumed that the intrauterine supply of nutrients to the fetus is the ideal model for parenteral infusate composition and used fetal retention

of amino acids as one basis for his recommendations. Fetal retention was based on differences in umbilical cord vein and artery amino acid concentration of 46 infants with gestational range of 35-42 weeks, median age 40 weeks. Estimates of fetal retention were calculated by multiplying the median difference between venous and arterial concentration by the rate of blood flow in cord vessels and assuming a hematocrit of 49%. The second basis for his recommendations was the response of 118 "mostly premature" infants to 7 different amino acid solutions. As with other investigators results from the two approaches were not always in agreement. In such cases, Pohlandt usually favored the data representing fetal retention.

CHAPTER III

EXPERIMENTAL PROCEDURE

Low birth weight infants admitted to the neonatal intensive care unit of The University of Tennessee Memorial Research Center and Hospital in Knoxville, Tennessee, from January to June 1982 were candidates for the study. Criterion for inclusion was that the infant would be given an intravenous glucose-amino acid solution for at least 24 hours with no enteral feeding. The decision regarding method of feeding was made by the neonatologist independent of the research. The study protocol was reviewed and approved by the the Research on Human Subjects Committee of The University of Tennessee. The proposed study was fully explained to at least one of each child's parents, and written consent was obtained before inclusion in the study. Consent was not sought when the mother's physical condition, the infant's prognosis or immediate treatment needs, the presence of obvious congenital anomalies, the death of a twin, or any other factor made a discussion of research participation inappropriate. For this reason, and because of their very small blood volume, the smallest and most immature infants were not included in the study. Gestational age was calculated from date of mother's last menstrual period and Dubowitz scoring (75).

Infants received infusions of glucose solutions from the time of admission until the amino acid-glucose solution (total parenteral nutrition) was initiated, usually on the second or third day of life.

Nitrogen was supplied as a crystalline L-amino acid mixture (Aminosyn, Abbott Laboratories, North Chicago, Ill.). The infusate was formulated to provide adequate minerals, trace metals, and vitamins, in addition to 15 amino acids. Changes in electrolytes were made as indicated by the infant's serum values.

The intravenous solutions, aseptically prepared by the hospital pharmacist under laminar flow, were delivered into umbilical artery catheters or peripheral lines through 0.22 micron inline filters. Rate of delivery was controlled by continuous flow infusion pumps, and intake was calculated and recorded hourly. Aseptic precautions were meticulously observed in caring for the catheter, daily changes of fluid and tubing, etc. All infusions were begun and blood samples collected between 4:00 and 6:00 p.m. to negate the effects of diurnal variation (76).

During the study period all infants were cared for under radiant overhead heaters or in servocontrolled incubators. They received no insulin during the study.

Infants were weighed daily between 7:00 and 8:00 a.m. Weights at initiation of amino acid infusion (4:00-6:00 p.m.) and 24 hours later were calculated by interpolation. The caloric and individual amino acid intakes were calculated from the composition and amount of infusate given each infant in the 24 hours before sample collections.

Samples of 0.8 ml of whole blood were collected in heparinized syringes from umbilical catheters just prior to initiation of amino acid

infusion and 24 hours later. Samples from heelsticks were also collected at one week of age from infants on total parenteral nutrition with no enteral intake. When possible cord blood samples were collected immediately after delivery and before placental separation from infants born at The University of Tennessee Memorial Hospital and Research Center but were not available from infants transferred to this regional facility.

Whole blood samples were centrifuged and the plasma decanted and frozen at -20 C. After thawing at room temperature and deproteinization with 1% picric acid, plasma amino acids were separated by one column ion exchange chromatography using the JCL-6AH Amino Acid Analyzer (JEOL Ltd., Tokyo, Japan), modified as previously described by Lin (77). This method allowed quantitative estimation of histidine, ornithine, lysine, arginine, taurine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine concentrations in the samples collected at delivery, just prior to initiation of amino acid infusion, and after 24 hours of infusion. Samples from one week old infants were analyzed by the same method, but at the time of their analysis an on-line computer system had been installed to assist in the calculation of amino acid concentrations. No differences in concentrations could be related to the change in instrumentation, but the new system did not allow consistent estimations of cysteine, taurine, or arginine. These values are consequently not available for the samples collected at one week of age.

Statistical analyses were performed with the Statistical Analysis System (SAS 79) maintained at The University of Tennessee Computer Center (78). Effects of independent variables on plasma amino acid concentrations were analyzed by analysis of variance (79). Linear regression was used to generate equations predicting plasma amino acid concentrations as functions of the significant independent variables. Pearson product-moment correlations were calculated to determine the relationships between gestational age and caloric and nitrogen intakes on the initial day of growth and between plasma branched chain amino acid concentrations and growth and caloric intake. P values of 0.05 or less were considered significant.

CHAPTER IV

RESULTS

Subjects

The 21 preterm infants included in the initial phase of this study are described in Table 2. The infants' gestational age ranged from 27-36 weeks, with a mean age of 31.2 ± 2.3 weeks. All had appropriate for gestational age birth weights ranging from 960-2608 g (mean = 1574 ± 432 g). There were 13 males and 8 females, 1 black infant and 20 caucasian. The sample included one set of twins (Sh1 and Sh2); the rest were single births. All infants survived except one (Yoa) who died at age 11 months of complications unrelated to parenteral infusion.

Results at Birth

Immediately after birth and before placental separation mixed umbilical cord blood was obtained from 10 subjects. The mean, range, and standard deviation of plasma amino acid concentrations in these samples is presented in Table 3.

Results Immediately Prior to Amino Acid Infusion and 24 Hours Later

After an initial period of infusion with solutions containing only glucose and electrolytes, each infant received intravenous fluids containing both amino acids and glucose as well as vitamins, minerals and electrolytes. Composition of the standard solution is shown in

TABLE 2

Description of subjects

Infants	Gestational age	Birthweight
	weeks	g
Dra	27	1000
Tab	28	960
Zim	28	1140
Lov	29	1200
Pol	29	1420
Tap	30	1140
Jar	30	1200
Yoa	30	1350
Vnc	31	1360
Kop	31	1620
Phi	31	1620
Scu	32	1440
God	32	1600
Dia	32	1620
Bws	32	1660
Bis	33	1900
Sh1	33	1760
Sh2	33	1790
Bro	34	2360
Bow	35	2608
Cla	36	2200
Means	31.2	1574
SD	2.3	432
Range	27-36	960-2608

TABLE 3

Amino acid concentrations in umbilical cord plasma

Amino acid	Mean	SD	Range
	mg/dl	mg/dl	mg/dl
Histidine	1.39	0.53	0.74-2.32
Ornithine	1.40	0.61	0.57-2.22
Lysine	4.32	1.27	2.89-6.57
Arginine	1.06	0.43	0.77-2.08
Taurine	2.59	1.17	1.31-4.86
Aspartic acid	0.36	0.05	0.16-0.74
Threonine	4.46	2.27	2.09-9.11
Serine	1.91	0.57	1.30-2.90
Glutamic acid	3.89	1.63	1.63-6.86
Proline	1.84	0.56	1.11-2.94
Glycine	1.42	0.45	0.93-2.19
Alanine	3.52	1.16	2.42-6.08
Cysteine	0.69	0.43	0.26-1.72
Valine	2.35	0.66	1.42-3.63
Methionine	0.42	0.19	0.23-0.87
Isoleucine	0.78	0.33	0.29-1.35
Leucine	1.14	0.55	0.65-2.48
Tyrosine	1.11	0.27	0.73-1.72
Phenylalanine	1.66	0.57	0.84-2.63

Table 4. This infusate contains no taurine, cysteine, glutamic acid or aspartic acid. Postnatal age at the time amino acid-supplemented infusion began varied from 24-75 hours. All infants had an umbilical artery catheter from which blood samples were drawn just prior to initiation of the infusion and 24 hours later. No other intravenous solutions were given during this time. The mean and standard deviations of the plasma concentrations of 19 amino acids before infusion and 24 hours later are presented in Table 5.

After 24 hours infusion there was a significant increase in plasma concentration of isoleucine, leucine, arginine, methionine, glycine, valine, alanine, serine, lysine, threonine, proline, and phenylalanine. Nonsignificant ($p > .05$) increases occurred in the concentrations of ornithine, aspartic acid, histidine, and cysteine, and nonsignificant decreases occurred in the concentrations of taurine, glutamic acid and tyrosine.

The absolute change in amino acid concentration (concentration after 24 hours infusion minus concentration before infusion), the percent of change in plasma concentration (absolute change / initial concentration) and the direction of individual change in plasma concentrations are presented in Table 6. Various factors were postulated to be related to the infant's plasma amino acid concentration after 24 hours infusion and/or to changes in concentration during that interval. Estimation of the effects of gestational age, postnatal age, weight, caloric intake, amino acid intake, and preinfusion concentration were determined by two-way analysis of variance.

TABLE 4

Composition of parenteral solution[1]

Component	Concentration/dl	Component	Concentration/dl
L-Isoleucine[2]	72 mg	Dextrose	10 g
L-Leucine	94 mg	Sodium	3 mEq
L-Lysine	72 mg	Chloride	3 mEq
L-Methionine	40 mg	Potassium	2.2 mEq
L-Phenylalanine	44 mg	Phosphate	1.5 mM
L-Threonine	52 mg	Magnesium	0.25 mEq
L-Tryptophan	16 mg	Calcium	1.92 mEq
L-Valine	80 mg	Multivitamin[3]	0.5 ml
L-Alanine	128 mg	Vitamin B-12	10 mcg
L-Arginine	98 mg	Folic acid	50 mcg
L-Histidine	30 mg	Zinc	22 mcg
L-Proline	86 mg	Copper	5.5 mcg
L-Serine	42 mg	Chromium	.055 mcg
L-Tyrosine	4.4 mg	Manganese	.44 mcg
Glycine	128 mg	Calories	38

[1]Prepared in hospital pharmacy.

[2]Amino acids supplied in 1 gm Aminosyn (Abbott Laboratories, Chicago, Ill.).

[3]MVI Pediatric (Armour Pharmaceuticals, Kankakee, Ill.).

TABLE 5

Plasma amino acid[1] concentrations before amino acid infusion and 24 hours later

	Before infusion			After 24 hours			
	Mean	SD	N	Mean	SD	N	P
	mg/dl	mg/dl		mg/dl	mg/dl		
Isoleucine	0.46	0.28	21	0.94	0.79	20	.0029
Arginine	0.68	0.34	17	1.26	0.88	20	.0160
Leucine	0.95	0.43	21	1.58	1.06	20	.0051
Methionine	0.38	0.21	20	0.60	0.38	19	.0096
Valine	1.40	0.57	21	2.11	0.82	20	.0003
Ornithine	1.13	0.87	20	1.24	0.47	20	.0611
Glycine	2.39	1.12	21	3.44	1.88	21	.0029
Alanine	2.08	1.43	21	2.63	1.56	21	.0039
Serine	1.93	0.85	21	2.82	1.66	21	.0042
Lysine	1.80	0.73	20	2.44	1.08	21	.0020
Threonine	3.12	1.70	21	3.96	2.45	21	.0346
Proline	1.98	1.26	21	2.43	1.11	21	.0088
Aspartic acid	0.34	0.25	21	0.36	0.23	19	.5421
Histidine	2.08	1.04	20	2.40	1.11	21	.1809
Phenylalanine	1.18	0.46	21	1.40	0.52	21	.0162
Cysteine	0.46	0.24	15	0.59	0.44	16	.6187
Taurine	2.49	1.15	13	2.36	1.06	13	.0662
Glutamic acid	4.70	3.70	21	3.70	2.47	21	.0969
Tyrosine	1.98	1.14	21	1.83	1.06	20	.1561

[1] Including the sulfonic amino acid taurine.

TABLE 6

Changes in amino acid concentration in the first 24 hours of infusion

Amino acid	Absolute change[1]	% change[2]	Decrease[3]	No change[3]	Increase[3]
	mean	mean	n	n	n
	mg/dl				
Isoleucine	0.50	136	1	2	17
Arginine	0.59	135	2	1	14
Leucine	0.65	83	5	1	14
Methionine	0.22	73	1	1	16
Valine	0.74	66	2	3	15
Ornithine	0.27	59	6	0	13
Glycine	1.06	50	4	1	16
Alanine	0.54	47	4	0	17
Serine	0.89	46	3	3	15
Lysine	0.69	45	3	2	15
Threonine	0.84	35	4	3	14
Proline	0.46	34	4	4	13
Aspartic acid	0.03	30	8	0	11
Histidine	0.35	29	6	2	12
Phenylalanine	0.22	27	5	0	16
Cysteine	0.13	27	5	1	7
Taurine	0.13	1	2	3	6
Glutamic acid	-0.99	-3	12	1	8
Tyrosine	-0.26	-9	13	0	7

[1]Concentration after 24 hours infusion - concentration before infusion.

[2]Absolute change/initial concentration.

[3]Decrease = number of infants whose plasma amino acid concentration decreased in the first 24 hours of amino acid infusion. No change = number whose concentration changed by less than 5%. Increase = number whose concentration increased.

Plasma amino acid concentrations after 24 hours infusion were directly associated with preinfusion plasma levels of leucine, isoleucine, valine, tyrosine, glycine, glutamic acid, methionine, lysine, histidine, threonine, serine, proline, aspartic acid and phenylalanine (Table 7). Post 24 hour infusion concentration of phenylalanine was also associated with postnatal age, younger infants tending to have higher plasma phenylalanine concentrations.

The absolute change in plasma phenylalanine concentration was similarly associated with postnatal age (Table 8). Proline, aspartic acid, glutamic acid and phenylalanine were the only amino acids whose preinfusion plasma levels had significant effects on absolute change in plasma concentration in the first 24 hours of infusion. In these 4 instances higher preinfusion concentrations were associated with smaller increases in plasma concentration.

The percent of change in plasma concentration of isoleucine, valine, phenylalanine, and histidine also tended to vary inversely with preinfusion concentration (Table 9). The percent of increase of valine concentration in the first 24 hours was associated with caloric intake and gestational age as well as with preinfusion concentration. Plasma valine concentration tended to increase more in more mature infants and in those with lower caloric intakes. The percent of increase in plasma isoleucine was also greater in infants with greater gestational age.

Neither weight at the beginning of amino acid infusion, nor the intake of each amino acid, nor the infant's sex were ever significantly

TABLE 7

Analysis of variance summary for factors affecting amino acid concentration after 24 hours of amino acid infusion

Amino acid	Intercept	Slope	Sum of squares	F-value	P
Preinfusion concentration:					
Leucine	0.377542	1.285439	5.59767986	6.92	.0170
Isoleucine	0.045574	2.003182	5.01801260	13.38	.0018
Valine	1.141767	0.704507	2.96411841	5.38	.0323
Tyrosine	0.364989	0.684520	12.15358264	23.53	.0001
Glycine	0.818811	1.099041	30.11522260	14.18	.0013
Glutamic acid	1.499299	0.465470	47.10963609	12.12	.0025
Methionine	0.276946	0.866330	0.59754204	5.33	.0347
Lysine	0.810852	0.930120	8.82217601	11.40	.0034
Histidine	1.356631	0.515445	5.41051949	5.05	.0375
Threonine	0.883852	0.995335	62.11972619	20.24	.0002
Serine	0.635086	1.142612	22.13503541	12.97	.0019
Proline	1.253910	0.606773	11.60728678	17.60	.0005
Aspartic acid	0.154573	0.616124	0.45014029	14.88	.0013
Phenylalanine	1.376448	0.705405	2.02803860	18.91	.0004
Postnatal age:					
Phenylalanine	1.376448	-0.017255	0.87769986	8.19	.0104

TABLE 8

Analysis of variance summary for factors affecting absolute change in plasma amino acid levels in the first 24 hours of amino acid infusion

Amino acid	Intercept	Slope	Sum of squares	F-value	P
Preinfusion concentration:					
Proline	1.253910	-0.393226	4.87488628	7.39	.0136
Aspartic acid	0.154573	-0.383875	0.17474029	5.78	.0279
Glutamic acid	1.499299	-0.534529	62.12529324	15.98	.0008
Phenylalanine	0.935636	-0.015536	0.72952701	6.07	.0235
Postnatal age:					
Phenylalanine	0.935636	-0.015022	0.58936237	5.48	.0335

TABLE 9

Analysis of variance summary for factors affecting percent of change in plasma amino acid levels in the first 24 hours of amino acid infusion

Amino acid	Intercept	Slope	Sum of squares	F-value	P
Preinfusion concentration:					
Isoleucine	-6.948225	-3.236195	10.69872200	10.02	.0231
Valine	-1.115742	-1.814252	22.15796342	10.40	.0061
Phenylalanine	1.918306	-0.599601	1.46529115	7.90	.0116
Histidine	0.832471	-0.258701	1.36292423	6.17	.0231
Gestational age:					
Isoleucine	-6.948225	0.341168	10.73816784	10.06	.0059
Valine	-1.115742	0.426272	12.98577452	6.10	.0270
Caloric intake:					
Valine	-1.115742	-0.146012	14.48733102	6.80	.0206
Postnatal age:					
Phenylalanine	1.918306	-0.020259	1.20993589	6.52	.0199

associated with plasma amino acid concentration after 24 hours infusion or with changes in that concentration, either absolute or percent.

Each infant's amino acid and caloric intake in the first 24 hours of infusion was calculated from the volume and composition of the solution infused. Volume varied as much as 230% (range = 75-171 ml/24 hours). Total intake was divided by body weight at the beginning of infusion to determine the intake in mg/kg/day for each amino acid (Table 10). Intakes were compared with those recommended by Steggink, Ghadimi, Pohlandt, Burger and Snyderman (10-13). The majority of infants received less than the minimum recommended intake of all amino acids except methionine, alanine, glycine, proline, and isoleucine. Methionine was the only amino acid received in amounts greater than the maximum recommended, i.e., 5 infants received more than the maximum methionine recommended while 16 had intakes within the recommended range. In contrast, no infant received even the smallest recommended amount of leucine, lysine, phenylalanine, tyrosine or histidine in the first 24 hours of infusion. For each of the other amino acids some infants had intakes within the recommended range while others had less than the minimum recommended intake. Caloric intake ranged from 19-51 kcal/kg/day with a mean of 35 ± 10 kcal/kg/day.

Change in plasma concentration per mg intake in the first 24 hours of infusion was calculated for each amino acid (Table 11). The rate of change was extremely variable. For example, the increase in concentration per mg of serine infused was almost 50 times greater

TABLE 10

Intake of amino acids in first 24 hours of infusion compared to literature recommendations

Amino acid	Mean	SD	Range	Recommended[1-6]	<Rec[7]	Rec[8]	>Rec[9]
	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day			
Leucine	87	22	47-121	125-400	21	0	0
Lysine	67	17	36-99	120-334	21	0	0
Methionine	37	9	20-54	15-45	0	16	5
Phenylalanine	41	10	22-57	59-104	21	0	0
Threonine	48	12	26-71	60-150	18	3	0
Valine	74	19	40-109	80-200	15	6	0
Alanine	118	30	64-174	100-700	8	13	0
Arginine	91	23	49-134	110-250	15	6	0
Glycine	118	30	64-174	50-255	0	21	0
Tyrosine	4	1	2-6	13-79	21	0	0
Histidine	28	7	15-41	50-120	21	0	0
Proline	80	20	43-117	50-135	2	19	0
Isoleucine	67	17	36-99	60-200	8	13	0
Serine	39	10	21-57	50-100	19	2	0
Tryptophan	15	4	8-23	30-120	21	0	0

[1]For each amino acid the first number is the smallest of the minimum recommended intakes of 4 investigators and the second is the largest of the maximum recommended intakes of these investigators.

[2]Pohlandt, F. (1979) Studies on the requirement of amino acids in newborn infants receiving parenteral nutrition. In: Nutrition and Metabolism of the Fetus and Infant (Visser, H. K. A., ed.), pp. 341-364, Martinus Nijhoff, the Hague.

TABLE 10 (continued)

[3]Burger, U. & Wolf, H. (1976) Investigations on the utilization of parenterally administered amino acids by premature and hypotropic neonates. Europ. J. Pediatr. 122, 169-175.

[4]Ghadimi, H. (1975) Newly devised amino acid solutions for intravenous administration. In: Total Parenteral Nutrition: Premises and Promises (Ghadimi, H., ed.), pp. 393-442, John Wiley & Sons, New York.

[5]Stegink, L. D. (1975) Development of a crystalline amino acid mixture for parenteral alimentation of young children. In: Intravenous Nutrition in the High Risk Infant (Winters, R. W. & Hasselmeyer, E. G., eds.), pp. 411-420, John Wiley & Sons, New York.

[6]Snyderman, S. E. (1975) Amino acid requirements. In: Intravenous Nutrition in the High Risk Infant (Winters, R. W. & Hasselmeyer, E. G., eds.), pp. 205-214, John Wiley & Sons, New York.

[7]< Rec = number of infants receiving less than minimum recommended intake.

[8]Rec = number of infants with intakes within the recommended range.

[9]> Rec = number of infants receiving more than the maximum recommended intake.

TABLE 11

Change in plasma amino acid concentration per mg intake in the first 24 hours of infusion

Amino acid	Change	Intake	Change/mg Intake
	mean mg/dl	mean mg/day	mean
Serine	0.89	53	.0168
Threonine	0.84	65	.0129
Histidine	0.35	38	.0092
Lysine	0.69	91	.0076
Valine	0.74	100	.0074
Glycine	1.06	161	.0066
Isoleucine	0.50	90	.0056
Leucine	0.65	118	.0056
Arginine	0.59	123	.0048
Methionine	0.22	50	.0044
Proline	0.46	108	.0043
Phenylalanine	0.22	55	.0040
Alanine	0.54	160	.0034

than that of alanine. The effects of amino acid intake were further analyzed by comparing the rates of change in plasma levels of amino acids infused in similar amounts (Table 12). Over the range of intakes received by these infants, changes in plasma concentration do not appear to bear a consistent direct relationship to intake. While similar rates of change occurred in methionine and phenylalanine and in leucine and arginine, rates were significantly different for the isoleucine-lysine and alanine-glycine pairs even though their intakes were identical.

Concentrations of plasma amino acids after 24 hours of infusion were compared with those of ad libitum breast fed term infants (80) and with the concentrations of the preterm fetus as reflected by umbilical cord concentrations determined in this laboratory (Table 13). The choice of reference data used for comparison is problematic. The goal of nutritional support is to provide the infant with nutrients essential for optimal growth and development while at the same time protecting the vulnerable nervous system from potentially damaging elevations in amino acid levels. There is, therefore, a need for estimating the minimal level reflecting adequate nutrition and the maximum level reflecting safe, nontoxic concentrations.

Although the preterm infant is a different biological entity, and the composition of milk from mothers of prematures differs from that of mothers who deliver at term (81), plasma concentration of amino acids of term breast fed infants was selected as one standard. It is presumed that these breast fed babies were being optimally nourished and

TABLE 12

Comparison of change in plasma levels of amino acids with similar intravenous intakes

Amino acid	Intake	Plasma change per mg intake	P
	mg/dl infusate	mg/dl	
Isoleucine	72	.0055	<.01
Lysine	72	.0076	
Alanine	128	.0034	<.001
Glycine	128	.0066	
Leucine	94	.0055	NS
Arginine	98	.0048	
Methionine	40	.0047	NS
Phenylalanine	44	.0040	

TABLE 13

Comparison of plasma amino acid concentrations in study infants after 24 hours of infusion, breast fed infants and umbilical cord blood samples

Amino acid	Subjects	Breast fed[1]	<Min[2]	=[2]	>Max[2]	Cord blood[3]	<Min[4]	=[4]	>Max[4]
	Range mg/dl	Range mg/dl				Range mg/dl			
Histidine	0.96-4.96	0.73-1.63	0	6	15	0.74-2.32	0	13	8
Ornithine	0.41-2.34	0.73-1.45	3	10	7	.044-6.21	1	19	0
Lysine	0.51-5.48	1.25-2.69	1	14	6	2.07-6.57	7	14	0
Phenylalanine	0.57-2.71	0.49-1.06	0	4	17	0.89-2.63	3	17	1
Tyrosine	0.56-4.46	0.60-2.28	1	15	4	0.69-1.72	2	12	6
Leucine	0.34-5.10	0.64-1.59	2	13	6	0.82-2.48	2	16	3
Isoleucine	0.21-3.79	0.26-0.68	1	8	12	0.29-1.35	1	16	3
Methionine	0.16-1.69	0.19-0.34	1	3	15	0.23-0.87	2	12	5
Valine	0.62-4.12	0.88-1.89	1	6	13	1.42-3.63	3	16	1
Alanine	0.83-7.85	1.19-3.31	1	16	4	1.93-6.08	6	14	1
Glycine	1.69-8.36	1.10-2.02	0	6	15	0.61-2.19	0	3	18
Glutamic acid	0.86-10.1	4.78-12.19	15	6	0	1.63-9.61	4	16	1
Serine	0.54-7.29	0.74-2.15	1	6	14	0.87-2.90	2	13	6
Threonine	0.50-10.93	0.61-1.72	1	1	19	2.09-9.11	4	15	2
Aspartic acid	0.10-0.92	---- ----	--	--	--	0.16-1.15	4	15	0
Proline	0.66-5.47	1.07-4.80	2	18	1	0.93-2.75	2	13	6
Cysteine	0.08-1.99	0.79-1.42	12	4	0	0.26-1.72	15	1	0
Taurine	0.69-4.86	0.31-1.09	0	1	12	1.31-4.86	2	11	0
Arginine	0.58-4.12	0.51-1.21	0	14	6	0.61-1.40	2	13	5

[1]Tikanoja, T. (1982) Plasma amino acids in term neonates after a feed of human milk or formula. Acta Paediatr. Scand. 71, 391-397.

[2]< Min = number of infants with levels less than minimum in breast fed term infants. = is number within range. > Max = number exceeding maximum.

[3]Plasma amino acids from umbilical cord samples from 16 premature infants (27-35 weeks gestation). Unpublished data from this laboratory.

[4]< Min = number of infants with levels less than minimum in premature cord plasma. = is number within range. > Max = number exceeding maximum.

would have no plasma concentration indicative of an amino acid deficiency or toxicity. It is likewise presumed that the amino acid supply to the fetus is optimal at each gestational age, making umbilical plasma concentrations an appropriate standard. Therefore, cord samples from 16 premature infants, including 10 study infants, (gestational age 27-35 weeks), obtained immediately after birth and before placental separation were analyzed. Umbilical plasma amino acid concentrations of these preterm infants were used as a second reference. It is recognized that the fetus has a net production of glutamine, glycine, and serine resulting in their transfer back to the placenta or mother via the umbilical artery and, therefore, an increase in their concentration in umbilical cord plasma (82). With the exception of these three amino acids, umbilical cord concentration is thought to reflect fetal supply and be devoid of toxic amino acid concentrations. Although there is wide variability in the concentrations reported in these two standards, it can be generally stated that umbilical cord plasma levels are higher than levels found in breast fed infants (exceptions being proline, glutamic acid, threonine and tyrosine). Minimal plasma levels of amino acids in breast fed infants are, consequently, probably a better standard for assessing adequacy while maximum concentrations in umbilical cord samples and/or breast fed infants are acceptable for determining possible toxicity.

Even at the low intakes received by infants in the first 24 hours of infusion, several amino acids were found in concentrations exceeding the maximum observed in either breast fed term infants or premature

umbilical plasma (Table 13). Several infants had comparatively high concentrations of glycine, methionine, arginine, histidine, phenylalanine, and serine. Conversely, very few of the infants could be considered to have an adequate cysteine level (1 of 16 when compared to cord values and 4 of 16 when compared to breast fed infants).

Results at One Week of Age

The eleven infants maintained on total parenteral nutrition with no oral supplementation for at least one week are described in Table 14. This group includes 9 of the original sample and 2 additional infants (Per and Dal). Not surprisingly, these infants tended to be smaller and younger in terms of gestational age than the infants in the original sample who had begun enteral feedings prior to the eighth postnatal day. Gestational age for the infants evaluated at one week of age ranged from 27 to 36 weeks with a mean age of 30.8 ± 2.8 weeks. Their weight at birth was appropriate for gestational age and averaged 1493 ± 397 g (range = 970-2200 g). This group included 7 males and 4 females and one set of twins (Sh1 and Sh2).

Blood samples from heel puncture sites were obtained on the eighth day of life (age one week) and analyzed as previously described. Results are presented in Table 15.

Each infant's amino acid and caloric intake in the 24 hours prior to sample collection was calculated from the volume and composition of the solution infused. Amino acid concentration in the infusate was either as described in Table 4 (p. 34) [one gram of the crystalline L-amino amino acid mixture Aminosyn (Abbott Laboratories, North

TABLE 14

Subjects receiving total parenteral nutrition exclusively for at least one week

Infants	Gestational age	Birthweight
	weeks	g
Per	27	970
Dra	27	1000
Dal	29	1160
Lov	29	1200
Yoa	30	1460
Vnc	31	1360
Kop	31	1620
Sh1	33	1760
Sh2	33	1790
Bis	33	1900
Cla	36	2200
Means	30.8	1493
SD	2.8	397
Range	27-36	970-2200

TABLE 15

Plasma amino acid concentrations at one week of age

Amino acid	Mean	SD	Range
	mg/dl	mg/dl	mg/dl
Histidine	2.29	0.43	1.54-2.77
Ornithine	3.48	1.27	2.21-6.20
Lysine	4.10	1.36	2.34-6.86
Phenylalanine	1.21	0.24	0.86-1.57
Tyrosine	1.23	0.41	0.73-1.82
Leucine	1.41	0.20	1.06-1.69
Isoleucine	1.00	0.20	0.64-1.41
Methionine	1.10	0.34	0.70-1.43
Valine	2.52	0.52	1.46-2.99
Glycine	3.97	0.97	2.23-6.21
Alanine	2.47	0.64	1.39-3.70
Glutamic acid	5.32	1.26	2.94-6.94
Serine	2.29	0.44	1.42-3.00
Threonine	3.34	1.30	1.49-6.41
Aspartic acid	0.43	0.33	0.07-1.06
Proline	2.86	0.80	1.64-4.22

Chicago, Ill.) per dl infusate] or was increased by adding 1.8 g of Aminosyn/dl. Relative concentration of amino acids did not differ however; for example intake of glycine and alanine remained equal, etc. Total amino acid intake in the 24 hours preceding sample collection was divided by the weight at one week to determine intake in mg/kg/day for each amino acid (Table 16). Intakes were again compared with the recommendations of Pohlandt, Burger, Stegink, Ghadimi, and Snyderman (10-13). In contrast to the intakes in the first 24 hours of infusion, the intake of most amino acids was now within the recommended range for most infants. The most obvious exception was methionine which was infused in excess of the maximum recommended amount in all 11 infants. In some cases this excess was marked; for example 5 infants received more than twice the maximum recommended intake and one 3 times. Eight (8) of the 11 infants also had intakes exceeding the maximum recommended for proline, 7 exceeded maximum for glycine and 5 each for serine and phenylalanine.

These very high intakes were at least partially reflected in plasma amino acid concentrations at one week (Table 17). All 11 infants had higher plasma glycine concentrations than the maximum reported in either breast fed term infants or premature umbilical cord samples. Methionine levels (measured in only 6 babies because of technical problems) exceeded maximum levels obtained from breast fed infants in all cases and exceeded the maximum level of cord plasma in 4 of 6 infants. Serine concentration exceeded the maximum observed in the breast fed standard in 8 of the 11 infants, but the great majority (10 of 11) were

TABLE 16

Intake of amino acids at one week compared to literature recommendations

Amino acid	Mean	SD	Range	Recommended[1-6]	<Rec[7]	Rec[8]	>Rec[9]
	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day			
Leucine	202	61	123-316	125-400	1	10	0
Lysine	155	47	94-243	120-334	3	8	0
Methionine	86	26	52-135	15-45	0	0	11
Phenylalanine	94	28	57-148	59-104	1	5	5
Threonine	112	34	68-176	60-150	0	10	1
Valine	172	52	105-269	80-200	0	7	4
Alanine	274	82	167-429	100-700	0	11	0
Arginine	210	63	128-328	110-250	0	8	3
Glycine	274	82	167-429	50-255	0	4	7
Tyrosine	35	11	21-55	13-79	0	11	0
Histidine	65	20	40-101	50-120	3	8	0
Proline	185	56	112-289	50-135	0	3	8
Isoleucine	155	47	94-243	60-200	0	10	1
Serine	92	28	54-144	50-100	0	6	5
Tryptophan	34	10	21-54	30-120	4	7	0

[1]For each amino acid the first number is the smallest of the minimum recommended intakes of 5 investigators and the second is the largest of the maximum recommended intakes of these investigators.

[2]Pohlandt, F. (1979) Studies on the requirement of amino acids in newborn infants receiving parenteral nutrition. In: Nutrition and Metabolism of the Fetus and Infant (Visser, H. K. A., ed.), pp. 341-364, Martinus Nijhoff, the Hague.

TABLE 16 (continued)

[3]Burger, U. & Wolf, H. (1976) Investigations on the utilization of parenterally administered amino acids by premature and hypotropic neonates. Europ. J. Pediatr. 122, 169-175.

[4]Ghadimi, H. (1975) Newly devised amino acid solutions for intravenous administration. In: Total Parenteral Nutrition: Premises and Promises (Ghadimi, H., ed.), pp. 393-442, John Wiley & Sons, New York.

[5]Stegink, L. D. (1975) Development of a crystalline amino acid mixture for parenteral alimentation of young children. In: Intravenous Nutrition in the High Risk Infant (Winters, R. W. & Hasselmeyer, E. G., eds.), pp. 411-420, John Wiley & Sons, New York.

[6]Snyderman, S. E. (1975) Amino acid requirements. In: Intravenous Nutrition in the High Risk Infant (Winters, R. W. & Hasselmeyer, E. G., eds.), pp. 205-214, John Wiley & Sons, New York.

[7]< Rec = number of infants receiving less than minimum recommended intake.

[8]Rec = number of infants with intakes within the recommended range.

[9]> Rec = number of infants receiving more than the maximum recommended intake.

TABLE 17

Comparison of plasma amino acid concentrations in study infants at one week, breast fed infants, and umbilical cord blood samples

Amino acid	Subjects	Breast fed[1]	<Min[2]	=[2]	>Max[2]	Cord blood[3]	<Min[4]	=[4]	>Max[4]
	Range mg/dl	Range mg/dl				Range mg/dl			
Histidine	1.54-2.77	0.73-1.63	0	1	8	0.74-2.32	0	5	4
Ornithine	2.21-6.20	0.73-1.45	0	0	11	0.44-6.21	0	11	0
Lysine	2.34-6.86	1.25-2.69	0	2	9	2.07-6.57	0	10	1
Phenylalanine	0.86-1.57	0.49-1.06	0	3	5	0.89-2.63	1	7	0
Tyrosine	0.73-1.82	0.60-2.28	0	9	0	0.69-1.72	0	7	2
Leucine	1.06-1.69	0.64-1.59	0	8	2	0.82-2.48	0	10	0
Isoleucine	0.64-1.41	0.26-0.68	0	1	9	0.29-1.35	0	10	0
Methionine	0.70-1.43	0.19-0.34	0	0	6	0.23-0.87	0	2	4
Valine	1.46-2.99	0.88-1.89	0	2	9	1.42-3.63	0	11	0
Alanine	1.39-3.70	1.19-3.31	0	9	2	1.93-6.08	1	10	0
Glycine	2.23-6.21	1.10-2.02	0	0	11	0.61-2.19	0	0	11
Glutamic acid	2.94-6.94	4.78-12.19	3	8	0	1.63-9.61	0	11	0
Serine	1.42-3.00	0.74-2.15	0	3	8	0.87-2.90	0	10	1
Threonine	1.49-6.41	0.61-1.72	0	1	10	2.09-9.11	1	10	0
Aspartic acid	0.07-1.06	---- ----	--	--	--	0.16-1.15	1	9	0
Proline	1.64-4.22	1.07-4.80	0	9	1	0.93-2.75	0	4	6

[1]Tikanoja, T. (1982) Plasma amino acids in term neonates after a feed of human milk or formula. Acta Paediatr. Scand. 71, 391-397.

[2]< Min = number of infants with levels less than minimum in breast fed term infants. = is number within range. > Max = number exceeding maximum.

[3]Plasma amino acids from umbilical cord samples from 16 premature infants (27-35 weeks gestation). Unpublished data from this laboratory.

[4]< Min = number of infants with levels less than minimum in premature cord plasma. = is number within range. > Max = number exceeding maximum.

within the range of concentrations found in umbilical plasma. The reverse was true for proline concentration which was within the range observed in breast fed infants in 9 of 10 cases and which exceeded the maximum concentration in the cord plasma of prematures in 6 of 10 infants. Although intake of histidine did not exceed recommendations in any infant and was, in fact, below the level recommended for 3 of the 11, the plasma concentration tended to be higher than reference values (8 of 9 > breast fed, 4 of 9 > cord). Nine infants had high lysine concentrations and 10 had high threonine concentrations when compared to values obtained from breast fed infants. The fact that the concentrations of these two amino acids did not exceed the range observed in umbilical cord plasma is not surprising considering the very high cord concentration.

Two-way analysis of variance was again used to test for the effects of gestational age and amino acid intake on plasma amino acid concentrations. Postnatal age, found previously to be associated with phenylalanine concentration, was not included because all the infants were evaluated on the eighth day of life. Histidine, tyrosine, and leucine concentrations were associated with gestational age, serine concentration with intake, and proline and valine concentrations with both intake and gestational age (Tables 18 & 19). Histidine, proline and valine increased linearly with gestational age . The plot of plasma tyrosine concentration versus gestational age, on the other hand, was curvilinear with the point of inflection at approximately 32 weeks

TABLE 18

Linear regression of plasma amino acids at one week of age with gestational age

Amino acid	Intercept	Slope	Sums of squares	F-value	P-value
Histidine	-1.969	0.1420	0.8067	8.03	0.0253
Proline[1]	-5.723	0.2248	3.2713	11.84	0.0108
Valine[2]	-1.898	0.0910	0.5535	6.14	0.0382
Tyrosine					
linear	61.5524	-4.0709	0.6692	7.05	0.0377
quadratic	61.5524	0.0683	0.6852	7.22	0.0362
Leucine					
linear	-17.0815	1.1607	0.0925	10.90	0.0131
quadratic	-17.0815	-0.0180	0.2100	24.76	0.0016

[1]Plasma value also varies with proline intake.

[2]Plasma value also varies with valine and caloric intakes.

TABLE 19

Linear regression of plasma amino acids at one week of age with intake of amino acid

Amino acid	Intercept	Slope	Sums of squares	F-value	P-value
Serine	1.3479	0.0101	0.8248	6.64	0.0299
Proline[1]	-5.7232	0.0091	2.0452	7.40	0.0297
Valine[2]	-1.8983	0.0092	1.9180	21.29	0.0017

[1]Plasma value also varies with gestational age.

[2]Plasma value also varies with gestational age and caloric intake.

(Figure 1). Plasma leucine concentration increased from 27 to approximately 32 weeks gestation, but then appeared to level off or actually decrease (Figure 2). This change after 32 weeks may be specious, however, because there was only one infant with gestational age greater than 33 weeks. Over the range of intakes in this study the plasma concentrations of serine, valine, and proline bore linear relationships to their intakes.

Relationships between Intakes, Growth and Plasma Amino Acid Concentration

While plasma amino acids give an immediate assessment of nutritional status, the best indicator of adequacy and appropriateness of nutrient intake is growth. Therefore, despite this study's limited time frame, an attempt was made to describe certain parameters of growth in the 11 infants.

As expected the infants lost weight in the first few days after birth. Maximum weight loss varied from 7 to 26% of birth weight with a mean loss of 16% (Table 20). This is slightly more than the reported normal weight loss for prematures of 10-15% (83), but these infants tended to be the smaller and less mature infants of the original sample. Weight gain was recorded as early as 5 days and as late as 10 days with a mean postnatal age of 6.9 days. At the time of first weight gain infants were receiving from 36-82 kcal/kg/day and from 0.20-0.55 g nitrogen/kg/day (Table 21). There was no correlation between gestational age and nitrogen intake on the first day of growth, but there was a tendency for more immature infants in this

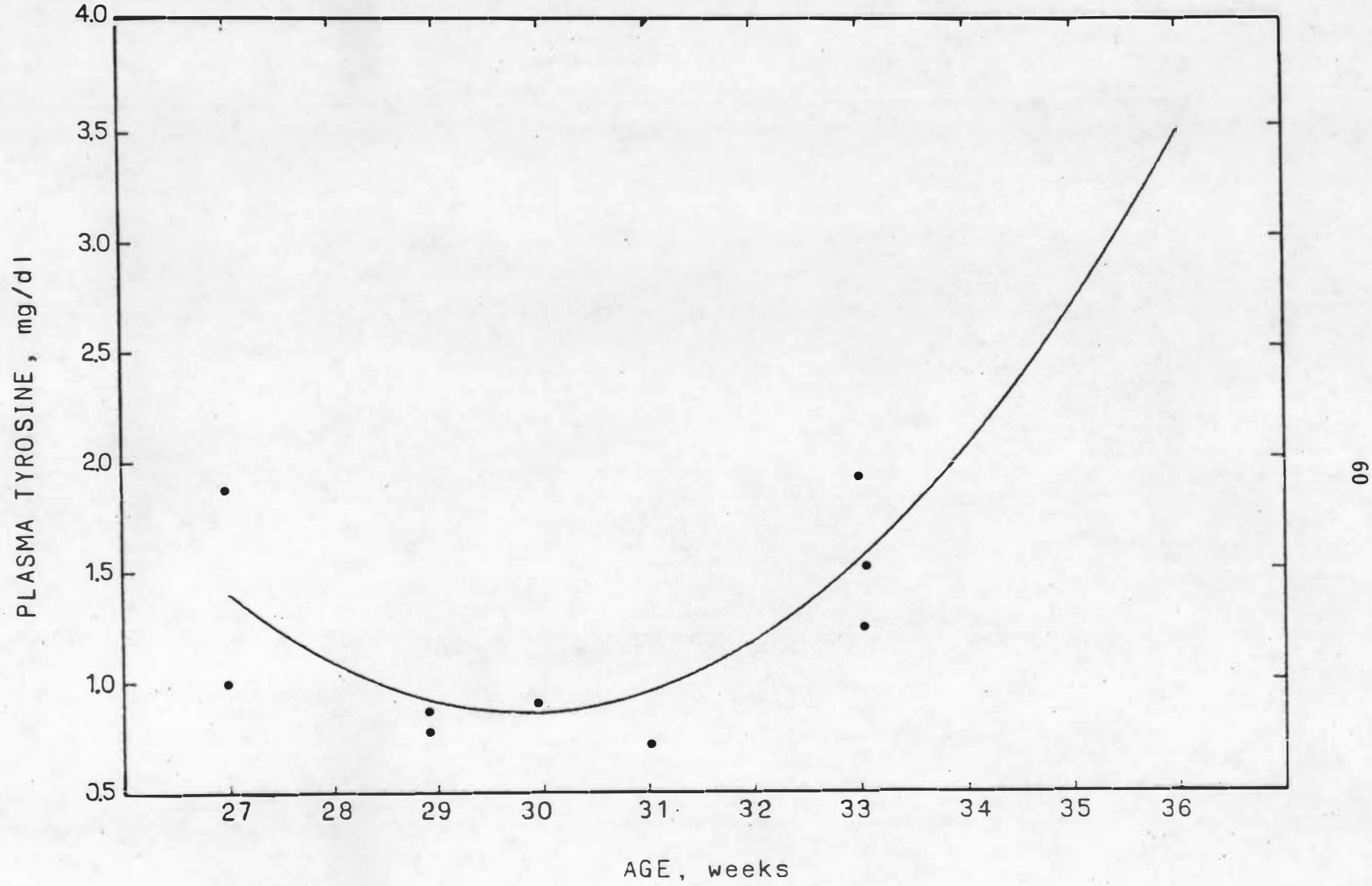


Figure 1. Plasma Tyrosine Concentration at One Week as a Function of Gestational Age.

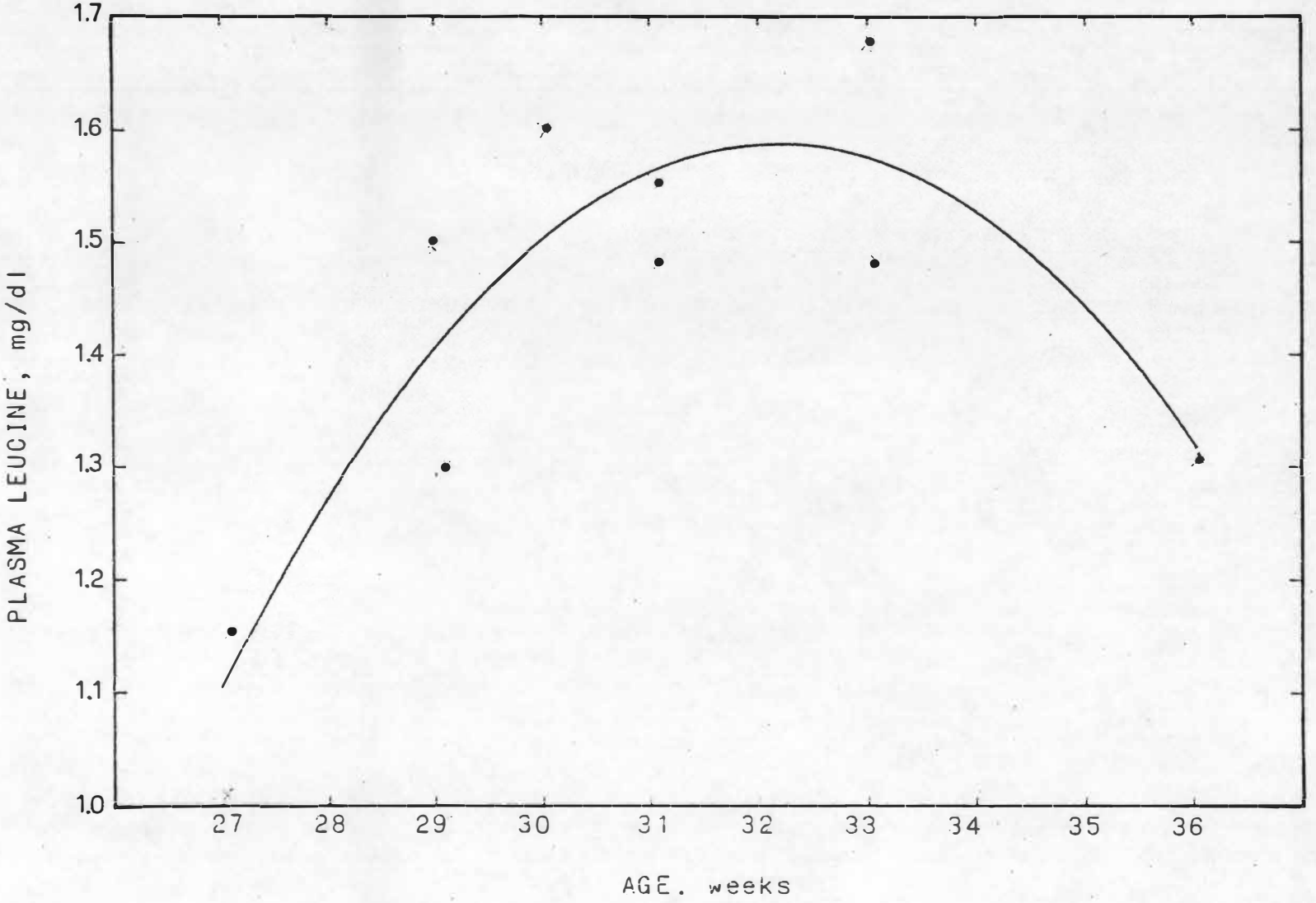


Figure 2. Plasma Leucine Concentration at One Week as a Function of Gestational Age.

TABLE 20

Nutrient intake on initial day of weight gain

Infant	Postnatal day	% Lost[1]	Calories	Nitrogen	Amino acids
			kcal/kg/day	g/kg/day	g/kg/day
Per	6	26	73	.55	3.45
Dra	7	13	59	.20	1.30
Dal	5	22	57	.21	1.33
Lov	6	21	82	.48	3.04
Yoa	8	17	56	.22	1.38
Vnc	10	20	57	.31	1.97
Kop	7	11	74	.40	2.53
Sh1	5	14	60	.35	2.18
Sh2	10	16	69	.35	2.16
Bis	7	13	38	.22	1.40
Cla	5	7	36	.21	1.32
Means	6.9	16.4	60.1	.32	2.00
SD			14.2	.12	

[1] % lost = maximum % of birth weight lost.

TABLE 21

Correlations between gestational age and selected nutrient intakes on initial day of weight gain

Subject	Age	Calories	Nitrogen
	weeks	kcal/kg/day	g/kg/day
Bis	33	38	.22
Cla	36	36	.21
Dal	29	57	.21
Dra	27	59	.20
Kop	31	74	.40
Lov	29	82	.48
Per	27	73	.55
Sh1	33	60	.35
Sh2	33	69	.35
Vnc	31	57	.31
Yoa	30	56	.22
Means	30.8	60.1	.32
SD	2.8	14.2	.12
Range	27-36	36-82	.20-.55
Correlation coefficient		-.57	-.32
Probability		.06	.33

sample to begin to grow at lower caloric intakes (correlation coefficient = -.57, $p < .06$).

Other investigators have reported a relationship between branched chain amino acid concentration and caloric intake and between branched chain amino acid concentration and growth (84). In this study concentration of valine, but not leucine or isoleucine, was significantly correlated with caloric intake in the preceding 24 hours (Table 22). Neither individual nor total branched chain amino acids were, however, associated with growth in the first week of life in these infants (Table 23).

TABLE 22

Correlations between plasma branched chain amino acids at one week and caloric intake in the preceding 24 hours

Subject	Calories	Leucine	Isoleucine	Valine
	kcal/kg/24 hours	mg/dl	mg/dl	mg/dl
Bis	38	--	--	1.68
Cla	44	1.32	0.82	2.49
Dal	73	1.30	1.03	2.90
Dra	54	1.06	0.99	1.46
Kop	65	1.48	1.12	2.86
Lov	61	1.48	1.41	2.53
Per	77	1.15	0.64	2.83
Sh1	57	1.48	0.94	2.95
Sh2	54	1.69	1.03	2.73
Vnc	68	1.56	0.96	2.99
Yoa	50	1.60	1.09	2.30
Means	58.5	1.41	1.00	2.52
SD	11.9	0.20	0.20	0.51
Range	38-77	1.06-1.69	0.64-1.41	1.46-2.99
Correlation coefficient		-.24	-.17	.65
Probability		.51	.64	.03

TABLE 23

Correlations between plasma branched chain amino acids at one week and change in weight in preceding 24 hours

Subject	Weight change	Leucine	Isoleucine	Valine	Total[1]
	g	mg/dl	mg/dl	mg/dl	mg/dl
Bis	20	--	--	1.68	--
Cla	-20	1.32	0.82	2.49	4.63
Dal	0	1.30	1.03	2.90	5.23
Dra	20	1.06	0.99	1.46	3.51
Kop	30	1.46	1.12	2.86	5.46
Lov	30	1.48	1.41	2.53	5.42
Per	30	1.15	0.64	2.83	4.62
Sh1	-10	1.49	0.94	2.95	5.37
Sh2	20	1.69	1.03	2.73	5.45
Vnc	0	1.56	0.96	2.99	5.51
Yoa	40	1.60	1.09	2.30	4.99
Means		1.41	1.00	2.52	5.02
SD		0.20	0.20	0.52	0.63
Correlation coefficient		0.09	0.36	-0.26	-0.05
Probability		.80	.31	.43	.90

[1]Total = leucine + isoleucine + valine.

CHAPTER V

DISCUSSION

The objective of this study was to describe the premature infant's plasma amino acid response to parenteral amino acid infusion and to determine the relation of that response to gender, plasma amino acid concentration at birth, amino acid status prior to infusion, postnatal age, caloric intake, weight, individual amino acid intake, and gestational age. The first part of the study examined the initial response (change in plasma amino acids in the first 24 hours of infusion with amino acids), and the second part evaluated plasma amino acid status at one week in infants still receiving total parenteral nutrition with no enteral intake.

Gender was not an influencing factor in the plasma concentration of any amino acid before infusion or 24 hours later or in the change in concentration during that interval. This is in agreement with Berry (85) who found no differences in plasma amino acid concentration at birth in infants of different sexes.

The amino acid status at birth, as reflected by concentration in umbilical cord blood, was not associated with plasma amino acid concentration at the time infusions began (age 24-75 hours). There was, however, no determination of circadian variations in cord values and the number of infants with available cord analyses was small (n = 10).

Anderson et al. (86) has reported that plasma amino acids levels rise only after a certain minimal intake of amino acids. They found no

plasma pool expansion of any amino acid at low levels of intake. In the first 24 hours of infusion in this study, however, there was a significant increase in concentration of all amino acids contained in the infusate except histidine and tyrosine (Table 5, p. 35). Increases occurred despite very limited intake of amino acids and calories (mean kcal/kg/day = 37 ± 11). Low plasma amino acid levels are thought to reflect deficiency states (87), suggesting that greater tissue uptake of infused amino acids and, therefore, smaller increases in plasma concentration would be found in infants with lower preinfusion amino acid levels. This was not supported by the data in this study, however, as the percent of change in plasma concentration showed either no association with preinfusion levels or was greater in infants with low preinfusion levels. It may be that the synthetic capacity of the most depleted infants (those with lowest plasma concentrations) was so limited that infused amino acids, in excess of those needed for energy, stayed in the plasma as part of the infant's amino acid pool rather than being taken up by the tissues for protein synthesis.

Postnatal age, ranging from 24-75 hours at the time infusion began, was evaluated as a factor in the infant's response to parenteral amino acids. Phenylalanine was the only amino acid significantly affected by postnatal age (Tables 7-9, pp. 38-40). Plasma phenylalanine concentration, absolute change in concentration in the first 24 hours of infusion, and the percent of change in concentration all decreased as postnatal age increased. This may indicate postnatal maturation of

phenylalanine hydroxylase, the enzyme catalyzing the conversion of phenylalanine to tyrosine (14). Filer (88) has reported a decrease in the concentration of threonine with increasing postnatal age, but that association was not confirmed in this study. Infants in the present study were tested at 2-4 days as compared to Filer's 11-35 days, possibly obscuring a significant slow decline in threonine concentration.

It has been suggested that gluconeogenic mechanisms are postnatally induced as the infant rapidly depletes his glycogen supply and is forced for the first time to supply his own carbohydrate needs (89). There was no indication in this study, however, that postnatal age had any bearing on the concentrations of alanine, serine, glycine, cysteine, or any other glucogenic amino acid. Induction of glucogenic enzymes may have occurred before amino acid infusion began or may have been masked by close monitoring and provision of appropriate glucose in both the amino acid solution and the glucose solution given since birth. There was also no association between caloric intake and the plasma concentration of major glucogenic amino acids at the level of caloric intakes in this study.

Weight, as a reflection of muscle mass, was evaluated as a factor in plasma concentration of individual amino acids. The amino acids most likely to be influenced by muscle mass would be the branched chain amino acids because they are metabolized primarily in muscle (29) and lysine because of its propensity for storage in muscle (90). After controlling for gestational age, however, weight was not associated

with the plasma concentration of any amino acid after 24 hours of infusion or at one week of age.

The composition and amount of amino acids infused have been found to be related to plasma amino acid concentration (88, 91-93), but the exact relationship is not well defined. Anderson et al. (86) concluded that "blood levels do not bear a simple direct relationship to intake but rather reflect the overall dynamics of the individual blood amino acid pool." Changes in amino acid concentration are thought to be affected by endogenous regulation as well as intake. The response of infants in this study to alanine and glycine, added to the infusate as sources of nonspecific nitrogen, is an example of this complex interaction.

Although recommendations for sources of nonspecific nitrogen have included glycine, alanine, and the branched chain amino acids, glycine has been most commonly used, at least partially because it is comparatively cheap. The infusate used in this study contained identical amounts of glycine and alanine (128 mg/g Aminosyn), but infant response to the two amino acids was different (Table 12, p. 46). Each mg of glycine infused in the first 24 hours resulted in an increase in plasma concentration roughly twice that of alanine (0.0066 vs. 0.0034 mg/dl). At one week of age plasma concentration of glycine in every infant exceeded the maximum observed in breast fed term infants and in preterm mixed cord blood. Maximum plasma concentration of alanine in study infants never exceeded that found in preterm cord samples

and was greater than the maximum level observed in breast fed term infants in only 2 cases (Table 17, p. 55). In contrast to glycine, alanine did not tend to accumulate in plasma at the concentration infused in this study. These observations were made, however, at a time of caloric deficit and a possible need for endogenous glucose synthesis. Although both alanine and glycine are glucogenic, glycine must first be converted to serine (14) and may not be as readily utilized as alanine. Dale (94) and Pohlandt (10) also found very high tolerance to alanine with plasma concentration increasing only at high rates of administration. This experience, along with the knowledge that fetal consumption of alanine is the greatest of any amino acid, prompted Pohlandt to recommend a maximum dose of 700 mg/kg/day of alanine, more than twice the maximum recommended for any other amino acid. This study supports the idea that alanine, rather than glycine, is the better source of nonspecific nitrogen in the premature infant, at least in times of caloric deprivation.

The high concentration of glycine in the infusate given infants in this study may have influenced the response of serine whose plasma concentration at one week was directly associated with intake (Table 19, p. 58). Serine and glycine can be interconverted through the glycine cleavage system (95) which is apparently functional in the preterm infant (26). In this study serine was present in relatively low concentration in the infusate (42 mg/dl, the lowest of any non-essential amino acid). It had, however, the highest increase in

plasma concentration per mg intake in the first 24 hours of infusion (Table 11, p. 44). Other investigators have reported significant increases in plasma serine concentration associated with both high intakes of serine and glycine (91, 94) and glycine alone (10, 94). The synthesis of serine from glycine requires the addition of a one carbon fragment which may be supplied by methionine, histidine or tryptophan (14). In infants in this study one carbon fragment donors are in short supply relative to glycine (Table 24). Therefore conversion of significant amounts of glycine to serine could result in deficiency of one carbon fragments needed for nucleic acid synthesis and thus result in depressed growth and erythropoesis. The failure of infants in this study to fully utilize infused amino acids (as evidenced by plasma accumulation) further compounds the potential problems.

In infants at one week of age plasma proline concentration was significantly associated with intake (Table 19, p. 58). Dale (94), studying postoperative neonates, found a similar linear increase in proline concentration with increases in the rate of infusion of a synthetic amino acid solution of undescribed composition. Increase in plasma proline concentration in infants in this study may have been related to the proline concentration of the infusate (86 mg/dl), which resulted in intakes greater than the recommended maximum in 8 of the 11 infants or the increase may have been related to immature renal function (96). The relationship between plasma concentration and urinary excretion is supported by the observation of Burger (97) that plasma proline concentrations were higher in infants who had

TABLE 24

Possible demand and sources of one carbon fragments from 100 ml intravenous solution containing 1 g Aminosyn[1]

Acceptor			Donors		
	mg	mmol		mg	mmol
Glycine	128	1.70	Methionine	40	0.27
			Histidine	30	0.19
			Tryptophan	16	0.08
Total:		1.70			0.54

[1]Abbott Laboratories, Chicago, Ill.

relatively little renal loss of proline. Maturation of the kidney resulting in decreased prolinuria might also be related to the association of increasing plasma proline with advancing gestational age (Table 18, p. 57).

Gestational age as a factor in the premature infant's response to parenteral amino acid infusion is of particular interest because of the possibility of enzymatic immaturity leading to either amino acid excess (when catabolic pathways are not developed) or to amino acid deficiency (when synthetic pathways are immature). For example, serious doubts have been raised about the ability of the preterm infant to synthesize cysteine and taurine from methionine precursors (67, 69). The absence of both cysteine and taurine from the infusate in this study and the presumed inability to synthesize either suggest that their plasma concentrations would fall over time. In fact, however, the decline in taurine concentration in the first 24 hours of infusion was insignificant and cysteine had a small but insignificant increase in concentration (Table 5, p. 35). Individual infants had as much as a 55% increase in taurine and a 114% increase in cysteine values. With no intake of either cysteine or taurine it appears that some infants have the enzymatic mechanisms for their synthesis. This ability may not be universal in this population, however, and cannot be predicted on the basis of gestational age, postnatal age, weight, caloric intake, or methionine intake under the conditions of this study.

Tyrosine concentration in one week old infants was found to increase significantly with gestational age (Table 18, p. 57, and

Figure 1, p. 60). This association is presumably due to the increased conversion of phenylalanine to tyrosine but the decline in phenylalanine levels with advancing gestational age did not reach significance ($p < .07$). The normal catabolic route for phenylalanine results in the synthesis of tyrosine in a reaction catalyzed by phenylalanine hydroxylase (8). The activity of this enzyme, although present in low levels since early in fetal development, normally shows a marked upsurge in the first day of life (7). This description is in agreement with the finding in this study of an association between phenylalanine concentration after 24 hours of infusion and postnatal age (Tables 7-9, pp. 38-40). Although plasma concentrations of phenylalanine and tyrosine in some infants exceeded maximum reference values none were suggestive of hyperphenylalanemia or tyrosinemia.

Plasma concentration of histidine in infants one week of age was directly related to gestational age (Table 17, p. 55). Supplied in the infusate in a concentration of 30 mg/g Aminosyn, histidine intake never exceeded the maximum recommended by other investigators, yet plasma concentrations tended to be high (Table 16, p. 53). Increased concentration in the plasma suggests either increased synthesis, decreased utilization, decreased urinary loss or decreased catabolism. Of these, decreased catabolism does not seem a likely explanation for the increase in histidine concentration. Histidine has 2 major catabolic routes (7). The preferred route in most cases begins with conversion of histidine to urocanate in a reaction catalyzed by histidase. In rats this enzyme is detectable at a very low level just before birth but

presumably develops postnatally. In this study histidine concentrations after 24 hours of infusion were not associated with postnatal age. The second catabolic reaction involves transamination of histidine by histidine-pyruvate transaminase, an enzyme developing in the late fetal period in rats but apparently not until 6-7 weeks after birth in humans (7). Although histidine has been considered a nonessential amino acid for healthy adults, too little is known about its biosynthesis to speculate on the maturation of synthetic pathways in premature infants. Like proline and glycine, histidine is normally excreted in large quantities in the urine of the premature infant. Decreasing renal loss with advancing gestational age may be the explanation for increased plasma histidine concentration, but this relationship could not be tested in this study.

The relationship between plasma leucine concentration and gestational age is confusing. The graph of this relationship (Figure 2, p. 61) shows an increase in plasma concentration from age 27 to approximately 32-33 weeks gestation followed by a decrease in concentration from 33-36 weeks, the upper limit of gestational age in the sample. During the study period infants were in a state of energy deficit and may have been utilizing branched chain amino acids for energy. It has been proposed that utilization of branched chain amino acids is facilitated by carnitine (98), which is found in higher concentration in cord blood of preterm than of term infants (99). If this is true, the utilization of leucine for energy would be facilitated by the more premature infant's higher carnitine levels. Decreased

utilization of leucine for energy by the more mature infants would result in an increasing plasma concentration of leucine with advancing gestational age. This is the situation depicted between 27 and 33 weeks gestation. The fall in plasma concentration after that time must be interpreted with caution because of the disproportionate influence of the one subject of 36 weeks gestational age.

Valine is the only amino acid whose concentration at one week was affected by caloric intake ($p < .04$). In contrast to the other branched chain amino acids which are purely ketogenic (leucine) or both ketogenic and glucogenic (isoleucine), valine is purely glucogenic (14). Infants in this study were in a state of energy deficit (mean caloric intake < 60 kcal/kg/day at one week), and the increase in plasma valine concentration associated with increased intake may be due to simple valine sparing,' i.e., decreased utilization for energy. Valine concentration is also associated with valine intake (Table 19, p. 58). Intake of valine at one week exceeded the maximum recommendation in 4 of 11 infants. Nine (9) of 11 infants had plasma concentrations exceeding the maximum observed in breast fed babies, however, while none exceeded the maximum measured in preterm cord samples. Literature reports on the relationship of parenteral valine intake to concentration in the plasma are similar. With intakes up to 200 mg/kg/day, Ghadimi (11) found all plasma values were within the range of cord blood. Pohlandt (10), however, found infants infused with 180 mg/kg/ day had plasma concentrations exceeding those of breast fed newborns. It would seem that the ever confusing question

of appropriate reference values for plasma amino acid levels in prematures is of particular importance in the case of valine. Response of premature infants to infusions of various amounts of valine should also be measured when caloric intake is controlled, a situation not found in this study.

Methionine is another amino acid whose plasma concentration is cause for concern. The maximum value reported in breast fed term newborns was exceeded in 15 of 19 infants after 24 hours of infusion and by all 6 infants whose methionine concentration was measured at one week of age. Concentration of methionine in the infusate in this study (40 mg/g Aminosyn) appears too high for this population as all infants received more methionine per kg/day at one week than recommended. In laboratory animals methionine was the most toxic amino acid, causing inhibition of growth and tissue damage when given in relatively low excess (100). Methionine has been shown to be particularly toxic in the young (101) and when protein intake is inadequate (102) as it was in this study. The amino acid source (Aminosyn) used in the infusate was not specifically formulated for infants. It is used with apparently few problems in adults who are able to synthesize cysteine and taurine from methionine precursors. The premature infant may lack this capacity, however, and be able to utilize relatively smaller amounts of methionine. These limitations are acknowledged by various investigators making recommendations for methionine intake by the premature infant (15-45 mg/kg/day), but are frequently ignored in practice.

CHAPTER VI

SUMMARY

This study investigated the response of premature infants to parenteral amino acids and related that response to various independent variables. Plasma samples were collected before the initiation of amino acid infusion and after 24 hours of infusion from 21 premature infants (gestational age 31.2 ± 2.34 weeks) admitted to the Intensive Care Nursery of The University of Tennessee Memorial Research Center and Hospital, Knoxville. Mixed umbilical cord blood samples were available from 10 of these infants. Blood samples were also obtained from 11 infants (gestational age 30.8 ± 2.8 weeks) receiving parenteral amino acids with no enteral supplementation at one week. Plasma amino acid concentrations were compared to those reported in breast fed term infants and in preterm umbilical cord samples. Intakes in the 24 hours prior to sampling were calculated and compared to published recommendations. The amino acid source was Aminosyn (Abbott Laboratories, Chicago, Ill.).

Neither plasma amino acid concentration nor change in concentration in the first 24 hours of infusion was affected by gender, plasma amino acid concentration at birth or weight. Changes in concentration of most amino acids appeared to be a result of endogenous regulation more than simple intake. Alanine infused at a relatively high concentration (128 or 230 mg/dl) showed relatively little plasma accumulation and appeared to be the best source of nonspecific nitrogen during the

hypocaloric conditions of the first week of life. The same concentration of glycine in the infusate lead to excessive plasma concentrations of glycine and may have contributed to high levels of serine. The high concentration of glycine in the infusate is not balanced by adequate sources of one carbon fragments for serine synthesis.

Intakes of histidine did not exceed recommendations, but plasma histidine was high in most infants. Concentration of plasma histidine varied directly with gestational age.

Plasma phenylalanine levels varied inversely with postnatal age and tyrosine concentration varied directly with gestational age, suggesting effects of both gestational and postnatal ages on the function of the phenylalanine hydroxylase system. In light of the very limited intakes, there was no evidence of tyrosine being an essential amino acid in most prematures.

Although the time period was very short, there were indications of cysteine and taurine synthesis in some infants. Methionine intakes exceeded recommendations in all infants at one week and were associated with excessive plasma concentrations.

Valine concentration varied directly with caloric intake, gestational age, and valine intake. Leucine concentration increased with gestational age from 27-32 weeks, but the relationship after that time is unclear. Isoleucine concentration was not affected by any of the variables tested.

Some overall conclusions can be reached. First, in these infants there is great variability in plasma concentration of amino acids and in

the response to infused amino acids. Preterm infants are not a homogenous group.

Secondly, interpretation of the data is only as good as reference values used for interpretation, and the fact remains that no one knows the "normal" plasma concentration of amino acids in the preterm infant. The premature infant is a unique biological entity, at a different maturational level than the term infant and in a different environment than the fetus of comparable gestational age. There is no prototype of optimal feeding, such as breast milk for the term infant, but evaluation of amino acid status of prematures fed their own mother's milk might provide a better standard for evaluating other feeding methodologies.

Finally, based on this study and the reference values used, Aminosyn does not appear to have the ideal amino acid composition for the preterm infant. For use during the first week of life when intake of both amino acids and calories is low, the infusate should contain less glycine and methionine and possibly less serine, proline, histidine, and valine. Alanine, isoleucine, and possibly leucine could be increased in concentration to provide the necessary nitrogen. In theory, the optimal practice would be to individually formulate solutions matched to the infant's changing energy intake, maturation, and metabolic demands. Much research needs to be done, however, before solutions can even be tailored to provide optimal nutrition to the preterm parenterally fed infant.

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VITA

Mitzi Wilkinson Davis was born in Maryville, Tennessee, on March 11, 1947. She attended elementary school in Blount County and graduated from Alcoa High School in June 1964. After one year at The University of Tennessee, Knoxville, she completed requirements for a Bachelor of Science degree in nursing at The University of Tennessee Medical Units in Memphis in 1968. The following year she taught and served for one quarter as acting director of the Associate Degree Nursing Program at Cleveland State Community College in Cleveland, Tennessee. In 1969 Ms. Davis moved to Atlanta where she worked as a staff nurse at the Veterans Administration Hospital and at Grady Memorial Hospital and was an instructor in Nursing at Dekalb Community College before receiving a Master's degree in maternal and child health from Emory University in March 1975. In September of that year she accepted a position as Assistant Professor in the College of Nursing at The University of Tennessee, Knoxville. She resigned from that position in September 1979 to begin work toward the Doctor of Philosophy degree in home economics. During her graduate work she held an appointment as graduate teaching assistant for one year and was Associate Professor of Nursing at Cumberland College for one year.

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