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Management of Inherited Disorders of Ureagenesis

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The conversion of ammonia to urea is known to require the function of at least nine proteins. Five of these are urea cycle enzymes (carbamyl phosphate synthetase I, ornithine transcarbamylase, argininosuccinate synthetase, argininosuccinate lyase, and arginase). A sixth enzyme (N-acetylglutamate synthase) catalyzes the formation of N-acetylglutamate, a cofactor for carbamyl phosphate synthetase I. The remaining three proteins are amino acid transporters for ornithine, aspartate/glutamate, and dibasic amino acids, respectively. An inherited deficiency in any of these proteins causes a block in the urea cycle and resultant hyperammonemia. Although the severity of these disorders varies, symptoms, diagnostic testing, and treatments are similar. Common symptoms include episodes of vomiting, lethargy, and coma associated with hyperammonemia. Diagnosis is based on a combination of measurement of plasma ammonia, plasma and urinary amino acids, urinary orotic acid, enzyme analysis, and molecular testing. Therapy involves a nitrogen-restricted diet, supplementation of essential amino acids and L-citrulline/L-arginine (except for hyperargininemia [L-citrulline is not approved by the United States Food and Drug Administration]), alternative pathway therapy with phenylbutyrate (or phenylacetate and benzoate), and, for the most severe cases, liver transplantation. ■

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Learning Objectives:

- Describe the major clinical findings in newborn infants, older children, and adults with disorders of the urea cycle.
- Explain the best way to establish diagnosis of urea cycle disorders.
- Identify and evaluate treatment options for disordered ureagenesis, emphasizing dietary management.
- Discuss the outlook for children with complete and partial urea cycle defects.

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Introduction

Urea Cycle and Related Disorders

Nine proteins within the liver are necessary to convert ammonium nitrogen, which is toxic to the brain, to urea, which is nontoxic (Fig. 1). Five enzymes comprise the urea cycle: carbamyl phosphate synthetase I (CPS), ornithine transcarbamylase (OTC), argininosuccinate synthetase (AS), argininosuccinate lyase (AL), and arginase (ARG) I [1]. Another enzyme, N-acetylglutamate synthase (NAGS), catalyzes the formation of N-acetylglutamate, the essential cofactor for CPS activity [2]. In addition, the generation of urea from ammonia requires at least three amino acid transporters: two located within the mitochondrial inner mem-

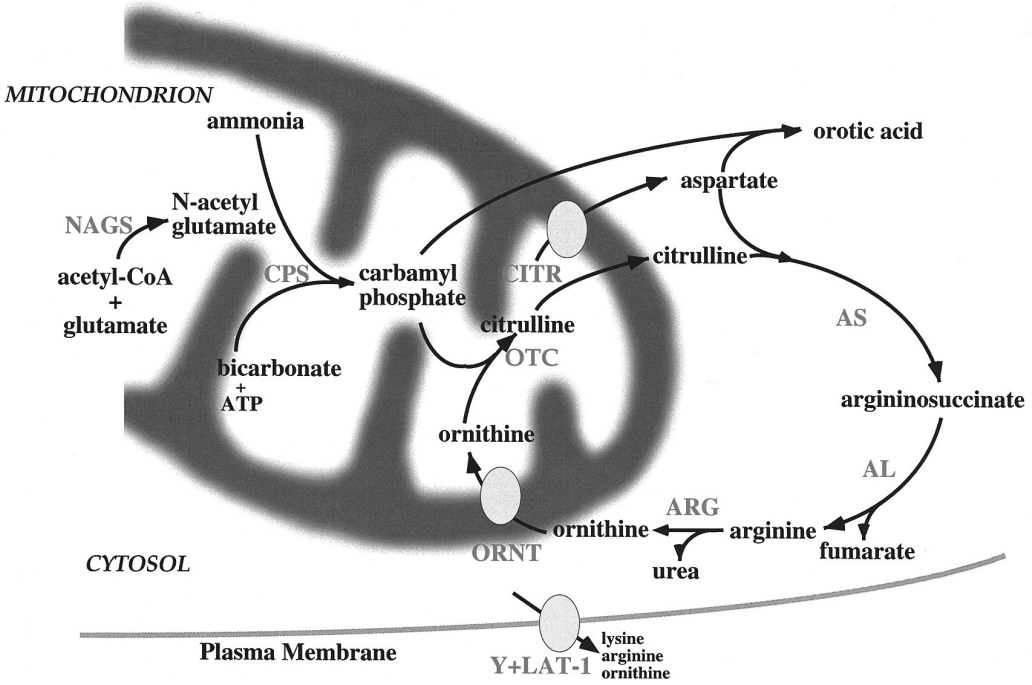

Figure 1. The urea cycle in a liver cell consists of six enzymes and three membrane transporters. NAGS = N-acetylglutamate synthetase; CPS = carbamyl phosphate synthetase I; OTC = ornithine transcarbamylase; AS = argininosuccinate synthetase; AL = argininosuccinate lyase; ARG = arginase; ORNT = ornithine mitochondrial membrane transporter; CITR = citrin calcium-dependent mitochondrial membrane aspartate/glutamate transporter; y+LAT-1 = dibasic amino acid plasma membrane transporter.

brane, the ornithine transporter [3] and the aspartate/glutamate transporter (called citrin or aralar II); [4] and one in the plasma membrane, the dibasic amino acid transporter (y+LAT-1) [5]. The two atoms of nitrogen incorporated into urea derive from free ammonia and aspartate. Defects in any one of the nine proteins involved in ureagenesis can cause hyperammonemia; however, the severity and the age at first presentation vary greatly between and even within the different disorders. Generally, the more proximal the block is, the more severe the disease. Thus, patients with complete CPS or OTC deficiency present almost invariably during the first few days of life with hyperammonemic coma, whereas patients with citrullinemia (AS deficiency) or argininosuccinic acidemia (AL deficiency) tend to present later, in the first month of life [6]. Arginase deficiency usually presents later in childhood [7] as do transporters' defects. Defects in the ornithine transporter cause hyperornithinemia, hyperammonemia, and homocitrullinuria (HHH) syndrome; [8] defects in the dibasic amino acid transporter cause dibasic amino aciduria, also called lysinuric protein intolerance (LPI) [5]. Defects in the citrin (aralar II) transporter cause citrullinemia type II [9]. Arginase deficiency leads to a condition termed argininemia. For more details on the bio- chemical and molecular basis of urea cycle disorders, please refer to "Advances in Inherited Urea Cycle Disorders" by Batshaw et al. [10].

Clinical Findings

The classic presentation of a complete defect in a urea cycle enzyme, other than ARG deficiency, is as a catastrophic illness. Typically, the affected baby is born after an uncomplicated full-term pregnancy, labor, and delivery with normal APGAR scores. Clinical symptoms in complete CPS and OTC deficiencies develop between ages 24 and 72 hours as poor sucking, hypotonia, vomiting, lethargy, and hyperventilation. This rapidly progresses to coma and seizures; if untreated, there is universal mortality, and even with treatment, mortality is common [11].

Individuals with mutations causing partial enzyme deficiencies have a spectrum of presentations, with hyperammonemic episodes developing in some during infancy, in others during later childhood, and sometimes not until adulthood in others [12–14]. In infants and young children, recurrent episodes of vomiting, lethargy, and irritability associated with failure to thrive are common. In older children, there may be prolonged episodes of

anorexia, ataxia, and behavioral abnormalities, including biting, self-injury, nocturnal restlessness, and hyperactivity [15–19]. In adults, the symptoms may mimic psychiatric or neurologic disorders and include headache, nausea, dysarthria, ataxia, confusion, hallucinations, and visual impairment [18, 20–25]. Neurologic findings may include increased deep tendon reflexes, papilledema, and decorticate or decerebrate posturing. Seizures are a late complication and occur after alterations in consciousness.

Symptoms may be delayed in onset by a mild deficiency or dietary self-selection, i.e., avoidance of meats, fish, eggs, milk, and other high-protein foods. Individuals with partial defects may even remain asymptomatic throughout life, depending on their protein intake and exposure to precipitants of hyperammonemia (e.g., intercurrent illnesses, certain drugs) [11].

In affected individuals, acute, life-threatening, hyperammonemic episodes have been precipitated by high-protein meals, parenteral nutrition, viral infections, medication, trauma, surgery, pregnancy and delivery, [26] and exposure to insect repellent [27]. In children with partial defects, it is not uncommon for the initial hyperammonemic episode to occur after weaning, when low-protein breast milk is replaced by higher-protein formula or cow's milk. Treatment with valproate or haloperidol has been associated with inducing hyperammonemic crises in patients with urea cycle disorders [28–31]. Valproate is believed to inhibit NAGS and thus causes secondary CPS deficiency with resultant aggravation of hyperammonemia [32].

Individuals with transporter defects and ARG deficiency present a somewhat different clinical picture. Although the degree of hyperammonemia and its symptoms are similar to partial urea cycle disorders, there are additional clinical features that complicate the picture. In citrullinemia type II, cholestatic jaundice and pancreatitis have been reported [33] (pancreatitis has also been recently reported in OTC deficiency). In ARG deficiency and HHH syndrome, there is a progressive spastic paraplegia, which is as yet unexplained. HHH syndrome also may be associated with growth failure and a bleeding tendency [34]. Lysinuric protein intolerance is even more complex, presenting as a multisystem disease, which may include skeletal, pulmonary, renal, and hematologic abnormalities. Osteoporosis, interstitial lung disease, glomerular disease, and growth failure have been described in this disorder [35].

Making the Diagnosis

The outcome in these disorders is a function of the specific enzymatic defect, its severity (complete or partial), age of onset, and the promptness of diagnosis and institution of therapy. To identify these disorders early, plasma ammonia levels should be measured in every acutely ill newborn. Plasma ammonia levels should also be measured in children and adults showing unexplained behavioral or neurological symptoms, recurrent vomiting, self-avoidance of high-protein foods, or atypical migraines.

To avoid factitious increase in plasma ammonia, the blood should be drawn into an ice-chilled tube, and the assay should be performed within 15 minutes of collection. Venous blood is adequate as long as the tourniquet is applied for only a short period of time or not at all [36]. Most hospitals now have automated analyzers that measure ammonia in less than 30 minutes and require less than 1 mL of blood. Normal plasma ammonia level is less than 35 μmol/L (63 μg/dL); it is slightly higher in premature infants. If testing is performed when clinical symptoms suggestive of hyperammonemia are present and plasma ammonia is found to be normal, the cause is unlikely to be a urea cycle or related disorder. However, plasma ammonia levels can be normal between episodes in such patients. In these instances, increased levels of plasma glutamine, and sometimes alanine and asparagine (obtained by a quantitative amino acid analysis), are suggestive of a high nitrogen load [37].

Typically, hyperammonemia resulting from urea cycle disorders is accompanied by a respiratory alkalosis. This distinguishes it from organic acidemias (e.g., propionic, methylmalonic, isovaleric acidemia), in which ketoacidosis is usually present along with hyperammonemia (Fig. 2). Although serum blood urea nitrogen concentration is lower than normal in urea cycle disorders (as a result of reduced ureagenesis), it can be subnormal in any patients with a decreased protein intake. Liver function test results are usually within normal limits in urea cycle disorders when the patient is clinically stable but could be transiently abnormal during hyperammonemic episodes.

Once hyperammonemia has been detected, the differential diagnosis requires the measurements of plasma and urinary amino acids, urinary organic acids, and orotic acid and plasma lactate (Fig. 3). Urinary organic acids are largely unremarkable in urea cycle and related disorders (except for orotic acid, which could be detected when massively increased), whereas they are abnormal in organic acidemias, congenital lactic acidoses, and fatty acid oxidation defects. Measurement of lactic acid and acylcarnitines can help distinguish between these three groups of disorders.

In differentiating among the primary urea cycle disorders, the citrulline level helps identify proximal versus distal defects. Citrulline is the product of CPS and OTC and is a substrate for AS and AL. As a result, plasma citrulline is absent or present in only trace amounts in complete-onset CPS and OTC deficiencies, but it may be low or

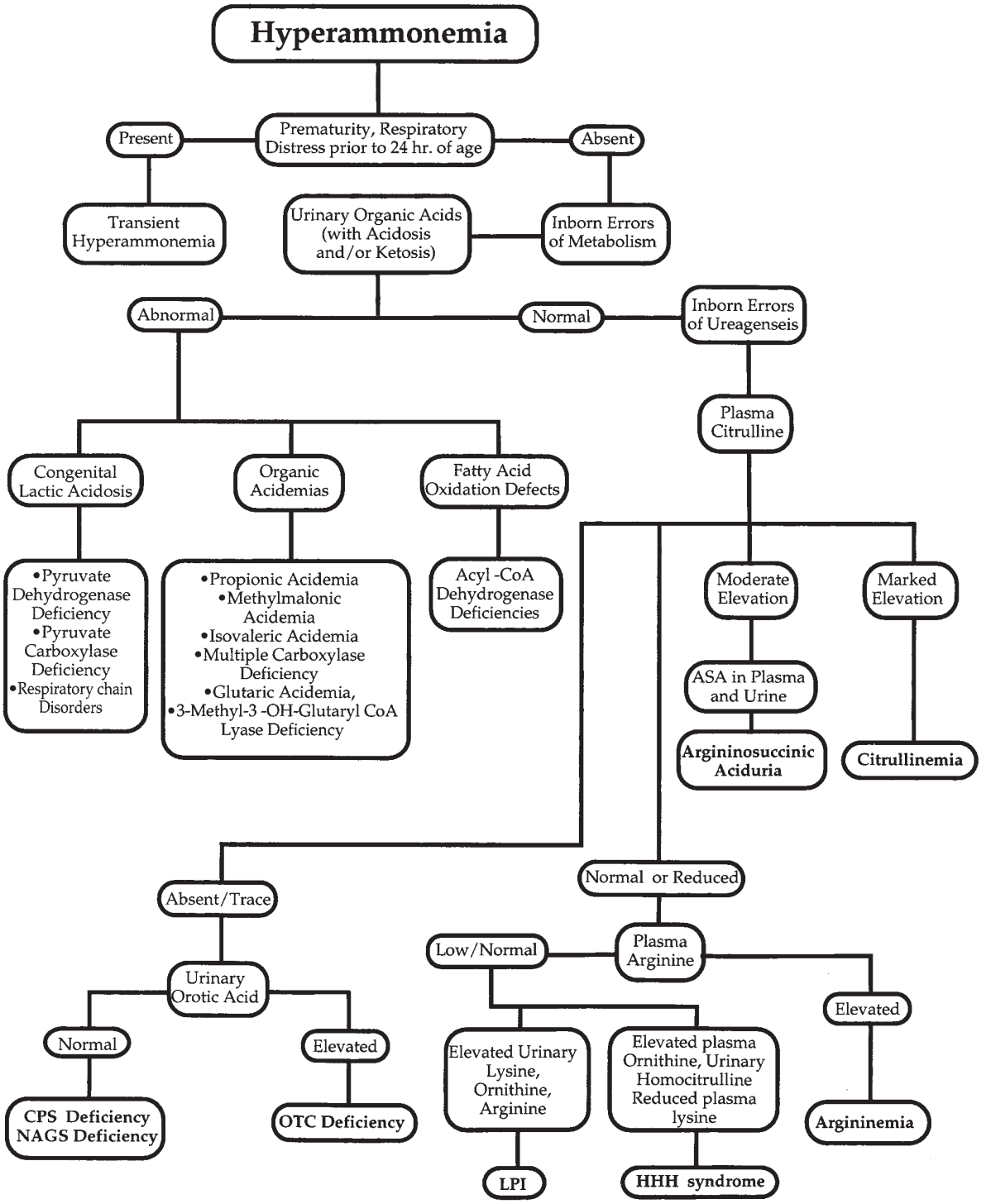

Figure 2. Algorithm for the differential diagnosis of inherited hyperammonemia.

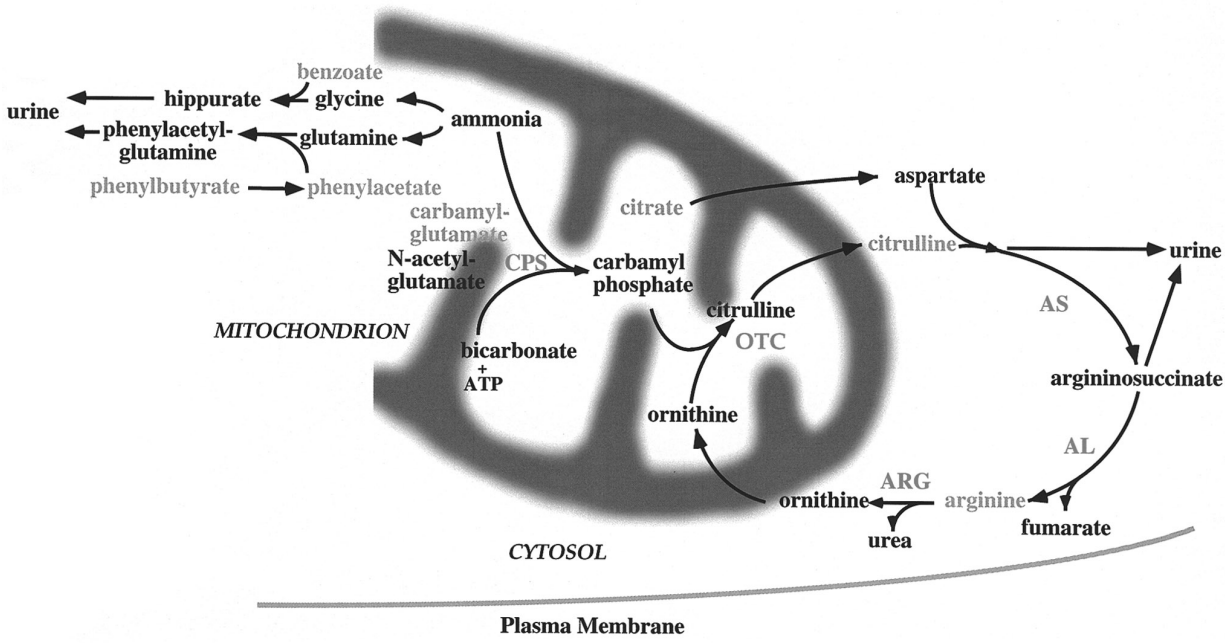

Figure 3. The urea cycle and drug therapy for urea cycle disorders. This therapy includes compounds that use alternative pathways to excrete waste nitrogen (phenylbutyrate, phenylacetate, benzoate, and arginine) and supplementation of essential components (arginine, citrulline, and carbamyl glutamate).

even normal in patients with milder, late-onset disorders. Citrulline is low or normal in NAGS deficiency. In contrast, AS deficiency (citrullinemia) is marked by up to a 100-fold increase in plasma citrulline, whereas citrullinemia type II and argininosuccinic acidemia (AL deficiency) show an approximately 10-fold increase.

Argininosuccinate lyase deficiency is distinguished by the presence of argininosuccinic acid (ASA) in blood and urine. It should be noted that the ASA chromatographic peak co-elutes with leucine or isoleucine, resulting in an apparent increase in one of these amino acids. The anhydrides of ASA elute later (approximately at the times of homocystine and γ-aminobutyric acid), and this should permit the correct identification of ASA.

To distinguish CPS from OTC deficiency, urinary orotic acid is measured; although low in CPS and NAGS deficiency, it is markedly increased in OTC and ARG deficiency. Urinary orotate excretion also can be mildly increased in AS and AL deficiencies and in the transport defects. Plasma arginine is decreased in all primary urea cycle disorders, except in argininemia, in which it is approximately increased 10-fold to 20-fold.

In the transport defects, HHH syndrome is distinguished by increases in plasma ornithine (200–1000 μmol/L) and urinary homocitrulline levels while plasma lysine is low. Markers for lysinuric protein intolerance include massively increased urinary excretion of lysine and moderate increases in arginine and ornithine excretion associated with low plasma lysine, arginine, and ornithine. As noted, in citrullinemia type II, plasma and urinary citrulline levels are increased, although less markedly than in AS deficiency.

Whereas AS, AL, and ARG deficiencies and the transport defects can be distinguished by their unique plasma and/or urinary amino acid profiles, a definitive diagnosis of CPS, OTC, and NAGS deficiencies depends on enzyme determination on a liver biopsy or molecular diagnosis on DNA (in OTC and CPS deficiency). In OTC deficiency, 75% to 80% of patients have been found to have an identifiable mutation by DNA studies [38].

Treatment

Severe hyperammonemia (>250 μmol/L) is almost always associated with altered consciousness, and levels more than 500 μmol/L cause swelling of astrocytes with resultant cytotoxic brain edema [39]. This life-threatening condition is best treated with hemodialysis (or, when this is not available, with hemofiltration) to remove the offending toxin as rapidly as possible. Otherwise, vascular compromise and/or brain herniation will ensue. During a hyperammonemic crisis, catabolism becomes more pronounced, ammonia production increases markedly, and the condition may quickly become irreversible. In less se-

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