# LIVER DISEASE IN CHILDREN

## SECOND EDITION

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# 35

# DISORDERS OF THE UREA CYCLE

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The urea cycle serves to incorporate waste nitrogen atoms into urea (the major waste nitrogen product), and it is required for the *de novo* biosynthesis and degradation of arginine.

A defect in the urea cycle has two consequences. First, it leads to hyperammonemia with the accumulation of nitrogen atoms in a variety of molecules, the pattern of which varies according to the specific enzymatic defect. Secondly, the amino acid arginine becomes an essential amino acid in all conditions except arginase deficiency.

## BIOCHEMISTRY

The enzymes, substrates, and cofactors required for ureagenesis are shown in Fig. 35.1. The urea cycle consists of five enzymes, and normal functioning depends on the presence of a number of mitochondrial membrane transporters (1). Carbamyl phosphate synthetase (CPS1) is a mitochondrial matrix enzyme that catalyzes the biosynthesis of carbamyl phosphate (CP) from ammonium and bicarbonate. *N*-acetylglutamate synthetase (NAGS) catalyzes the formation of NAG from glutamate and acetyl-CoA. NAG is an essential cofactor for CPS1 and may be an important regulator of ureagenesis. Ornithine transcarbamylase (OTC) is a mitochondrial matrix enzyme that catalyzes the biosynthesis of citrulline from ornithine and CP. Citrulline is then exported to the cytosol, where it condenses with aspartate via argininosuccinic acid synthase (AS) to form argininosuccinate. This in turn is cleaved to arginine and fumarate by argininosuccinase. Arginine is subsequently hydrolyzed by arginase (ARG1) to urea and ornithine. Ornithine can then be again transcarbamylated to citrulline. In addition to the enzymes mentioned above, ORNT1, a mitochondrial membrane ornithine transporter, and Citrin, a mitochondrial membrane citrulline transporter, are required for normal functioning of the urea cycle (2,3).

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## NITROGEN SOURCES FOR UREA

Waste nitrogen disposal is a complex process requiring interorgan, intrahepatic, and intracellular compartmentation for the conversion of nitrogen not used for synthetic purposes to urea (Fig. 35.2). Free ammonium and aspartic acid are the sole sources of nitrogen for ureagenesis. However, the pathways from amino acid nitrogen to ammonium and aspartate are less clear. Within the liver, both alanine and glutamate are transaminated to aspartate and incorporated into urea (4,5). These reactions take place predominantly in the periportal hepatocytes, emphasizing the role of metabolic zonation within the liver (6). Perivenous hepatocytes predominantly contain enzymes that catalyze the amidation of glutamate to glutamine or, alternatively, the deamination of glutamate to ammonium and ketoglutarate. A number of other amino acids may provide ammonium for ureagenesis by deamination including histidine, tryptophan, threonine, and lysine.

Extrahepatic sources of nitrogen for ureagenesis are derived from the gastrointestinal tract, the kidney, and muscle. Within the intestines, glutamine is converted to ammonium, citrulline, and alanine, all of which are released into the portal circulation (7,8). Ammonium and alanine are taken up by the liver, whereas citrulline is not, but rather is transported to the kidney for conversion to arginine (9). The kidney provides a waste nitrogen atom by catalyzing the synthesis of arginine from citrulline and aspartate via renal AS activity (10–13). Renal glutaminase also may supply ammonium directly for the CPS reaction. Within the muscle, alanine production via transamination of pyruvate represents an important nitrogen precursor for ureagenesis. The muscle is also a major source of glutamine production (14–16).

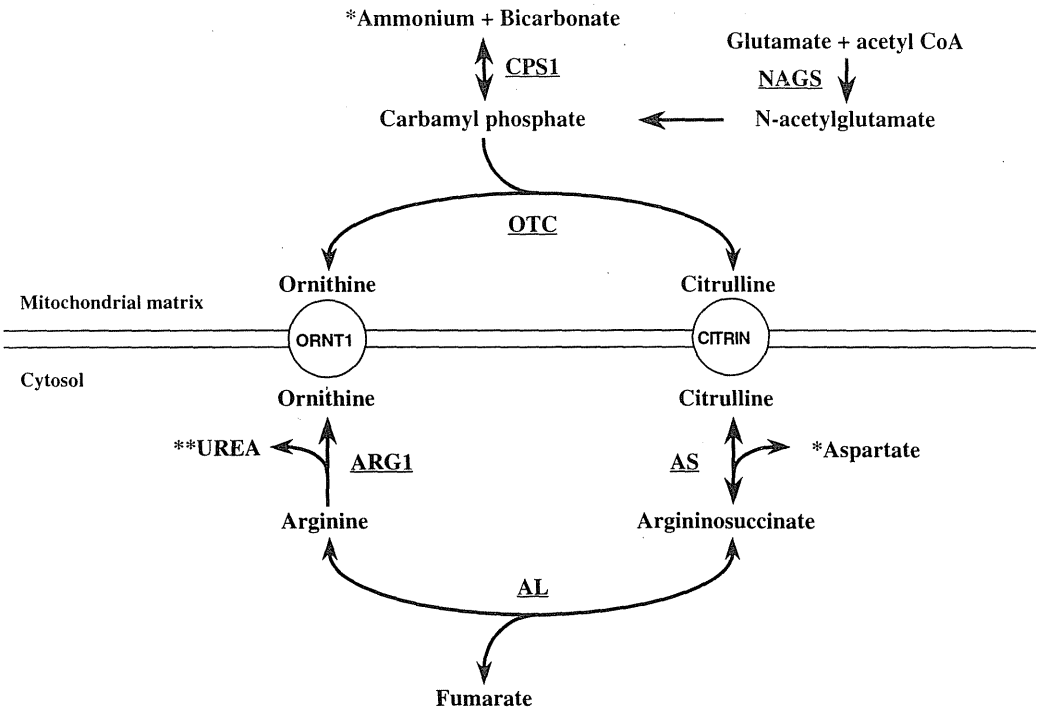


**FIGURE 35.1.** Substrate and products involved in the urea cycle. The asterisks denote the waste nitrogen atoms. *CPS1,* mitochondrial carbamyl phosphate synthetase; *NAGS, N*-acetylglutamate synthetase; *OTC,* ornithine transcarbamylase; *CITRIN,* presumed citrulline mitochondrial carrier protein; *AS,* argininosuccinic acid synthetase; *AL,* argininosuccinic acid lyase; *ARG1,* hepatic arginase; *ORNT1,* mitochondrial ornithine transporter.

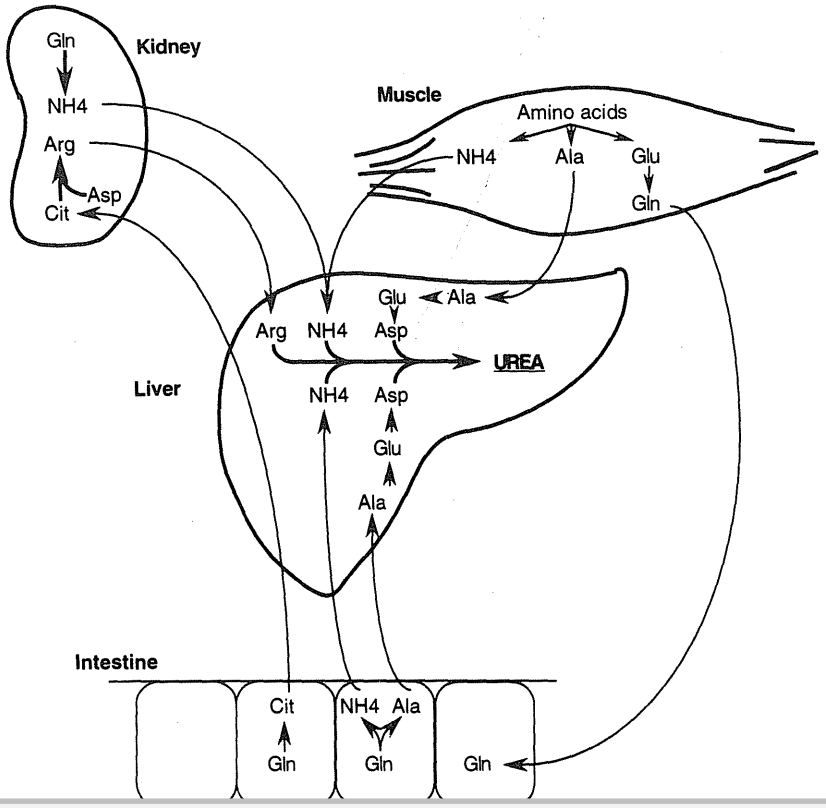


**FIGURE 35.2.** Pathways of waste nitrogen synthesis from amino acids. Muscle is the major source of nitrogen for ureagenesis through its production of alanine and glutamine. $NH_4$, ammonium; *Gln,* glutamine; *Ala,* alanine; *Asp,* aspartic acid; *Cit,* citrulline; *Glu,*

## GENETICS

### Carbamyl Phosphate Synthtase

The gene encoding the CPS1 gene in humans has been partially characterized. A full-length complementary DNA (cDNA) of 5,215 bp has been cloned and encodes an open reading frame predicted to encode a protein of 1,500 amino acids, including a mitochondrial leader sequence (17,18). Although the genomic structure has not been reported, the gene has been localized to chromosome 2q35 (19,20). Several mutations in affected individuals have been described (17,21).

### Ornithine Transcarbamylase

The gene encoding ornithine transcarbamylase activity has been characterized in humans (22,23). It is located at chromosome Xp21.1 (24). It spans 85 kb, contains 10 exons, and generates a 1,600-bp messenger RNA (mRNA). The predicted protein has 354 amino acids, the first 32 of which are a mitochondrial leader sequence. The protein is active as a homotrimer. The OTC gene is subject to X chromosome inactivation in females, contributing to the wide variation and severity seen in carrier females. Molecular analysis of affected individuals has demonstrated a wide variety of mutations (25–28). Direct mutation analysis is accomplished by Southern blot analysis (~10% of patients have deletions) or polymerase chain reaction amplification of the 10 exons and their intron boundaries followed by sequencing. Linkage analysis can be used to exclude or include the inheritance of the mutant gene where direct mutation analysis is unsuccessful and where the pedigree structure is suitable.

### Arginosuccinic Acid Synthase

The gene encoding arginosuccinic acid synthase activity maps to chromosome 9q34. It covers 63 kb, has 16 exons, and generates an mRNA of 1,600 bp (29,30). The predicted protein has 412 amino acids and is active as the homotetrameric form. There are a large number of processed pseudogenes (i.e., homologous, intronless, and inactive sequences) mapped to several loci, including chromosomes 2, 3, 4, 5, 6, 7, 9, 11, 12, X, and Y (31). These probably arose by reverse transcription of mRNA followed by integration of copied sequences into genomic DNA. A large number of mutations have been described in the structural gene, the majority of which produce mRNA and no immunoreactive protein, suggesting most mutations in this disorder produce unstable protein products (32–34).

### Arginosuccinase

The gene encoding arginosuccinase maps to chromosome 7cen-q11.2. It is 35 kb long, divided to 16 exons, and encodes a predicted protein of 463 amino acids (35,36). The active enzyme is the homotetrameric form. Disease causing

### Arginase

There are two forms of arginase activity. ARG1 is expressed in the liver and red cells and is deficient in arginemia. The gene encoding ARG1 maps to chromosome 6q23, is 11.5 kb in size, and contains 8 exons (39–42). The mRNA is 1.4 kb and encodes a predicted protein of 312 amino acids. Molecular studies have shown that arginase deficiency is produced by a variety of mutations (43). A second arginase (ARG2) is expressed in a wide variety of tissues, including the kidney, brain, and gastrointestinal tract. ARG2 maps to chromosome 14q24.1-24.3 and encodes a mitochondrial protein of 355 amino acids (44,45). ARG2 appears to play a role in nitric oxide (NO) biosynthesis, as well as in ornithine, proline, and polyamine metabolism (46). To date, disorders due to deficiency of this enzyme have not been described.

### *N*-Acetylglutamate Synthetase

To date, the gene encoding this enzyme has not been identified or characterized.

## CLINICAL FEATURES

The clinical presentation of patients with CPS, OTC, AS, and *argininosuccinase* deficiencies is very similar in that they all present with hyperammonemia. However, considerable variability occurs within and among each of these diseases. This variability relates to the different mutations found in each of the genes encoding these enzymes, to the pattern of inheritance (OTC is X linked), and finally to the relative toxicity of accumulated substrates or deficient products (Table 35.1). It is convenient to divide the presentation of these disorders into neonatal presentation and late presentation.

## NEONATAL ONSET

In the neonatal period the presentation of these disorders is stereotypic and characterized by encephalopathy, respiratory alkalosis, and hyperammonemia. Infants are usually the product of a full-term normal pregnancy with no known prenatal or perinatal risk factors. Labor and delivery are normal, and the patient appears to be normal for at least 24 hours. Between 24 and 72 hours, the patient shows decreased feeding, which usually proceeds to vomiting, increasing lethargy, hypothermia, and hyperventilation. The latter often leads to a suspicion of pulmonary disease. However, chest X-rays are often normal. Routine laboratory data generally show a respiratory alkalosis. Electrolytes are uninformative, and their very normality serves to exclude other causes of hyperammonemia. Serum urea nitrogen may be very helpful because it is often either absent or as low as 1 mg/dL. Without treatment, the infant becomes comatose, requiring mechanical ventilation. Computed tomography scan of the brain often

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