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Not So Rare: Errors of Metabolism during the Neonatal Period

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Abstract

During the neonatal period, the diagnosis of an error of metabolism (EM) was once thought to portend a poor prognosis or lethality. Over the past two decades, the prognosis of many EMs has changed. The critical aspect of the metabolic evaluation in a sick newborn is to rapidly identify whether there may be a metabolic problem. If there is a metabolic problem, the goal is to minimize the sequelae of the specific disorder. This review will explore how to approach and evaluate a newborn suspected of having an EM. A discussion of clinical and laboratory findings that often accompany EM will be included.

Not So Rare: Errors of Metabolism During the Neonatal Period

By Sandra A. Banta-Wright, MN, RNC, NNP, and Robert D. Steiner, MD

At delivery, a full-term newborn may appear active and healthy. However, over a course of hours, days, or weeks, nonspecific signs and symptoms of distress may develop. In some cases, there will be a gradual decline in the infant's condition with slow deterioration. Alternatively, the onset of distress can be sudden, catastrophic, and life threatening. The symptoms usually indicate a differential diagnosis of respiratory, cardiac, gastrointestinal, neurological, and infectious disease. There is a tendency to not include consideration of an error of metabolism (EM), formerly referred to as an inborn error of metabolism, until the above "more common causes of distress" have been excluded–this approach can be fatal. During the first year of life, EMs mimic or even predispose to these conditions. Individual EMs are, for the most part, indeed rare. Collectively, however, EM disorders are not an infrequent cause of distress in the neonatal period. It is now estimated that 1 in 1,000 newborns is at risk for being affected by an EM.[1] Due to the large numbers of EM that can present during the neonatal period, some fear that, to appropriately diagnosis EM, the clinician must understand numerous biochemical pathways and their interrelationships. Fortunately, approaching a suspicious illness in a newborn based on even a limited knowledge of biochemistry and metabolic laboratory studies can lead to effective diagnostic testing and management. The recognition of a neonate with EM can be accomplished using an organized and rational approach based on diagnostic algorithms presented in this review. When a newborn presents acutely with signs of an EM, appropriate therapy needs to be initiated rapidly due to the high risk of morbidity and mortality. Thus, EM must be considered concurrently in the differential diagnosis that includes the more common disease processes. Increasingly, disorders of metabolism are being managed successfully with acceptable outcomes. Appropriate therapy, in many cases, can result in prevention of mental retardation and death.

It is the purpose of this review to provide an outline for an approach to the evaluation of a newborn suspected of having an EM. In addition, the major clinical and laboratory findings of EMs presenting during the neonatal period will be reviewed. A limited discussion of treatment will focus on the stabilization and acute management of newborns with EM. The discussion will be limited to those EM that present during this early period of life, rather than later

during infancy, childhood, or adulthood. Detailed biochemical pathways of EMs are not provided. More comprehensive discussions of each of these topics can be found in recent editions of reference textbooks.[2-4]

Errors of Metabolism during the Neonatal Period

There are over 300 EM that have been identified in man.[4] The disorders of metabolism diagnosed during the newborn/infancy period can be categorized, for the most part, into 1 of 6 areas. These are disorders of: 1) amino acids, 2) carbohydrates, 3) fatty acid oxidation, 4) lysosomal and peroxisomal function, 5) mitochondrial energy metabolism, and 6) organic acids. A summary of EM that have been diagnosed during the neonatal period is listed in Table 1. This summary should not be considered all inclusive. New disorders of EM continue to be elucidated with the advances in clinical research.

Clinical Presentation of EM in the Neonate

As previously discussed, the clinical manifestations of EM are similar to the presentation of several other newborn disorders. Newborns have only a few ways to respond to an acute insult, such as an EM.[5,6] These responses include signs and symptoms of respiratory, cardiac, gastrointestinal, and neurological distress. These symptoms are summarized in Table 2.

Laboratory Evaluaition of EM in a neonate

The laboratory evaluation for a newborn suspected of having an EM can be illustrated as a pyramid in Fig 1. The specific details of collection, handling, processing, and shipping, if needed, of laboratory samples must be clearly understood before the samples are obtained. In addition, during this time period, frequent communication and collaboration with laboratory personnel in more than one laboratory may be needed. This will expedite testing and avoid needless delay and repetition of sample collection and processing from an already compromised newborn. The first tier includes broadly focused screening tests that can provide a relatively rapid and inexpensive diagnostic evaluation. Several of these tests are routinely performed during the care of a sick newborn. A significant imbalance may be identified, which when promptly treated may prevent long-term sequelae. Additional tests for metabolic disease are listed in tier 2. Tier 2 tests of the pyramid provide more information as to differential diagnosis. The studies in tier 1 and 2, when combined, can help direct care of the patient and the need for consultation with a regional medical center where clinical biochemical genetics/metabolic disease expertise is available. Tier 3 of the pyramid focuses on the metabolic disease process and provides information for consultation with the metabolic disease specialist. These tests are not routinely available in many local hospital laboratories. As these studies require highly specialized laboratory skills and equipment, these tests are generally available at larger, regional medical centers. In addition, some commercial laboratories also offer these tests. Many of the commercial laboratories, with some notable exceptions, cannot provide STAT analyses and/or expert result interpretation. It can be difficult to interpret results of laboratory tests performed at commercial laboratories where the techniques used can be different from academic laboratories, though recently the quality of testing offered by several commercial laboratories has improved greatly. The fourth tier of the pyramid involves highly specialized tests in the diagnosis of EM. Definitive diagnostic testing can require specific enzymatic analysis using white blood cells, cultured skin fibroblasts, or other tissue specimen, such as muscle. Only a few laboratories perform many of the enzyme and mutation analyses that are required for confirmation of the diagnosis. The results may not be available immediately, but can take days, weeks, or months to be reported by the specific biochemical genetic laboratory. The metabolic disease consultants can assist in identifying which specific tests to order, the priority of the tests, and can provide direction in the initial treatment of the neonate.

Tandem Mass Spectrometry

With increasing use of tandem mass spectrometry (MS/MS), the analysis of acylcarnitines and amino acids in dried filter paper blood spot (Guthrie) specimens has proven useful as a method for mass newborn screening for a number of amino acid, fatty acid oxidation, organic acid, and urea cycle disorders.[7] This technique has been referred to as expanded newborn screening. The analysis usually requires only one or two dried filter paper blood spots. In addition, it is automated and usually takes only 1 to 2 minutes per sample. When a newborn is suspected of having an EM, the regional academic or state laboratory should be notified to facilitate a more rapid screening result. However, the result is dependent on the sample being in the laboratory and acceptable quality of the blood spot. The ability to use MS/MS to provide direction toward the differential diagnosis and the collection of confirmatory laboratory tests for many metabolic disorders has been life saving for many newborns. However, not every state or region has MS/MS available for expanded newborn screening. In addition, the test is not 100% sen-

Table 1. Summary of Errors of Metabolism that May Present in the Neonatal Period

Amino Acids	Carbohydrate	Fatty Acid Oxidation	Lysosomal and Peroxisomal Function	Mitochondrial Energy Metabolism	Organic Acids
Argininemia	Galactosemia, Classical	Carnitine/acylcarnitine translocase deficiency (CACT)	Farber disease Fucosidosis	Hypertrophic cardiomyopathy and myopathy (infantile onset)	2-Methylbutryrl-CoA dehydrogenase deficiency (2MBCD or SBCAD)
Argininosuccinic aciduria (ASA lyase deficiency)	Hereditary fructose intolerance	Carnitine palmitoyl transferease deficiency type I (CPT-I)	Gaucher disease, infantile form		
Carbamyl phosphate synthetase deficiency (CPS)	Fructose-1,6-diphosphatase deficiency		Glycogen storage disease, type II (Pompe disease)	Lethal infantile cardiomyopathy (LIC)	
Citrullinemia (ASA synthetase deficiency)	Glycogen storage disease, type I	Carnitine palmitoyl transferase deficiency type II (CPT-II)	GM_1 gangliosidosis	Lethal infantile cardiomyopathy	2-Methyl-3OH butyric aciduria
Homocystinuria (HCU)	Glycogen storage disease, type III	Carnitine transport defect	I-cell disease	X-linked cardioskeletal myopathy (Barth syndrome)	3-Methylcrotomyl-CoA carboxylase deficiency (3MCC)
Hyperornithinemia–hyperammonemia–homocitrullinuria (HHH) Syndrome	Other glycogen storage diseases	Glutaric aciduria, type I (GA-I)	Mucopolysaccharidosis type VII		
Hyperphenylalaninemias (PKU, HPhe) Classical PKU, Hyperphenylalaninemia, Biopterin Cofactor Defects	Pyruvate carboxylase deficiency (PCD)	Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD)	Neonatal adrenoleukodystrophy	Lethal infantile mitochondrial disease (LIMD) Pearson syndrome	3-Methylgluta conyl-CoA hydratase deficiency
	Phosphoenolpyruvate carboxykinase deficiency (PEPCK)	Trifunctional protein deficiency Medium chain acyl-CoA dehydrogenase (MCADD)	Niemann–Pick disease type A, C/Sialidosis, type II/Wolman disease/Zellweger syndrome	Progressive infantile poliodystrophy (Alpers Disease)	3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG, coAlyase)
Lysinuric protein intolerance (LPI)		Multiple acyl-CoA dehydrogenase deficiency (MADD or GAII, Glutaric acidemic, type II)		Pyruvate dehydrogenase deficiency (Leigh syndrome)	Adenosylcobalamin synthesis defects/ Biotinididase deficiency
Maple syrup disease (MSD)					
Nonketotic Hyperglycinema (NKG)					Isovaleric acidemia (IVA)
Ornithine transcarbamylase deficiency (OTC)		Very long chain acyl-CoA dehydrogenase deficiency (VLCADD)			Methylmalonic acidemias (MMA)
Pyroglutamic Aciduria/5-oxoprolinuria					Mitchondrial acetoacetyl-CoA thiolase deficiency (BKT, 3-deficiency)
Sulfite oxidase Deficiency/Molybdenum Cofactor deficiency					
Tyrosinemia Transient neonatal, type I, type II, type III					Multiple carboxylase deficiency (MCD) Propionic acidemia (PA)

Table 2. Common Clinical Manifestations of Errors of Metabolism Presenting in the Newborn/Infancy Period

Gastrointestinal Signs
Cholestasis
Diarrhea
Jaundice
Poor feeding
Vomiting
Neurological Signs
Abnormalities of tone
Irritability
Lethargy (progressing toward coma)
Poor/weak suck
Seizures
Organomegaly
Heart
Liver
Spleen
Respiratory Signs
Apnea
Respiratory failure
Tachypnea/hyperpnea

sitive for some disorders. The result may be available in some cases only after the neonate has already presented clinically and is in the neonatal intensive care unit in critical condition.

Differential Diagnosis of EM

The acute onset of EM in the neonate can be divided into two main categories of presentation: metabolic acidosis and hyperammonemia. A third category of presentation is hypoglycemia, but this is not as common as metabolic acidosis and hyperammonemia. For discussion of hypoglycemia and EM, several reviews are available.[3-5] An approach to the differential diagnosis with each of these clinical findings will be presented. The algorithms direct an approach to care that must be individualized to meet the newborn's specific needs. It is important to remember that EM can present with catastrophic illness in the neonatal period with no acidosis and a normal ammonia level. Examples include nonketotic hyperglycinemia (NKH), Zellweger syndrome, Cobalamin C defects, and pyridoxine dependency.[8-12]

Metabolic Acidosis

Metabolic acidosis is defined as a low pH (<7.35) due to an excess of acid (H^+) or a deficit of base (HCO_3^-). This occurs when there is an increased production of fixed or nonvolatile acids with a simultaneous decrease in the buffering capacity. The anion gap should be calculated to elucidate whether the metabolic acidosis is caused by an accumulation of organic acids or a decrease in bicarbonate. This is important in establishing the diagnosis of suspected EM. The anion gap is calculated by Sodium – (Chloride + Bicarbonate).[13] Hyperchloremic acidosis is a hallmark of metabolic acidosis due to bicarbonate wasting and usually occurs in newborns with intestinal or renal disorders. When the anion gap is increased (≥ 16) with a normal chloride level, the acidosis is from excess acid production.

Metabolic acidosis is a common finding in the sick newborn. In some cases, the metabolic acidosis is acute, severe, and life threatening, as in shock. In other cases, the acidosis is mild, but persistent and, in still others, may be intermittent. The most common approach to metabolic acidosis in neonates is to correct the acidosis by administering bicarbonate. Metabolic acidosis caused by EM presenting acutely in the neonatal period, almost without exception, will not respond to bicarbonate, except transiently. The effective treatment of acidosis in EM is correction of the cause of the acidosis, such as decreasing the endogenous overproduction of specific acids.

Several categories of EM may present with metabolic acidosis and an increased anion gap (Table 3). A simplified flowchart for the evaluation of a newborn with metabolic acidosis and an increased anion gap is presented in Fig 2. The largest group associated with metabolic acidosis during the neonatal period is organic acidemias.[14] In addition, this situation occurs also with some amino acid disorders. Other disorders, such as defects in pyruvate metabolism or in the respiratory chain, may present with metabolic acidosis and an elevated lactate during the neonatal period, but these may not always have an increased anion gap.[15] However, these newborns will often not have specific diagnostic elevations of organic acids on urine organic acid analysis. A lactate/pyruvate ratio less than 25 suggests pyruvate dehydrogenase deficiency (PDH) or other disorders of pyruvate metabolism, whereas a ratio greater than 25 suggests a respiratory chain defect.[16-20] In addition, fatty acid oxidation disorders, such as medium-chain acyl-CoA dehydrogenase deficiency (MCADD), may present with metabolic acidosis and hypoglycemia.[21]

The organic acidemias, such as methylmalonic acidemia (MMA), propionic acidemia (PPA), and isovaleric acidemia (IVA), form the most common category to be considered in the evaluation of metabolic acidosis.[22-25] All three disorders are the result of defects in branched chain amino acid metabolism, affecting the catabolism of isoleucine (ILE), leucine (LEU), and valine (VAL). All disorders are autosomal recessive. These three disorders are almost clinically indistinguishable from one another when they present in the neonatal period. The clinical finding is

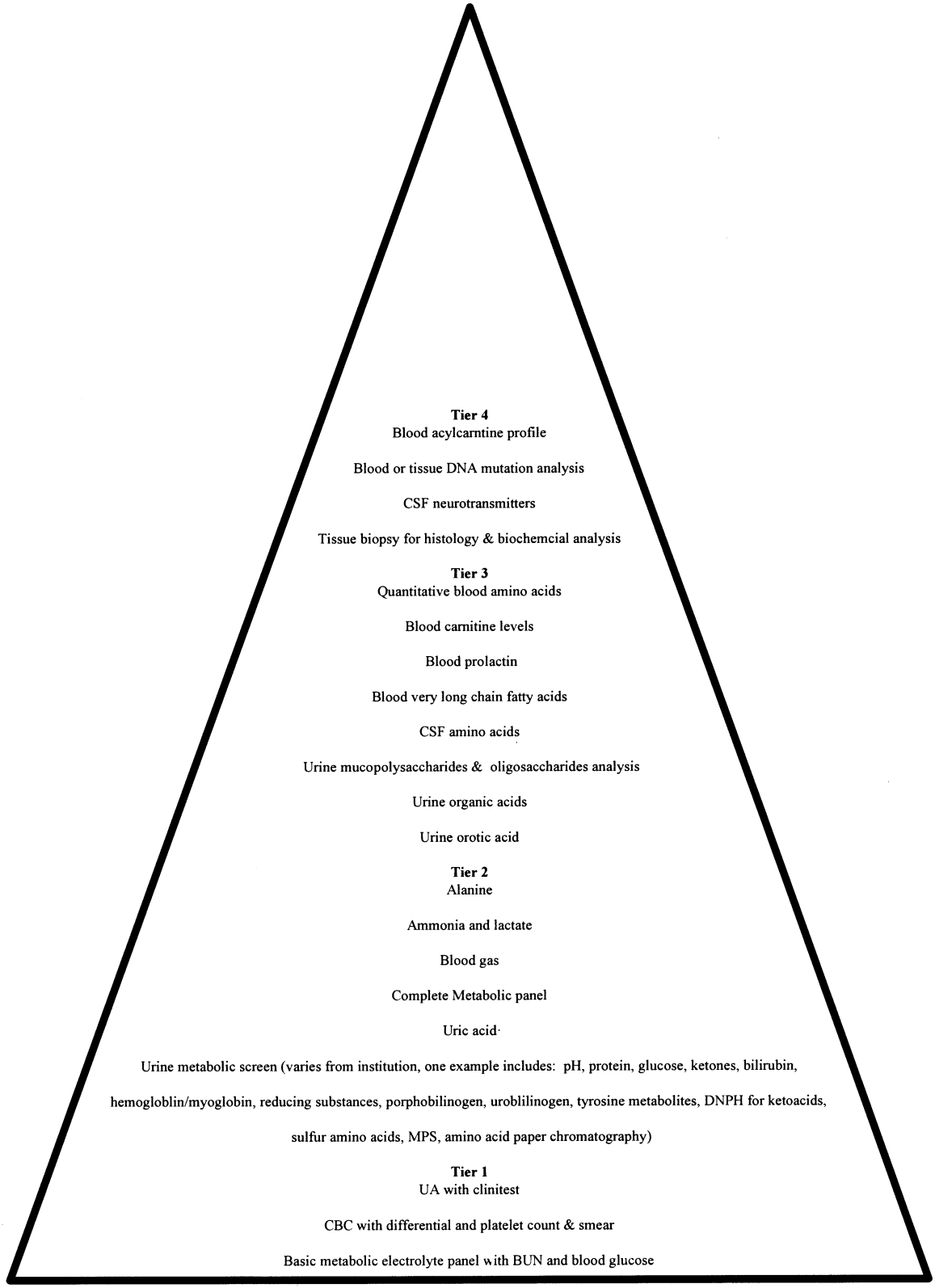

Fig 1. Pyramid of laboratory tests for suspected metabolic disease in a newborn.

Table 3. Errors of Metabolism with Metabolic Acidosis and Increased Anion Gap in Neonates

Amino acids
Maple Syrup Disease (MSD)
Carbohydrate disorders
Fructose-1,6-diphosphatase deficiency
Fatty acid oxidation
Carnitine/acylcarnitine translocase deficiency (CACT)
Carnitine palmitoyl transferase deficiency, type II (CPT-II)
Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD)
Medium chain acyl-CoA dehydrogenase deficiency (MCADD)
Trifunctional protein deficiency
Very long chain acyl-CoA dehydrogenase deficiency (VLCADD)
Organic acidemias
2-Methyl-3-OH butyric aciduria
3-Hydroxy-3-methylglutaryl-CoA (HMG) lyase deficiency
3-Methylcrotonyl CoA carboxylase deficiency (3MCC)
Isovaleric academia (IVA)
Methylmalonic acidemias (MMA)
Multiple carboxylase deficiency (MCD)
Propionic acidemia (PA)
Pyroglutamic aciduria

a healthy newborn at birth who becomes rapidly ill after the first day of life. Clinical signs include ketoacidosis, poor feeding, vomiting, dehydration, hypotonia, lethargy, tachypnea/hyperpea, seizures, coma, and an unusual odor (Table 4). The main laboratory diagnostic tool is urinary organic acid analysis. The urine organic acid analysis will reveal the specific elevated urinary organic acids.[26] In addition, ketonuria will be present. When associated with metabolic acidosis, particularly in the neonatal period, ketonuria is almost always pathognomic of an error of metabolism. The plasma lactate is often elevated in organic acidemias as a result of secondary interference with coenzyme A (CoA) metabolism. Notably, neutropenia and thrombocytopenia are common and mimic neonatal sepsis. Hyperammonemia is common, but is not usually as impressive in organic acid disorders as in urea cycle defects. There will be an increased acylcarnitine-to-free carnitine ratio, which is accompanied by abnormal patterns of blood acylcarnitines.[27,28]

The management of a newborn with a presumed diagnosis of an organic acidemia is to first stabilize the newborn. Within 24 to 48 hours, the results of quantitative amino acid and organic acid analysis should be available. If the laboratory cannot provide results in this time frame, alternative arrangements with another laboratory should be pursued. Treatment at this time is directed toward the removal of the accumulating metabolites, such as ammonia or organic acid intermediates, with hemodialysis.[29] If a center is unable to provide hemodialysis to a newborn, transfer should be strongly considered. Exchange transfusions, peritoneal dialysis, or hemofiltration are less efficient than hemodialysis in managing these disorders.[30] Insulin can be used to augment the anabolic state.[31] Carnitine is used to remove toxic metabolites during the acute phase.[32-34] During an acute illness, intravenous carnitine is preferred either as a continuous drip or in six divided daily doses over oral administration. After the newborn has been stabilized, oral feedings should be reinitiated. A wide range of specialty formulas are available that restrict certain amino acids.[35] The assistance of a dietitian with experience in managing EM and the metabolic specialist is vital. Efforts to reduce the endogenous production of toxic metabolites may include antibiotic suppression of gut flora that produce metabolites that enter the newborn's bloodstream, such as the long-term use of metronidazole in the treatment of methylmalonic and propionic acidemias.[36] In addition, specific vitamins, such as hydroxocobalamin, may be provided as cofactors for certain enzyme deficiencies. Several abnormalities in the reduction of cobalamin result in impaired methylmalonly-CoA mutase (MMM) activity in MMA. Some newborns with decreased MMM activity will respond to pharmacologic doses of hydroxocobalamin with enhancement of enzyme activity and increased tolerance of ILE, methionine (MET), threonine (THR), and VAL.[37,38] The success in the acute management of the organic acidemias has led to improved survival and outcome. The long-term prognosis varies widely for different disorders and within disorders due to the genetic heterogeneity.

Hyperammonemia

In the newborn period, a normal ammonia level is less than 50 μmol/L.[39] A blood ammonia level between 70 and 100 μmol/L should be viewed in conjunction with clinical findings. An elevated ammonia level $\geq$ 100 μmol/L indicates an abnormality in nitrogen balance. A typical clinical presentation of neonatal hyperammonemia is a full-term newborn who is initially well the first day of life. After 24 hours, the newborn becomes a poor feeder, lethargic with hypotonia, vomits frequently, and is hyperpneic.[40] Hyperammonemia is a life-threatening condition, as elevated ammonia is a toxin especially on the central nervous system. If not identified and treated rapidly, the newborn will have irreversible neurological sequelae.

There are several disorders that can cause hyperammonemia in the neonatal period (Table 5). When hyperammonemia is present in the newborn/infancy period, an attempt to identify a diagnosis is essential. A systematic

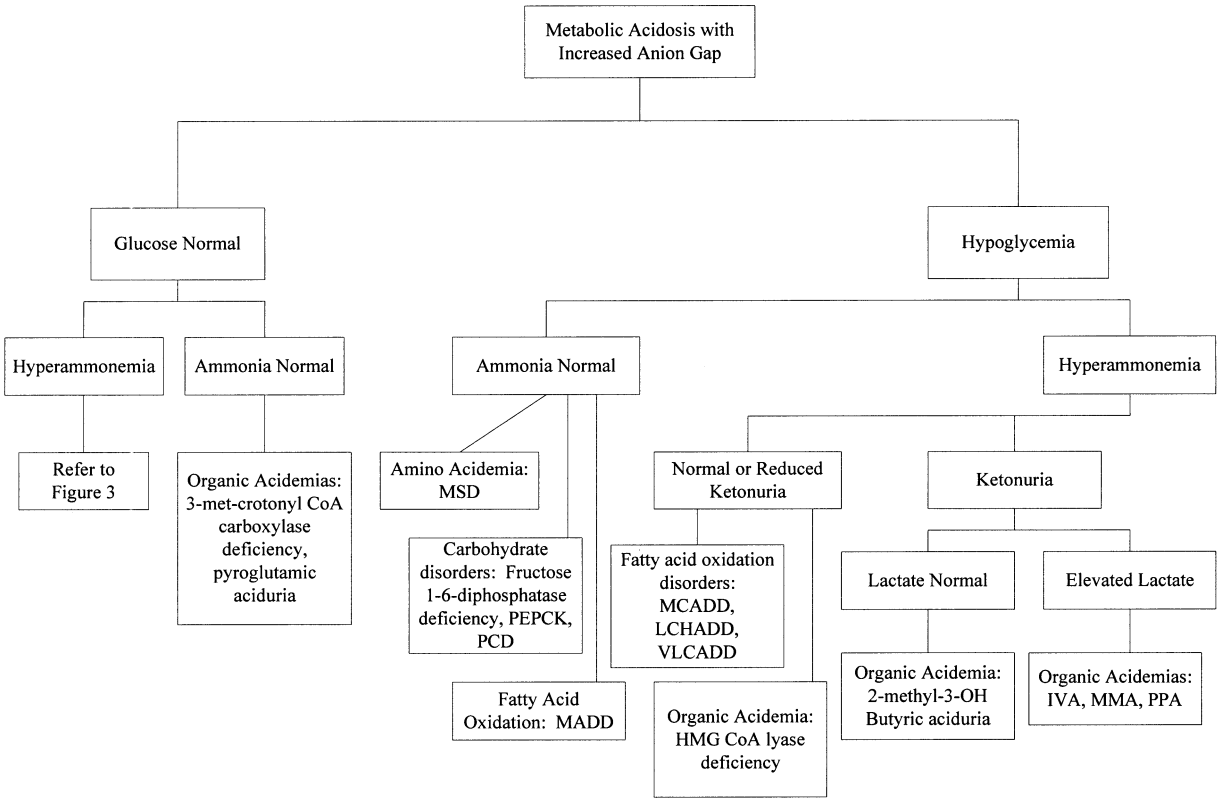

Fig 2. Simplified flowchart of the evaluation of a newborn with metabolic acidosis and an increased anion gap. HMG, 3-hydroxy-3-methylglutaryl-CoA lyase deficiency; IVA, isovaleric acidemia; LCHADD, long chain 3-chydroxyacyl-CoA dehydrogenase deficiency; MSD, Maple syrup disease; MCADD, medium chain acyl-CoA dehydrogenase deficiency; MMA, methylmalonic acidemia; MADD, multiple acyl-CoA dehydrogenased deficiency (GAII, glutaric acidemia, type II); PEPCK, phosphoenolpyruvate carbosykinase deficiency; PPA, propionic acidemia; PCD, pyruvate carboxylase deficiency; VLCADD, very long chain acyl-CoA dehydrogenase deficiency. Adapted with permission from Ward JC. Inborn errors of metabolism of acute onset in infancy. Pediatr Rev 11:205-216, 1990.

approach for the differential diagnosis of hyperammonemia is presented in Fig 3. It is very important to remember that the first step in evaluating suspected hyperammonemia is to obtain an accurate blood ammonia level.[41] The venous ammonia sample must be collected, stored, and transported on ice to the laboratory. Once in the laboratory, this specimen must be rapidly analyzed and not run as a routine and must be reported with an adjustment for the newborn age.

Newborns that present with hyperammonemia during the first day of life usually are either premature or full-term newborns with a secondary hyperammonemia, such as a pyruvate carboxylase deficiency (PCD).[42,43] The severely elevated ammonia level associated with prematurity is known as transient hyperammonemia of the newborn (THAN).[42] THAN typically presents in a preterm infant of approximately 36 weeks' gestation, who has respiratory distress and significant hyperammonemia. Survivors of THAN do not experience recurrent episodes of hyperammonemia. The neurological outcome of THAN is dependent on the extent of the neonatal insult. As a result, this disorder should not be ignored, rather rapid and vigorous medical intervention is required. The authors have had experience with premature infants with ammonia levels greater than 2,000 μmol/L and normal outcome in THAN.

Hyperammonemia after the first 24 hours following birth is more characteristic of a primary hyperammonemia, which includes mainly urea cycle defects.[44] Other categories of EM that also present after 24 hours of age with hyperammonemia include organic acidemias and fatty acid oxidation disorders.[45] The key to differentiation is the presence or absence of acidosis, ketosis, or hypoglycemia.[46] The urea cycle defects usually present with respiratory alkalosis due to the hyperpnea, induced by the hyperammonemia. In contrast, the initial presentation of many organic acidemias is severe metabolic acidosis with an increased ion gap. Also, fatty acid oxidation disorders tend to present with mild metabolic acidosis. The second

Table 4. Odors Associated with Errors of Metabolism

Odor	Disorder
Acid like	Methylmalonic acidemia (MMA)
Cabbage like	Tyrosinemia, type 1
	Methionine malabsorption
Cat urine	3-Methylcrontonylglycinuria (MCC)
	Multiple carboxylase deficiency (MCD)
Curry	Maple syrup disease (MSD)
Fish market	Trimethylaminuria
Musty, mousy	Classical phenylketonuria (PKU)
Maple syrup or burnt sugar	Maple syrup disease (MSD)
Rancid butter	Tyrosinemia, type 1
Sulphurous like	Cystinuria
Sweaty feet	Isovaleric acidemia (IVA)
	Multiple acyl-CoA dehydrogenase deficiency (MADD or glutaric acidemia, type II, GAII)

Note: Adapted with permission from Blau N, Blaskovics et al: Simple tests in urine and blood in Blau N, Duran M, Blaskovics ME, Gibson KM (eds.): Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases (ed 2). Berlin, Springer, 2003; pp 3-10.

distinguishing feature of the urea cycle defects is the absence of ketosis, which is easily determined by a urine dipstick to check for the presence of ketones.[44] The last variable is the absence or presence of hypoglycemia. The classical presentation of fatty acid oxidation disorders is nonketotic hypoglycemia or hypoketotic hypoglycemia.[47] Lastly, ketotic hypoglycemia with acidosis is characteristic of PCD in addition to organic acidemias.[48]

The largest category of disorders with hyperammonemia is urea cycle defects.[43] In the absence of acidosis, ketosis, and hypoglycemia, a tentative diagnosis of urea cycle defect should be considered. Disorders to be included in the differential diagnosis of the urea cycle defect include: N-Acetylglutamate synthetase (NAGS), carbamyl phosphate synthetase (CPS), ornithine transcarbamylase (OTC), argininosuccinic acidemia (AS), and argininosuccinic lyase deficieny (AL) deficiencies.[46] NAGS, CPS, AS, and AL are autosomal recessive. OTC is X-linked. Clinically, the presentations in the neonatal period are virtually identical due to the hyperammonemia, which is the common variable. The laboratory evaluation will include the quanitative analysis of plasma amino acid and urine organic acids/urine orotic acid analyses.[26,49,50] The laboratory results will determine the compounds within the urea cycle that are increased and decreased. This is based on the detoxification of ammonia as a five-step process (Fig 4).[51] It begins with the formation of carbamyl phosphate from ammonia by carbamyl phosphate synthetase. Carbamyl phosphate is added to ornithine to form citrulline by ornithine transcarbamylase. Citrulline is converted to argininosuccinate by argininosuccinate synthetase, which is then cleaved to produce arginine by argininosuccinate lyase. Arginine is cleaved by arginase to form urea and ornithine. The urea is excreted in the urine. The ornithine is now available to restart the cycle again.

The plasma citrulline and argininosuccinate provide the distinguishing features among the urea cycle defects. If citrulline is absent or decreased, urine orotic acid will differentiate between NAGS, CPS, and OTC deficiency. In NAGS and CPS, the urine orotic acid will be low; while in OTC it is elevated.[52,53] After OTC, CPS and NAGS have been excluded and, if the citrulline level is normal, the disorder to consider is hyperornithinemia–hyperammonemia–homocitrullinuria (HHH) syndrome.[54] If the citrulline level is elevated, the agrininosuccinic acid level will aid in the diagnosis between AL, also known as argininosuccinic aciduria and AS, also known as citrullinemia. In citrullinemia, argininosuccinic acid is deficient.[55] Thus, citrulline is produced in massive quantities. In AS, there will be a moderate increase in citrulline with increased levels of argininosuccinic acid and its anhydrides.[56] A defect in the last step in the urea cycle, arginase, does not usually present in the neonatal period, but increasing

Table 5. Errors of Metabolism with Hyperammonemia in Newborns

Amino acids defects
Argininosuccinic acid lyase deficiency (AL)
Argininosuccininc acid synthetase deficiency (AS)
Carbamyl phosphate synthetase deficiency (CPS)
Hyperornithinemia–hyperammonemia–homocitrullinemia syndrome (HHH)
Lysinuric protein intolerance (LPI)
Ornithine transcarbamylase deficiency (OTC)
N-Acetylglutamate synthetase deficiency (NAGS)
Fatty acid oxidation defects
Carnitine acylcarnitine translocase deficiency (CACT)
Carnitine palmitoyltransferase II deficiency (CPT-II)
Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD)
Very long chain acyl-CoA dehydrogenase deficiency (VLCADD)
Organic acidemia defects
3-Hydroxy-3-methylglutaryl-CoA (HMG) lyase deficiency
Isovaleric acidemia (IVA)
Methylmalonic acidemia (MMA)
Multiple acyl-CoA dehydrogenase deficiency (MADD)
Propionic acidemia (PA)

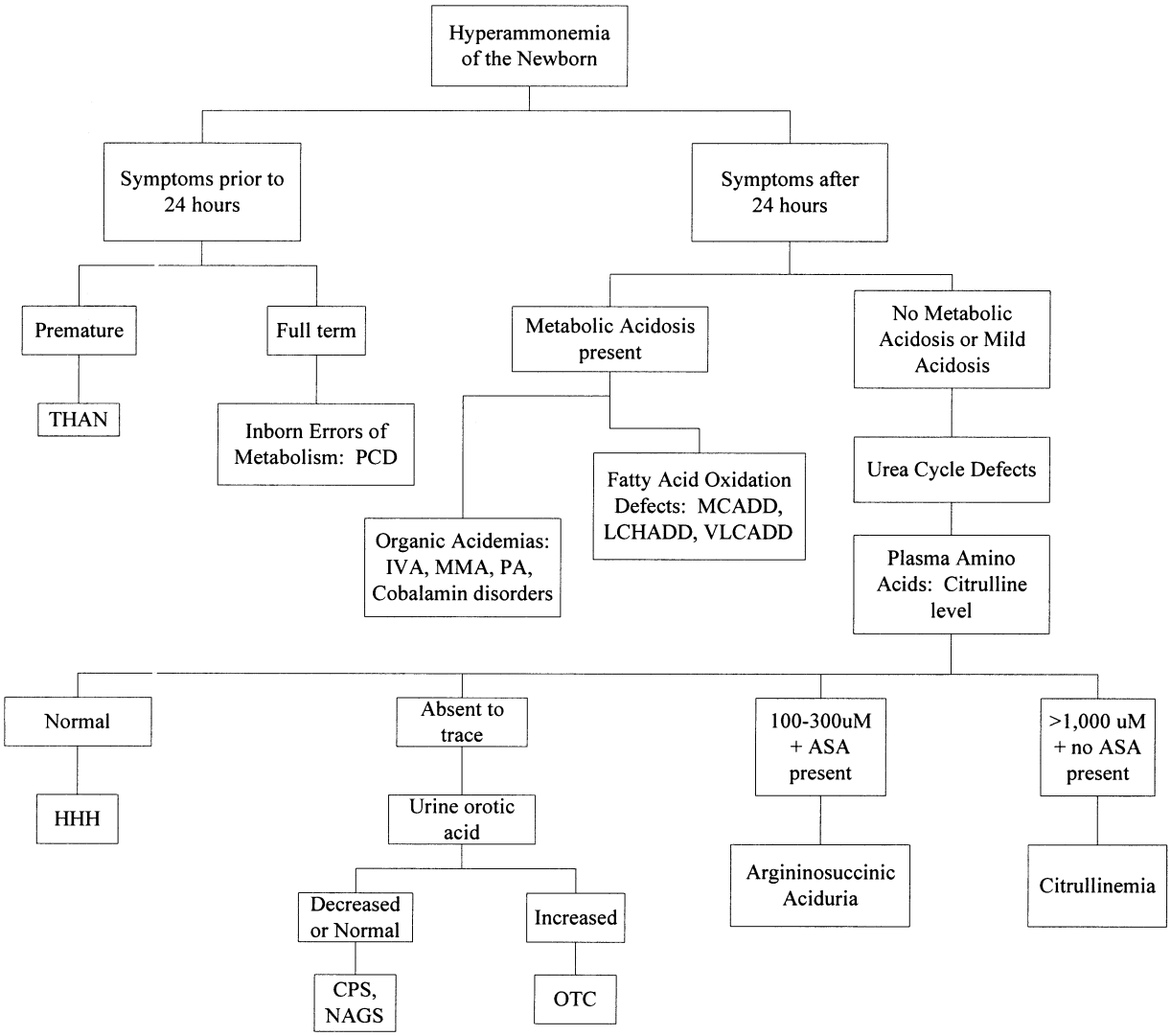

Fig 3. Simplified flowchart for evaluation of hyperammonemia in the newborn. CSPD, carbamyl phosphate synthetase deficiency; HHH, hyperornithinemia–hyperammonemia–homocitrullinuria syndrome; IVA, isovalemic acidemia; LCHADD, long chain 3-chydroxy-acyl-CoA dehydrogenase deficiency; MCADD, medium chain acyl-CoA dehydrogenase deficiency; MMA, methylmalonic acidemia; NAGS, *N*-acetylglutamate synthetase deficiency; OTC, ornithine transcarbamylase deficiency; PCD, pyruvate carboxylase deficiency; PA, propionic acidemia; THAN, transient hyperammonemia of the newborn; VLCADD, very long chain acyl-CoA dehydrogenase deficiency. Adapted with permission from Hudak ML, Jones MD, Brusilow SW. Differentiation of transient hyperammonemia of the newborn and urea cycle enzyme defects by clinical presentation. J Pediatr 107:712-719, 1985.

knowledge of this disorder and use of tandem mass spectrometry may result in earlier diagnosis during the neonatal period.[57-59]

Neonates with profound and prolonged hyperammonemia with coma due to urea cycle defect will have had a neurological insult to the brain that may be significant. If so, the option of withdrawal of support rather than aggressive intervention should be discussed with the parents.[60]

During the acute phase of treatment of a newborn with a urea cycle defect, the therapy is multifocused. Initial treatment at this time is directed toward the removal of the accumulating metabolites, such as ammonia. Intravenous glucose is administered to restore hydration, as the majority of the newborns with urea cycle defect are dehydrated as a consequence of poor oral intake and vomiting. With hydration, tissue perfusion is increased, which protects renal function and blunts further catabolic production of nitrogen. The preferred choice of fluids is 10% dextrose with salts until definitive therapy is available.[60] All dietary protein may be discontinued for 24 hours to decrease the

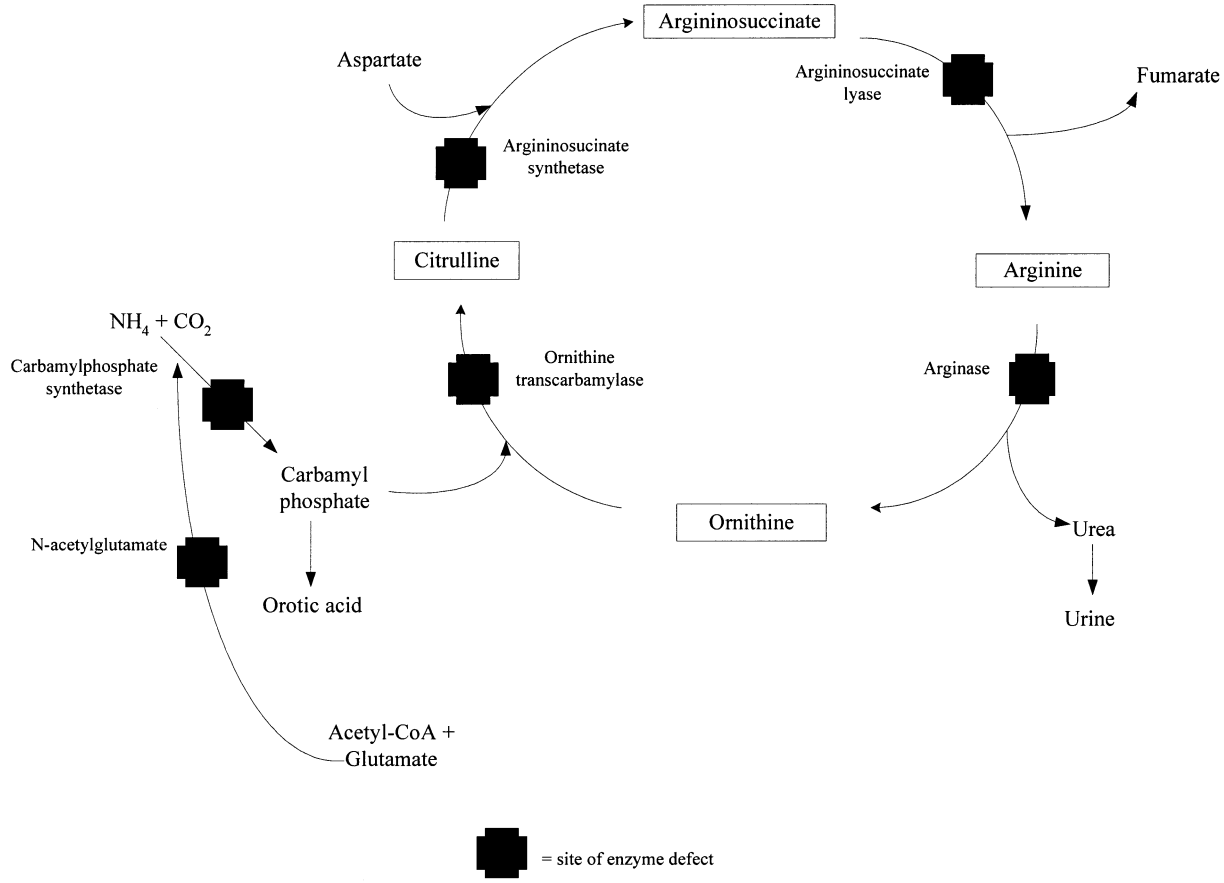

Fig 4. Simplified schematic of urea cycle with enzyme defects.

protein load.[60] Caloric intake is provided as carbohydrate and fat. During this period, hemodialysis most often is needed.[29] As with a newborn with an organic acidemia, if the hospital is unable to offer hemodialysis, transfer of the newborn to another medical center with hemodialysis capability should be strongly considered. Exchange transfusions, peritoneal dialysis, or hemofiltration are less efficient than hemodialysis in managing these disorders.[30] Drug therapy is administered to provide an alternative path for nitrogen excretion. Intravenous sodium phenylacetate combines with glutamine to produce phenylacetylglutamine, which is excreted in the urine.[61,62] Sodium benzoate combines with glycine to produce hippurate. Hippurate is rapidly cleared by the kidneys.[63,64] With the removal of the excessive nitrogen, there is a decrease in arginine synthesis. As a result, arginine becomes an essential amino acid that must be replaced.[65-67] The assistance of a dietician with experience with EM is essential. The diet involves protein restriction for life to an amount tolerated without causing hyperammonemia and preventing excessive protein catabolism while at the same time providing the body with the nutritional needs for growth and development.[68,69]

The overall prognosis for newborns with a urea cycle defect is guarded. Without treatment, these newborns will die. Even with aggressive medical intervention, many will die. Those newborns that do survive may have neurological insult and recurrent life-threatening metabolic crises. The long-term outcome into adulthood is largely unknown, as the oldest children are just surviving past childhood. Many of the children eventually die and have developmental delays in physical growth and cognitive skills.[70-72] The long-term correction of a urea cycle defect is the correction of the enzymatic defect within the hepatocytes. Liver transplantation has been successful in infants with CPS and OTC.[73-75] After transplantation, the serum ammonia levels fall to normal levels without the need for protein restriction or medications. With advances in liver transplantation, the indications for liver transplantation for urea cycle defects are changing.[76,77] Because of the limited number of livers available for transplantation, gene therapy could provide the ultimate correction of the urea cycle defect. This area of laboratory and clinical research has the potential to reduce the morbidity and mortality seen in urea cycle defects.[78-83]

Table 6. Collection of Blood, Urine, and Cerebrospinal Fluid for Metabolic Evaluation in the Dying Newborn

Blood	
Amino acids	1 ml in EDTA or heparin tube
Ammonia	1 ml in lithium heparin tube on ice
Blood gas	0.3 ml in heparin-coated syringe
Carnitine (free and total)	1 ml in lithium heparin tube on ice
Comprehensive metabolic panel	0.5 ml in lithium heparin tube
Fatty acid profile	0.3 ml in fluoride heparin tube
Hematology	0.5 ml in EDTA tube (no capillary)
Lactic acid	0.6 ml in 0.6 ml sodium fluoride tube on ice
Urine	
ph, ketone bodies, reducing substances, amino acids, organic acids	5 ml, freeze at −20°C
Cerebrospinal fluid	
Amino acids	0.5 ml in plastic tube
Cells, protein, glucose	0.5 ml in plastic tube
Culture	0.5 ml in sterile plastic tube
Lactic acid	0.5 ml in plastic tube

Note: Adapted with permission from Oregon Health and Science University Laboratory Services Manual, 2003.

Evaluation of the Severely Ill Neonate Near Death

In some situations, such as severe early onset, late-diagnosed OTC, survival of the newborn may be impossible. In most neonatal intensive care units, there is protocol for dealing with a dying newborn. However, there are several steps that must be taken to increase the probability of a specific diagnosis postmortem, when an EM is suspected.[84-86] The collection of blood, urine, and cerebrospinal fluid is outlined in Table 6. Many of these samples may be collected before death. Parental permission for skin biopsy and autopsy must be requested and obtained. A full- thickness skin sample by 3 to 4 mm punch biopsy should be obtained, placed in tissue culture medium, kept at room temperature, and sent within 24 hours to the appropriate laboratory where the fibroblasts can be grown in culture. A skin biopsy is optimal if it can be done before death. A discussion with the parents regarding an autopsy for the purpose of investigation of a suspected EM needs to occur if possible before the newborn's death. Ideally, the autopsy should be performed less than 4 hours after death before postmortem changes can compromise the integrity of the enzyme system. At autopsy, samples of liver, spleen, muscle, heart, kidney, and brain should be obtained and stored in liquid nitrogen or at –70°C. Also readily available at autopsy, bile should be collected and frozen. A small amount of bile should be spotted on newborn filter paper to be used in the postmortem assay of acylcarnitines.[87] If there is a lack of urine, postmortem vitreous humor from the eye is easily collected by aspiration and subsequently frozen for electrolyte and organic acid-analysis.[89] Before shipment to laboratories, the laboratories should be notified of the precious samples that are being shipped to facilitate proper processing, storing, and analysis. These specimens are vital to provide resolution to the family for the cause of death. Parents need to be counseled regarding the recurrence risk for most of these disorders. In addition, they need to be made aware that, without a specific diagnosis, future prenatal diagnosis is impossible.

Summary

The ability to diagnose more and more EM during the neonatal period is due to advances in biochemical genetics. After initial laboratory studies, certain conditions, such as metabolic acidosis and hyperammonemia, will be key clues to signal the need for further consultation with a metabolic disease specialist and more specific laboratory studies, though absence of these derangements does not preclude an EM diagnosis. Initial therapy is directed toward stabilization of the neonate. Long- term care is dependent and based on a definitive EM diagnosis. The majority of therapies are lifelong and require continual nutritional, medical, and laboratory monitoring. As children with EM are now surviving long term, the ability to provide them and their families anticipatory guidance as to their long-term physical, mental, and emotional needs is becoming increasingly important. As these children reach adulthood, their reproductive abilities and the teratogenic effect that their disease or its treatment can or will have on their children is unknown. In virtually all cases of probable EM, the diagnosis is possible even if it is established postmortem. A diagnosis is essential to provide the family,

who may have already suffered the loss of a newborn, with thoughtful genetic counseling.

References

1. Enns GM, Steiner RD. Diagnosis and treatment of children with suspected metabolic disease. Compr Pediatr (in press)
2. Nyhan WL, Ozand PT: Atlas of Metabolic Diseases. London, UK, Chapman & Hall, 1998
3. Rimoin DL, Connor JM, Pyeritz RE: Emery and Rimoin's Principles and Practice of Medical Genetics, (ed 3) Vols I and II. New York, NY, Churchill-Livingstonm, 1997
4. Scriver CR, Beaudet AL, Sly WS, et al: The Metabolic and Molecular Bases of Inherited Disease (ed 8) Vols I–IV. New York, NY, McGraw-Hill, 2001
5. Burton BK: Inborn errors of metabolism in infancy: A guide to diagnosis. Pediatrics 102(E69), 1998
6. Leonard JV, Morris AAM: Inborn errors of metabolism around time of birth. Lancet 583-587, 2000
7. Millington DS: Tandem mass spectrometry in clinical diagnosis, in Blau N, Duran M, Blaskovics ME et al (eds): Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases (ed 2). Berlin, Germany, Springer, 2003, pp 57–85
8. Carson NAJ: Non-ketotic hyperglycinemia: A review of 70 patients. J Inherited Metab Dis 2(Suppl):126–138, 1982
9. Goldfischer S, Moore CL, Johnson A, et al: Peroxisomal and mitochondrial defects in the cerebro–hepato–renal syndrome. Science 182:62–64, 1973
10. Wilson GN, Holmes RD, Hajra AK: Peroxisomal disorders: Clinical commentary and future prospects. Am J Med Genet 30:771–792, 1998
11. Bankier A, Turner M, Hopkins IJ: Pyridoxine dependent seizures: A wider clinical spectrum. Arch Dis Child 58:415–418, 1983
12. Harding CO, Pillers DM, Steiner RD, et al: Potential for misdiagnosis due to lack of metabolic derangement in combined methylmalonic aciduria/hyperhomocysteinemia (cblC) in the neonate. J Perinatol 23:384–386, 2003
13. Oh MS, Carroll HJ: The anion gap. N Engl J Med 297:814–817, 1977
14. Ozand PT, Gascon GG: Organic acidurias: A review. J Child Neurol 6:288–303, 1991
15. Robinson BH, Taylor J, Sherwood WG: The genetic heterogeneity of lactic acidosis: Occurrence of recognizable inborn errors of metabolism in a pediatric population with lactic acidosis. Pediatr Res 14:956–962, 1980
16. Blass JP, Cederbaum S, Gibson GE: Clinical and metabolic abnormalities accompany deficiencies in pyruvate oxidation, in Hommes FA (ed): Normal and Pathological Development of Energy Metabolism. New York, NY, Wiley-Liss, 1983, pp 210–250
17. Stansbie D, Sherriff RJ, Denton RM: Fructose load test: An in vivo screening test designed to assess pyruvate dehydrogenase acitivity and interconversion. J Inherited Metab Dis 1:163–165, 1978
18. Coude FX, Saudubray JM, DeMaugre F, et al: Dichloroacetate as treatment for congenital lactic acidosis. N Engl J Med 29924:1365–1366, 1978
19. Munnich A, Rustin P, Rotig A, et al: Clinical aspects of mitochondrial disorders. J Inherited Metab Dis 15:448–455, 1992
20. Rotig A, Cormier V, Blanche S, et al: Person's marrow-pancreas syndrome: A multisystem mitochondrial disorder in infancy. J Clin Invest 86:1601–1608, 1990
21. Treem WR, Witzleben CA, Piccoli DA, et al: Medium-chain and long-chain acyl CoA dehydrogenase deficiency: clinical, pathologic and ultrastructural differentiation from Reye's syndrome. Hepatology 6:1270–1278, 1986
22. Berry GT, Yudkoff M: Segal S: Isovaleric academia: Medical and neurodevelopmental effects of long-term therapy. J Pediatr 113:58–64, 1988
23. Fenton WA, Gravel RA, Rosenberg LE: Disorders of propionate and methylmalonate metabolism, in Scriver CR, Beaudet AL, Sly WS et al (eds): The Metabolic and Molecular Bases of Inherited Disease (ed 8). New York, NY, McGraw-Hill, 2001, pp 2165–2194
24. Matsui SM, Mahoney MJ, Rosenberg LE: The natural history of the inherited methylmalonic acidemias. N Engl J Med 308:857–861, 1983
25. Sweetman L, Williams JC: Branched chain organic acidurias, in Scriver CR, Beaudet AL, Sly WS et al (eds): The Metabolic and Molecular Bases of Inherited Disease (ed 8). New York, NY, McGraw-Hill, 2001, pp 2125–2164
26. Hoffmann GF, Feyh P: Organic acid analysis, in Blau N, Duran M, Blaskovics ME et al (eds): Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases (ed 2). Berlin, Germany, Springer, 2003, pp 27–44
27. Gibson KM, Elpeleg ON, Morton DH, et al: Disorders of leucine metabolism, in Blau N, Duran M, Blaskovics ME et al (eds): Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases (ed 2). Berlin, Germany, Springer, 2003, pp 165–189
28. Nyhan WL, Gibson KM: Disorders of valine–isoleucine metabolism, in Blau N, Duran M, Blaskovics ME et al (eds): Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases (ed 2). Berlin, Germany, Springer, 2003, pp 191–213
29. Brusilow SW, Maestri NE: Urea cycle disorders: Diagnosis, pathophysiology and therapy. Adv Pediatr 43:127–170, 1996
30. Donn SM, Swartz RD, Thoene JG: Comparison of exchange transfusion, peritoneal dialysis and hemodialysis for the treatment of hyperammonemia in an anuric newborn infant. J Pediatr 951:67–70, 1979
31. Kalloghlian A, Gleispach H, Ozand PT: A patient with propionic academia managed with continuous insulin infusion and total parenteral nutrition. J Child Neurol 7S:88–91, 1992
32. deSous C, Chalmers RA, Stacey TE, et al: The response of L-carnitine and glycine therapy in isovaleric academia. Eur J Pediatr 144:451–456, 1986
33. Stumpf DA, Parker WD, Engelini C: Carnitine deficiency, organic acidemias and Reye's syndrome. Neurology 35:1041–1045, 1985
34. Suglycama N, Matsuda I, Wada Y, et al: Urinary propionylcarnitine analysis for monitoring carnitine supplementation in inherited disorders of propionate metabolism. J Inherited Metab Dis 17:611–615, 1994
35. Acosta PB, Yannicelli S: Nutrition Support Protocols ed 4. Columbus, Ohio, Ross Products Division, 2001
36. Thompson GN, Chalmers RA, Walter JH, et al: The use of metronidazole in management of methylmalonic and propionic acidemias. Eur J Pediatr 149:792–796, 1990
37. Cooper BA, Rosenblatt DS: Inherited defects of vitamin B_{12} metabolism. Annu Rev Nutr 7:291–320, 1987
38. Morrow G, Burkel GM: Long term management of a patient with vitamin B_{12}-responsive methylmalonic academia. J Pediatr 96:425-426, 1980
39. Barsotti RJ: Measurement of ammonia in blood. J Pediatr 138: S11–S20, 2001
40. Summar M, Tuchman M: Proceedings of a consensus conference for the management of patients with urea cycle disorders. J Pediatr 138:S6–S10, 2001
41. Green A: When and how should we measure plasma ammonia? Annu Clin Biochem 25:99–209, 1988
42. Ballard RA, Vinocur B, Reynolds JW, et al: Transient hyperammonemia of the preterm infant. N Engl J Med 299:920–925, 1978
43. Coude FX, Ogier H, Marsac C, et al: Secondary citrullinemia with hyperammonemia in four neonatal cases of pyruvate carboxylase deficiency. Pediatrics 68:914, 1981
44. Burton BK: Urea cycle disorders. Clin Liver Dis 4:815–830, 2000
45. Saudubray JM, Charpentier C: Clinical phenotypes: diagnosis/algorithms, in Scriver CR, Beaudet AL, Sly WS et al (eds): Metabolic and Molecular Bases of Inherited Disease (ed 8). New York, NY, McGraw-Hill, 2001, pp 1327–1403
46. Brusilow SW, Horwich AL: Urea cycle enzymes, in Scriver CR, Beaudet AL, Sly WS et al (eds): Metabolic and Molecular Bases of

Inherited Disease (ed 8). New York, NY, McGraw-Hill, 2001, pp 1909–1964

47. Sim KG, Hammond J, Wilcken B: Strategies for the diagnosis of mitochondrial fatty acid beta-oxidation disorders. Clin Chim Acta 323: 37–58, 2002

48. Nyhan W, Ozand PT: Pyruvate carboxylase deficiency, in Atlas of Metabolic Disease. New York, NYpp. 265-272, Chapman & Hall, 1998

49. Batshaw ML, Thomas GH, Brusilow SW: New approaches to the diagnosis and treatment of inborn errors of urea synthesis. Pediatrics 68:290–297, 1981

50. Bachmann C, Colombo JP: Diagnostic value of orotic acid excretion in heritable disorders of the urea cycle and in hyperammonemia due to organic acidurias. Eur J Pediatr 134:109–113, 1980

51. Coomes MW: Amino acid metabolism, in Devlin TM (ed): Biochemistry with Clinical Correlations (ed 5). New York, NY, Wiley-Liss, 2002, pp 779–821

52. Bachmann C: Inherited hyperammonemias, in Blau N, Duran M, Blaskovics ME, et al (eds.): Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases (ed 2). Berlin, Germany, Springer, 2003, pp 261–276

53. Inoue E, Loura M, Saheki T, et al: Abnormality of citrulline synthesis in liver mitochondria from patients with hyperornithinaemia, hyperammonaemia and homocirtullinuria. J Inherited Metab Dis 10:227–280, 1987

54. Nyhan W, Ozand PT: Ornithine transcarbamylase deficiency, in Atlas of Metabolic Disease. New York, NYpp 168-177, Chapman & Hall, 1998

55. Buist NRM, Kennaway NG, Hepburn CA, et al: Citrullinemia: Investigation and treatment over a four year period. J Pediatr 85:208–214, 1974

56. Nyhan WL, Sakati NO: Argininosuccinic acidura, in Diagnostic Recognition of Genetic Disease. Philadelphia, PApp 165-167, Lea & Febiger, 1987

57. Jorda A, Rubio V, Portoles M, et al: A new case of arginase deficiency in a Spanish male. J Inherited Metab Dis 9:393–397, 1986

58. Braga AC, Vilarinho L, Ferreira E, et al: Hyperargininemia presenting as persistent neonatal jaundice and hepatic cirrhosis. J Pediatr Gastoenterol Nutr 24:218–221, 1997

59. Picker JD, Puga AC, Leve HL, et al: Arginase deficiency with lethal neonatal expression: Evidence for the glutamine hypothesis of cerebral edema. J Pediatr 142:349–352, 2003

60. Summar M: Current strategies for the management of neonatal urea cycle disorders. J Pediatr 138:S30–S39, 2001

61. Batshaw ML, Brusilow SW: Treatment of hyperammonemic coma caused by inborn errors of urea synthesis. J Pediatr 97:893–900, 1980

62. Brusilow SW, Valle DL, Batshaw M: New pathways of nitrogen excretion in inborn errors of urea synthesis. Lancet 2:452–454, 1979

63. Batshaw ML: Sodium benzoate and arginine: Alternative pathway therapy in inborn errors of urea synthesis. Clin Biol Res 127:69–83, 1983

64. Green TP, Marchessault RP, Freese DK: Disposition of sodium benzoate in newborn infants with hyperammonemia. J Pediatr 102:785–790, 1983

65. Brusilow SW: Arginine, an indispensable amino acid for patients with inborn errors of urea synthesis. J Clin Invest 74:2144–2148, 1984

66. Kline JJ, Hug G, Schubert WK, et al: Arginine deficiency syndrome: Its occurrence in carbamyl phosphate synthetase deficiency. Am J Dis Child 135:437–442, 1981

67. Brusilow SW, Batshaw ML: Arginine therapy of argininosuccinase deficiency. Lancet 1:124–127, 1979

68. Acosta PB, Yannicelli S: Protocol 24: Urea cycle disorders in Nutrition Support Protocols (ed 4) Columbus, Ohio: Ross Products Division, 2001, pp 418-432

69. Leonard JV: The nutritional management of urea cycle disorders. J Pediatr 138:S40–S45, 2001

70. Msall M, Batshaw ML, Suss R, et al: Neurologic outcome in children with inborn errors of urea synthesis. N Engl J Med 310:1500–1505, 1984

71. Maestri NE, Clissold DB, Brusilow SW: Long term survival of patients with argininosuccinate synthetase deficiency. J Pediatr 127:929–935, 1995

72. Nyhan WL, Ozand PT: Orthinthine transcarbamylase deficiency, in Atlas of Metabolic Diseases. London, UKpp 168-177, Chapman & Hall, 1998

73. Todo S, Starzl Tel Tzakis A, et al: Orthotopic liver transplantation for urea cycle enzyme deficiency. Hepatology 15:419–422, 1992

74. Hasegawa T, Tzakis AG, Todo S, et al: Orthotopic liver transplantation for ornithine transcarbamylase deficiency with hyperammonemic encephalopathy. J Pediatr Surg 30:863–865, 1995

75. Saudubray JM, Touti G, Gelonlay P, et al: Liver transplantation in urea cycle disorders. Eur J Pediatr 158(Suppl 2):S55–S59, 1999

76. Dunn S, Weintraub W, Vinocur CD, et al: Is age less than 1 year a high risk category for orthotopic liver transplantation? J Pediatr Surg 28:1048–1050, 1993

77. Van der Werf WJ, D'Alessandro AM, Knechtle SJ, et al: Infant pediatric liver transplantation results equal those for older pediatric patients. J Pediatr Surg 33:20–23, 1998

78. Demarquoy J: Retroviral-mediatred gene therapy for the treatment of citullinemia: Transfer and expression of argininosuccinate synthetase in human hemopoietic cells. Experientia 49:345–348, 1993

79. Kiwaki K, Kanegae Y, Saito I, et al: Correction of ornithine transcarbamylase deficiency in adult spf (ash) mice and in OTC-deficient human hepatocytes with recombinant adenoviruses bearing the CAG promoter. Hum Gene Ther 7:821–830, 1996

80. Batshaw ML, Robinson MB, Ye X, et al: Correction of ureagenesis after gene transfer in an animal model and after liver transplantation in humans with ornithine transcarbamylase deficiency. Pediatr Res 47: 588–593, 1999

81. Jones SN, Grompe M, Munir MI, et al: Ectopic correction of ornithine transcarbamylase deficiency in sparse fur mice. J Biol Chem 265:14684–14690, 1990

82. Patejunas G, Lee B, Dennis JA, et al: Evaluation of gene therapy for citrullinaemia using murine and bovine models. J Inherited Metab Dis 21(Suppl 1):138–150, 1998

83. Batshaw ML, Wilson JM, Raper S, et al: Recombinant adenovirus gene transferin adults with partial ornithine transcarbamylase deficiency (OTCD). Human Gene Therapy 10:2419–2437, 1999

84. Kronick JB, Scriver CR, Goodyear RP, et al: A perimortem protocol for suspected genetic disease. Pediatrics 71:960–963, 1983

85. Helweg-Larsen K: Postmortem protocol. Acta Paediatr 389(Suppl):77–79, 1993

86. Steiner RD, Cederbaum SD: Laboratory evaluation of urea cycle disorders. J Pediatr 138:S21–S29, 2001

87. Rashed MS, Ozard PTl, Bennett J, et al: Inborn errors of metabolism diagnosed in sudden death cases by acylcarnitine analysis of postmortem bile. Clin Chem 41:1109–1114, 1995

88. Bennett MJ, Ragni MC, Hood I, et al: Comparison of postmortem urinary and vitreous humour organic acids. Am Clin Biochem 29:541–545, 1999