Prospective treatment of urea cycle disorders

Nancy E. Maestri, PhD, Elizabeth R. Hauser, MHS,* Dennis Bartholomew, MD,** and Saul W. Brusilow, MD

From the Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland

We present a diagnostic and therapeutic protocol designed to prevent clinical expression of inborn errors of urea synthesis in the neonatal period, and discuss the long-term developmental outcome of survivors. The families of 32 infants, among 43 identified prenatally as being at risk for a urea cycle disorder, chose to have their infants treated according to a diagnostic and therapeutic protocol, beginning at birth. The therapy was effective in avoiding neonatal hyperammonemic coma and death in seven patients with carbamoyl phosphate synthetase deficiency, argininosuccinate synthetase deficiency, and argininosuccinate lyase deficiency. When treated prospectively, five of eight patients with ornithine transcarbamylase deficiency avoided severe hyperammonemia and survived the neonatal period. Two patients with carbamoyl phosphate synthetase deficiency and two with ornithine transcarbamylase deficiency have subsequently died; three additional patients with the latter disorder have received orthotopic liver transplants. Our experience suggests that these surviving patients have had a more favorable neurologic outcome than patients rescued from neonatal hyperammonemic coma. However, all of them require a burdensome medical regimen and may have handicaps that include impairment of development and recurrent episodes of hyperammonemia. Further, those with deficiency of carbamoyl phosphate synthetase or ornithine transcarbamylase have a high mortality rate. (J PEDIATR 1991;119:923-8)

Hyperammonemia occurs with varying severity in all patients with inborn errors of urea synthesis; these errors include deficiencies of carbamoyl phosphate synthetase, ornithine transcarbamylase, argininosuccinate synthetase, argininosuccinate lyase, and arginase. There is considerable variability in severity within each enzyme deficiency, presumably a function of genetic variability, and among the enzyme deficiencies as a result of their different metabolic consequences. The most severe expression of these diseases, excluding arginase deficiency, is neonatal hyperammonemic coma, the consequences of which include death, if untreated, or mental retardation and cerebral palsy in the surviving treated infants.

ALD	Argininosuccinate lyase deficiency
ASD	Argininosuccinate synthetase deficiency
CPSD	Carbamoyl phosphate synthetase deficiency
OTCD	Ornithine transcarbamylase deficiency

Prenatal diagnosis of at-risk fetuses, by DNA or bio-

Supported by the National Institutes of Health (grants HD11134, HD 26358, and RR-00052), the Kettering Family Foundation, the T. D. and M. A. O'Malley Foundation, and the National Organization for Rare Disorders.

The opinions expressed in this article are those of the authors and do not necessarily reflect those of the United States Air Force or the Department of Defense.

Submitted for publication Feb. 12, 1991; accepted June 24, 1991.

Reprint requests: Nancy E. Maestri, PhD, Department of Pediatrics, Johns Hopkins Hospital, Park 336, 600 North Wolfe St., Baltimore, MD 21205.

*Now at the Department of Biostatistics, University of Michigan, Ann Arbor, MI 48109-2029.

**Now at Air Force Medical Genetics Center, U.S. Air Force Medical Center, Keesler Air Force Base, Biloxi, MS 39534-5300.

9/20/32004

Table I. Prospective neonatal treatment protocols for "at risk" infants as described in this study

	Diagnosis		
	CPSD or OTCD	ASD	ALD
Diet (per kg per day)			
Natural protein (gm)	0.7	1.5	1.5
Essential amino acids (gm)	0.7	—	—
Calories (kcal)	120	120	120
Medications (mg/kg/day)*			
Arginine freebase	170	700	700
Sodium benzoate	250	250	—
Sodium phenylacetate†	250	—	—

*Oral dosage.
†Sodium phenylacetate was added to the protocol in 1984.

chemical analysis, and subsequent termination of the pregnancies can prevent these diseases. However, termination is not an option for all patients, either because of ethical concerns or because of a lack of 100% specificity of fetal diagnosis. We have developed a prospective therapeutic protocol as an alternative for parents who do not wish to abort an affected fetus; it is implemented within 12 hours of birth, before a definitive diagnosis is possible. The goal of this therapy is to prevent the development of hyperammonemia and its dire consequences in the neonatal period and thus improve the long-term developmental outcome of affected survivors.

We present here the outcome of 32 at-risk infants who were treated prospectively at birth according to a therapeutic protocol.

METHODS

Subjects. From 1981 to 1988, a total of 43 fetuses were identified as being at risk for a urea cycle defect on the basis of the diagnosis of a previously affected sibling with neonatal onset. Before conception or delivery, parents were counseled regarding three options available after the birth of an at-risk infant: the experimental prospective diagnostic and therapeutic protocol described below; no therapy unless hyperammonemia occurred; and comfort care without therapy after the development of hyperammonemia. As part of the counseling procedure, the medical burdens associated with treatment were also explained. These include the requirement of an artificial diet, the need for medication for an indefinite period, and the risk of subsequent episodes of hyperammonemia, brain damage, and death. Parents were encouraged to discuss these concerns with other parents who were familiar with the therapy. The option for orthotopic liver transplantation when the child reaches an appropriate age was also discussed.

Thirty-two parents (ALD, 2; ASD, 4; CPSD, 6; OTCD, 20) chose to have their at-risk fetus treated prospectively at birth. Prenatal DNA analysis indicated that three at-risk male fetuses had OTCD. One patient with ALD and three patients with ASD were identified after amniocentesis and enzyme analysis of cultured cells. The genotype of the remaining fetuses was unknown at birth. Parental consent was granted under procedures approved by the Johns Hopkins Joint Committee on Clinical Investigation. This study was performed in collaboration with physicians enrolled in the trials of sodium benzoate, sodium phenylacetate, and sodium phenylbutyrate approved by the U.S. Food and Drug Administration.

Prospective therapeutic protocols. Therapeutic protocols were designed to prevent hyperammonemia in neonates known to be at risk for a urea cycle defect. The original protocols for patients in this study are summarized in Table I. The most recent modifications of these protocols are described below. For all patients, the protocol included implementing therapy within 2 hours of birth and obtaining cord blood and plasma samples every 12 hours thereafter to monitor levels of urea, ammonium, and amino acids. Blood pH and partial pressure of carbon dioxide should also be measured at least daily.

Deficiency of ornithine transcarbamylase or carbamoyl phosphate synthetase. Hemodialysis is occasionally necessary to control the plasma ammonium level of infants with OTCD or CPSD if medical therapy should fail to do so. Promptly after birth, size 7F or larger catheters should be placed in an artery and in a vein. The importance of a large line cannot be overemphasized. With blood flow of 50 ml/min, ammonium clearance may be similar to the blood flow through the dialyzer; virtually all the ammonium may be removed in a single pass. Because the clearances of ammonium by peritoneal dialysis and continuous arteriovenous hemofiltration are only 10% of the hemodialysis clearance of ammonium, the latter is the procedure of choice.

Within 2 hours of birth, a priming infusion of sodium benzoate and sodium phenylacetate, 250 mg/kg each, and of 10% arginine HCl, 2 ml/kg, in 10% glucose, 35 ml/kg, should be given for a period of 2 hours. After this priming dose is completed, a sustaining infusion containing the same doses of each drug in 10% glucose, 60 ml/kg, should be administered for 24 hours and continued until the medications can be given orally. A generous caloric intake should be provided as glucose and a lipid emulsion (Intralipid, from KabiVitrum AB, Stockholm, Sweden [Franklin, Ohio]) if feasible, to minimize endogenous proteolysis.

After 24 to 48 hours of life, if the infant's plasma ammonium level is within normal limits, a 0.5 gm infusion (per kilogram) of a nitrogen–amino acid–protein combination (Trophamine, from Kendall-McGaw Laboratories, Santa Ana, Calif.) may be administered daily, supplemented daily

with at least 80 kcal/kg from glucose and Intralipid. If plasma ammonium levels remain within normal limits or nearly so, the daily intake of Trophamine may be increased to 1.2 gm/kg for the next 48 to 72-hour period.

If the diagnosis of OTCD or CPSD is confirmed on the basis of plasma and urine substrate analysis,[1] oral medications and enteral nutrition may continue. The recommended diet for neonates on the protocol described above consists of natural protein (from standard infant formula), no more than 2 gm/kg per day, supplemented with Mead Johnson product No. 80056 (4.9 kcal/gm) to supply a total daily caloric intake of 120 to 130 kcal/kg. This protein intake should be attained in staged increments of 0.25 to 0.5 gm/kg per day. The plasma levels of ammonium, glutamine, branched-chain amino acids, and protein should be monitored.

Argininosuccinate synthetase deficiency. As soon as possible after birth, the infant at risk for ASD should receive the following intravenously: 10% arginine HCl, 6 ml/kg per day (3 mmol/kg per day), sodium benzoate, 250 mg/kg per day, and sodium phenylacetate, 250 mg/kg per day, in 10% glucose to provide as high a caloric intake as is practicable. Sodium bicarbonate should be added to the infusate to buffer the hydrochloride. Formula feeding may begin shortly after birth, starting with protein, 0.5 g/kg per day; Mead Johnson product No. 80056, made up to contain 0.7 kcal/ml, may be given orally as a caloric supplement. If plasma ammonium levels remain normal, protein intake may be gradually increased up to 2.0 gm/kg per day, supplemented with Mead Johnson product No. 80056 to supply a total daily caloric intake of 110 to 120 kcal/kg. If plasma citrulline levels remain normal for 48 hours, a diagnosis of normalcy may be made and therapy discontinued.

Argininosuccinate lyase deficiency. Within 2 hours of birth, all infants at risk for ALD should be fed (by gavage, if necessary) a mixture of arginine freebase, 600 mg/kg per day, plus normal formula feeding, starting with protein, 0.5 gm/kg per day. If oral feeding is not tolerated, it is essential that the infant promptly be treated intravenously with 10% arginine hydrochloride at a dosage of 6 ml/kg per day (3 mmol/kg per day), with generous amounts of sodium bicarbonate to buffer the hydrochloride. As he or she is able to tolerate oral feedings, the intravenous administration of arginine may be discontinued in favor of arginine (freebase).

Additional calories can be supplied by using Mead Johnson product No. 80056 to provide the infant with a total of 110 to 120 kcal/kg per day. Water should be added as tolerated. The formula must be adjusted so that the infant receives a full day's dose of arginine each day. For a period of several days, the formula may be gradually increased to provide no more than 2.0 gm of protein per kilogram. If argininosuccinate and its anhydrides do not appear in plasma within 48 hours, a diagnosis of normalcy may be made and therapy discontinued.

Diagnosis. The diagnostic algorithm for urea cycle disorders, as previously described,[1] was employed for these at-risk neonates with the following modifications. For infants at risk for CPSD and OTCD, the absence of plasma citrulline, or its presence in trace concentrations, at 48 and 72 hours of age was considered diagnostic of these disorders. A citrulline level greater than 5 μmol/L at 48 hours of age, unaccompanied by hyperammonemia or hyperglutaminemia, was considered normal. Currently, percutaneous liver biopsy for assay of enzyme activity is recommended if these substrate levels are ambiguous. For neonates at risk for ASD and ALD, the appearance of a high plasma level of citrulline in the former, or of argininosuccinate lyase in the latter, was considered diagnostic of the disease. Normal levels of these amino acids at 48 hours of age were considered diagnostic of normalcy of the infant.

Long-term therapy. The disease-specific protocols were modified during the 10-year period of this study as a consequence of the availability of investigational new drugs. The first-generation protocol included the administration of sodium benzoate and arginine or citrulline; patients born before 1984 were maintained on this protocol. The second-generation protocol added sodium phenylacetate; it was first used to treat patients in 1984. The third-generation protocol included high doses of sodium phenylacetate or phenylbutyrate and excluded sodium benzoate[2]; patients were changed to this protocol as it became available in 1987. New patients were usually converted from standard to high-dose therapy within the first 3 to 6 months of life. Sodium phenylbutyrate is now recommended as the drug of choice at a dosage of 450 to 500 mg/kg per day (9.9 to 13 gm/m^2 per day).[3] Current protocols also include a higher intake of natural protein during the first few months of life.[3]

Follow-up study. The medical record of each affected infant was obtained periodically to collect metabolic, clinical, and developmental data. Long-term metabolic control was monitored by periodic measurement of plasma ammonium and plasma amino acid levels in conjunction with nutritional and anthropometric assessments. Long-term clinical control was estimated by the number of hospitalizations for intercurrent hyperammonemic episodes, duration of the episode, and the peak plasma ammonium level. Developmental progress and intelligence after 6 months of age were evaluated by private physicians or psychologists, who used standardized tests.

RESULTS

Implementation of therapy. Each of the pregnancies resulted in the birth of an infant with normal 1- and

Table II. Survival history of prospectively treated patients with urea cycle disorders

ID No.	Diagnosis	Birth year	Age at start of protocol*			Status	Survival (mo)	Developmental assessment	Other medical problems
			Protocol 1	Protocol 2	Protocol 3				
1	ALD†	1982	—	—	—	Alive	102	Significant delay; no expressive language	Aortic stenosis, asthma, seizures
2	ASD	1984	1 wk	5 mo	37 mo	Alive	83	Educable, mentally impaired	Seizures (9 mo)
3	ASD	1985		At birth	37 mo	Alive	69	Some speech delay	
4	ASD	1987		At birth	12 mo	Alive	42	Mild delay in language, cognitive functioning	
5	CPSD	1981	At birth	11 mo	76 mo	Alive	125	Borderline functioning	Seizures (8 mo)
6	CPSD	1987		At birth	5 mo	Dead	46	Borderline functioning	
7	CPSD	1987		At birth	3 mo	Alive	49	Low normal intelligence	
8	OTCD	1981	At birth			Dead	7		
9	OTCD	1983	At birth			Dead	33	Language disorder	
10	OTCD	1984		At birth		Tx	21 (+68)‡	Within normal range	
11	OTCD	1986		At birth	14 mo	Tx	47 (+5)	Within normal range	
12	OTCD	1987		At birth		Tx	18 (+32)	Within normal range	Seizures, after transplant

Tx, Received a liver transplant.
*Protocol 1 consisted of administration of sodium benzoate and arginine or citrulline; protocol 2 consisted of protocol 1 plus administration of sodium phenylacetate; protocol 3 consisted of high doses of sodium phenylacetate or phenylbutyrate plus arginine or citrulline (sodium benzoate is excluded).
†Received arginine from birth.
‡Months of survival after liver transplant.

5-minute Apgar scores and of normal size. The therapeutic protocols were implemented successfully within 12 hours of birth. Cord blood and serial plasma samples were obtained for the measurement of ammonium and amino acids.

Diagnostic protocol and neonatal outcome. Three of the four infants at risk for ASD were identified on the basis of extremely elevated citrulline levels, ranging from 2692 to 2893 μmol/L. One infant at risk for ALD had an abnormal citrulline concentration of 262 μmol/L at 120 hours; this level is typical of patients with ALD. Elevated levels of argininosuccinate and its anhydrides were also present in this patient. Of 26 infants at risk for OTCD or CPSD, 11 had plasma chromatograms that showed no detectable peak at the retention time of citrulline, indicating that they were affected. The mean (±SD) peak plasma citrulline level in the first 72 hours among the remaining 17 unaffected infants in this study was 13.7 ± 11.08 μmol/L (range 1 to 51 μmol/L; normal range for neonates, 9 to 29 μmol/L[4]).

Plasma ammonium levels measured between 48 and 72 hours after birth showed considerable variability, reflecting diagnostic category, efficacy of treatment, and differing normal reference values at various institutions. The infants affected with ASD or ALD had peak ammonium levels that ranged from 29 to 82 μmol/L, compared with a mean level of 50.1 μmol/L in unaffected infants (range 5 to 112 μmol/L). One infant affected with CPSD had plasma ammonium levels that remained at less than 50 μmol/L; the two other infants with CPSD had elevated ammonium levels (125 and 219 μmol/L) but no symptoms of hyperammonemia. All of these infants survived the neonatal period.

Hyperammonemia developed in the eight patients with OTCD. In five infants the mean peak ammonium level (115 μmol/L) was moderately elevated for a brief period, and they survived the neonatal period. However, three infants had ammonium levels that remained greater than 500 μmol/L despite aggressive therapy, including intravenous infusion of arginine, sodium benzoate, and sodium phenylacetate; exchange transfusion in one patient; and hemodialysis in the second. These three infants died at days 4, 5, and 12, respectively. Inasmuch as the 13 mutations identified at the ornithine transcarbamylase locus[5-7] differ, it is likely that subtle differences in severity of disease in these patients affected their response to treatment and therefore the outcome.

Liver biopsy samples were obtained from three patients with OTCD for enzyme assay. Because plasma citrulline and ammonium levels remained within the normal range for 3 to 5 days, a urea cycle defect was ruled out in 17 infants and the therapeutic protocol was discontinued.

Long-term follow-up study. Twelve prospectively treated affected infants survived the neonatal period, free of symptoms of hyperammonemia and developmental delay. All

were discharged from the hospital on protein-restricted diets and medication regimens to stimulate excretion of waste nitrogen and to maintain nitrogen homeostasis (Table II).

Deaths. There have been three deaths among the 12 survivors (Table II): CPSD patient 6, and OTCD patients 8 and 9. Patient 6 died at 46 months of age during a hyperammonemic episode that did not respond to peritoneal dialysis or hemodialysis. He had been maintained on protocol 3 since his fifth month of life and had had four prior hyperammonemic episodes.

Two patients with OTCD also died as a result of hyperammonemia that did not respond to treatment at 7 months and 33 months, respectively. Patient 8 had been treated with protocol 1 and had had nine hyperammonemic episodes in his 7 months of life; although his physicians reported that his development was normal, the frequency of hyperammonemic episodes suggests that his nitrogen metabolism was not adequately controlled. Patient 8 was also treated with protocol 1. He had had four hyperammonemic episodes, each associated with a viral infection, before his death.

The three surviving OTCD patients have received orthotopic liver transplants at ages 18, 21, and 47 months, respectively; they are on normal diets and are no longer maintained on these therapeutic protocols.

All patients have had intercurrent hyperammonemic episodes, often associated with common viral or bacterial infections. Treatment of these episodes has involved overnight hospitalizations for intravenous drug therapy and, in two cases, dialysis. Patients 1, 2, and 5 each had seizures during a hyperammonemic episode and required long-term treatment with anticonvulsant agents.

The single patient with ALD has had developmental delay despite having had arginine supplementation since birth and no severe episode of hyperammonemic encephalopathy. At 10 weeks of age he had excessive regurgitation, poor weight gain, and possible neurologic deterioration. Aortic stenosis was diagnosed when he was 35 months of age. He has had five hyperammonemic episodes, and a seizure disorder that developed at 35 months of age has required treatment with carbamazepine. Repeated testing has indicated psychomotor delay, and at 87 months of age he lacks expressive language (Table II).

Anthropometric measurements. The weight of most of the patients appears to have tracked consistently. The weight of patients with ASD is at or above the mean after 1 year of age, but there is greater variability in the weight of patients with CPSD or OTCD. In most patients with CPSD, weight tracks between the 5th and 50th percentiles. The weight gain of patient 6 appeared to level off in the months preceding his death.

There was no apparent relationship between weight and outcome in patients with OTCD. The weight of patient 9 was between the 5th and 10th percentiles up to the time of his death. Patient 12 had weight measurements below the 5th percentile preceding his liver transplantation at 18 months of age, whereas the weight of patient 10 had increased to the 50th percentile before transplantation at 21 months. Their most recent reported measurements (38 months and 52 months) indicate that weight is below the 5th percentile after transplantation in both. There has been less variability in height measurements of these patients, which also track consistently. None of these patients has height measurements above the mean for age.

Developmental progress. Treatment of these patients was not part of a strictly controlled clinical trial. Many different standardized tests were used to assess development; the timing and type of tests were at the discretion of parents and private physicians. Children who appeared to be developing normally were tested less frequently than children who showed evidence of developmental delay. Most patients have had some developmental delay (Table II), and almost all are reported to have problems with expressive or receptive language resulting in referral for speech therapy, occupational therapy, and/or physical therapy in structured preschool programs.

DISCUSSION

The protocols were successful in averting neonatal hyperammonemic coma and death in all patients (n = 7) affected with ALD, ASD, and CPSD. However, among eight patients affected with OTCD, three died as a result of hyperammonemic coma. The remaining five had moderately elevated levels of plasma ammonium but no symptoms of hyperammonemia.

Therapy did not interfere with the diagnosis of normalcy in 17 unaffected infants. Their medications were discontinued within 96 hours, and regular formula feedings were instituted immediately. This brief period of therapy does not appear to have had any effect on growth and development of the unaffected infants.

We reported previously that during the first 2 years of life the overall survival rate for patients with CPSD and OTCD who survived rescue from neonatal hyperammonemic coma is approximately 73%[8]; the one prospectively treated patient who died before 2 years of age in this study had been treated with sodium benzoate alone. Survival after age 2 years appears to be comparable in the rescue and prospectively treated groups; both have a mortality rate of approximately 29%. However, small numbers of patients, changes in treatment protocol during the study period, and liver transplantation in three patients with OTCD preclude direct comparison of survival times.

All four of the prospectively treated patients with ASD

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.