85: Urea Cycle Enzymes

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Abstract

- The urea cycle, which consists of a series of five biochemical reactions, has two roles. In order to prevent the accumulation of toxic nitrogenous compounds, the urea cycle incorporates nitrogen not used for net biosynthetic purposes into urea, which serves as the waste nitrogen product in mammals. The urea cycle also contains several of the biochemical reactions required for the *de novo* synthesis of arginine.

- Urea cycle disorders are characterized by the triad of hyperammonemia, encephalopathy, and respiratory alkalosis (the earliest objective evidence of encephalopathy). Five well-documented diseases (each with considerable genetic and phenotypic variability) have been described, each representing a defect in the biosynthesis of one of the normally expressed enzymes of the urea cycle. Four of these five diseases—deficiencies of carbamyl phosphate synthetase (CPS) (OMIM 237300), ornithine transcarbamylase (OTC) (OMIM 311250), argininosuccinic acid synthetase (AS) (OMIM 215700), and argininosuccinate lyase (AL) (OMIM 207900)—are characterized by signs and symptoms induced by the accumulation of precursors of urea, principally ammonium and glutamine. The most dramatic clinical presentation of these four diseases occurs in full-term infants with no obstetric risk factors who appear normal for 24 to 48 hours and then exhibit progressive lethargy, hypothermia, and apnea all related to very high plasma ammonium levels.

- Milder forms of these diseases occur; they may present with signs of encephalopathy at any age from infancy to adulthood. The most common of these late-onset diseases occurs in female carriers of a mutation at the OTC locus of one of their X chromosomes. The late-onset cases present with respiratory alkalosis and episodic mental status changes progressing, if not emergently treated, to cerebral edema, brainstem compression, and death. The acute encephalopathy is characterized by brain edema and swollen astrocytes, the cause of which is attributed to intraglial accumulation of glutamine resulting in osmotic shifts of water into the cell. Axons, dendrites, synapses, and oligodendroglia are normal. A fifth disease, arginase deficiency (OMIM 107830), is characterized by a clinical picture consisting of progressive spastic quadriplegia and mental retardation; symptomatic hyperammonemia, which can be life-threatening, occurs neither as severely or as commonly as in the other four diseases. Apart from OTC deficiency, which is inherited as an X-linked disorder, the other four diseases are inherited as autosomal-recessive traits. Carrier status of OTC mutations in women is determined by pedigree analysis and molecular methods. For fetuses at risk, antenatal diagnosis is available by a number of methods, particular to each disease, including enzyme analysis of fibroblasts cultured from aminocytes, as well as molecular (DNA) methods.

- Molecular genetic analysis of the urea cycle enzymes has addressed their structure and expression and has permitted DNA-based diagnosis of deficiency, in many cases by direct analysis of mutations. Using the cloned complementary DNA as probes, expression in liver of RNA for all the enzymes has been observed to be increased severalfold by starvation. RNA coding for the 160-kDa subunit of the CPS I homodimer is detected almost exclusively in the liver and translates a precursor protein representing the product of fusion of two ancestral prokaryotic subunits, joined with an N-terminal mitochondrial targeting sequence. Few mutations have been identified in this large coding sequence in affected pedigrees so far, but a restriction fragment-length polymorphism (RFLP) in the human CPS locus is useful in prenatal diagnosis of deficiency. OTC is also expressed principally in the liver, and its subunit is also translated as a precursor, comprising an N-terminal mitochondrial targeting sequence that functions via an α-helical structure and net positive charge, joined with a mature portion that resembles prokaryotic transcarbamylases. Mitochondrial import requires the action of a variety of components in the cytosol to maintain an import-competent conformation, in the outer mitochondrial membrane for recognition of the precursor, in both outer and inner membrane for protein translocation, and in the matrix for proteolytic processing and folding to the active conformation. Gene deletions have been observed in approximately 15 percent of affected males. More than 100 different single base substitutions have been identified, producing amino acid substitution in many cases, involving either of the two domains of the OTC subunit. In other cases, splicing is affected, either destabilizing the messenger RNA (mRNA) or frameshifting the subunit. Prenatal diagnosis can be offered to most women who are established as heterozygous carriers by pedigree analysis, allopurinol testing, or DNA analysis, using direct DNA analysis of fetal DNA where the mutation is known, or using RFLPs. Recombinant OTC retroviruses have transduced cultured hepatocytes of mice with inherited OTC deficiency, and recombinant OTC adenoviruses have been injected into newborn mutant animals with evidence of rescue of deficiency. These gene transfer experiments aim toward achieving stable long-term OTC expression. Argininosuccinate synthetase (AS) is programmed from a single locus, but a large number of homologous processed pseudogenes are localized throughout the genome. Expression of AS mRNA has been studied in cultured cells, where the level of mRNA is greatly increased in response to canavanine treatment and repressed by the presence of arginine. The AS coding sequence has been successfully transferred into both cultured cells and mouse bone marrow cells as an approach to AS deficiency of supplying enzyme activity outside the liver. Analysis of AS mutations reveals considerable heterogeneity in the position of mutation, with most composed of codon substitutions that produce unstable protein products. Where direct mutation analysis is not possible, a number of polymorphisms at the AS locus enable linkage study of affected pedigrees. Human AL is similar to avian δ-crystallins, in which a virtually identical protein is apparently used as a structural component. Analysis of AL mutants also reveals considerable heterogeneity. Arginase in human liver and red cells is a cytosolic enzyme distinct from a second mitochondrial-localized enzyme. Deficient patients have shown heterogeneity in the site of mutation. Two RFLPs at the locus have been identified.

- Treatment requires restriction of dietary protein intake and activation of other pathways of waste nitrogen synthesis and excretion. For patients deficient in CPS, OTC, and AS, treatment with sodium phenylbutyrate activates the synthesis of phenylacetylglutamine, which has a dual effect. By providing a new vehicle for waste nitrogen excretion, which suppresses residual urea synthesis in the late-onset group, a reserve urea synthetic capacity is

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generated that may support nitrogen homeostasis when required. In patients deficient in AS and argininosuccinase, supplementation of the diet with arginine promotes the synthesis of citrulline in the former and argininosuccinate in the latter, both of which serve as waste nitrogen products.

Outcome of treatment of neonatal-onset disease has been disappointing. Even those neonates treated prospectively prior to the onset of hyperammonemia are at high risk for neurologic deficits. Parents should be realistically counseled as to the likely outcome if the infant is rescued. Treatment of late-onset disease appears to preserve the neurologic status found at the start of therapy.

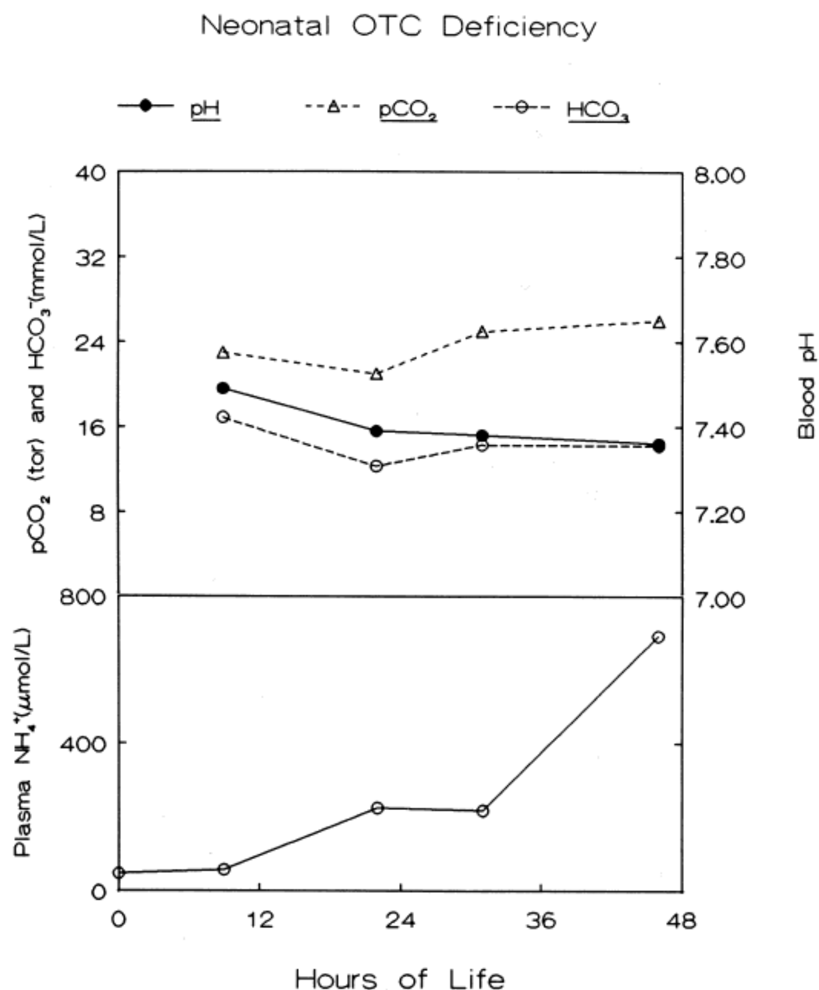
Introduction

The urea cycle serves two purposes: (1) it contains, in part, the biochemical reactions required for the *de novo* biosynthesis and degradation of arginine, and (2) it incorporates nitrogen atoms not retained for net biosynthetic purposes into urea, which serves as a waste nitrogen product. Campbell's review[1] of the comparative biochemistry of nitrogen metabolism describes other waste nitrogen products (ammonium and purines) found in other animals.

It also has been proposed[2] that the urea cycle plays an important role in the disposal of bicarbonate and hence on pH homeostasis. A number of arguments against this view have been offered.[3] Perhaps the strongest case against this function of the urea cycle can be found in patients with complete, or nearly so, defects in one of the enzymes of the urea cycle; apart from respiratory alkalosis related to the stimulatory effect of ammonium on respiration, these patients have little evidence of a disorder of pH homeostasis. For example, 28 ornithine transcarbamylase (OTC)-deficient neonates who presented with hyperammonemia had a respiratory alkalosis as manifested by the following blood gases: pH 7.5; pCO_2, 24 torr; HCO_3, 19.3 mM.[4] As shown in Fig. 85-1, a respiratory alkalosis develops very early in the course of untreated hyperammonemia in an OTC-deficient neonate. Furthermore, a decrease in ureagenesis caused by partial hepatectomy did not influence acid–base balance.[5] These data suggest that hepatic urea synthesis plays little or no role in maintaining acid–base balance, as has been proposed.[6]

Fig. 85-1

Course of blood pH, PCO_2, plasma bicarbonate, and ammonium concentration of untreated OTC-deficient neonate. Early onset and persistence of hyperventilation and respiratory alkalosis are apparent.



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A defect in the ureagenic pathway has two consequences: arginine becomes an essential amino acid[7] (except in arginase deficiency, where the enzyme defect results in a failure of degradation of arginine) and nitrogen atoms accumulate in a variety of molecules, the pattern of which varies according to the specific enzymatic defect, although plasma levels of ammonium and glutamine are increased in all urea cycle disorders not under metabolic control.

Waste Nitrogen Disposal

The biochemical pathway of urea synthesis is described in Fig. 85-2 and in Table 85-1. Waste nitrogen disposal is far more complex, requiring interorgan, intrahepatic, and cellular compartmentation relationships in the conversion to urea of nitrogen not used for net biosynthetic purposes. Although it has been known for decades that ammonium and aspartate are the sources of nitrogen for ureagenesis, the pathways from amino acid nitrogen to ammonium and aspartate have been less clear.

Fig. 85-2

Substrates, products, and cofactors required for ureagenesis. The asterisks denote waste nitrogen atoms. AS = argininosuccinic acid synthetase; AL = argininosuccinase; CPS = carbamyl phosphate synthetase; NAGS = *N*-acetylglutamate synthetase; OTC = ornithine transcarbamylase.

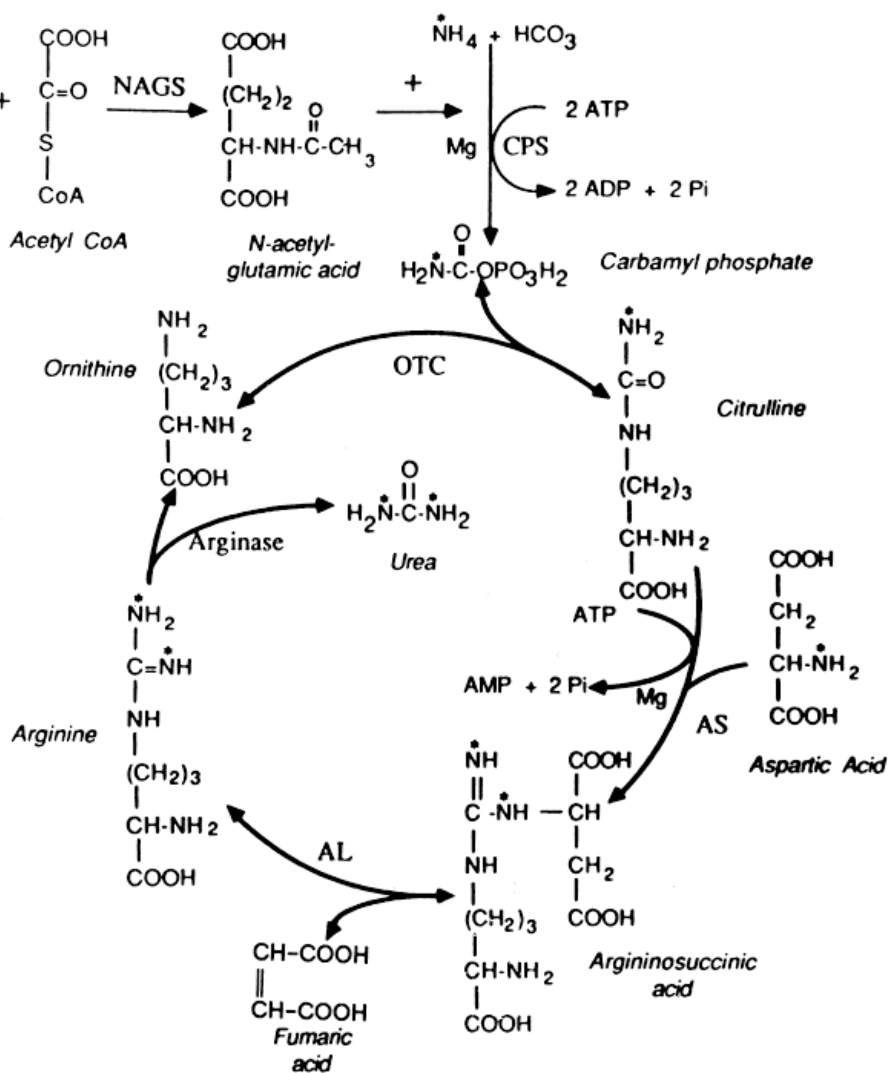

Table 85-1

Table 85-1: The Enzymes of the Urea Cycle*

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Table 85-1 The Enzymes of the Urea Cycle*

Enzyme	Compartment	Activity	M_r	pH opt	K_m, mM	Equilibrium Constant	Tissue Distribution
N-acetyl glutamate synthetase, EC 2.3.11	Mitochondrial matrix	0.30–1.49	200,000	8.5	Glu, 3.0 Ac CoA 0.7 Arg, 0.01	Irreversible	Liver, intestine, kidney (trace), spleen
Carbamyl phosphate Synthetase EC 6.3.4.16	Mitochondrial matrix	279†	310,000 dimer	6.8–7.6	NH_4, 0.8 HCO_3, 6.7 Mg ATP, 1.1 NAG, 0.1	Irreversible	Liver, intestine, kidney (trace)
Ornithine transcarbamylase, EC 2.1.3.3	Mitochondrial matrix	6600	108,000 trimer	7.7	CP, 0.16 Orn, 0.40	$\frac{(Cit)(p)}{(Orn)(CP)} = 10^5$	Liver, intestine, kidney (trace)
Argininosuccinic acid synthetase, EC 6.3.4.5	Cytosol	90	185,000 tetramer	8.7	Asp, .03 Cit, .03	$\frac{(ASA)(AMP)(Mg\,PP)(2H)}{(Cit)(Asp)(Mg\,ATP)} = 0.89$‡	Liver, kidney, fibroblasts, brain (trace)
Argininosuccinase, EC4.3.2.1	Cytosol	220	173,200 tetramer	7.5	Asp, 0.017 Cit, 0.016 ATP, 0.041	$\frac{(Arg)(fumarate)}{(ASA)} = 11.4 \times 10^{-3}$	Liver, kidney, brain, fibroblasts
Arginase, EC 3.5.3.1	Cytosol	86,600	107,000 tetramer	9.5	Arg, 10.5	Irreversible	Liver, erythrocytes, kidney, lens, brain (trace)

... wet weight ‡AT pH = 7.0

Intrahepatic Sources of Nitrogen for Ureagenesis

It was proposed on theoretical grounds[15] that intramitochondrial ammonium for the carbamyl phosphate synthetase (CPS) reaction was derived from the oxidative deamination of glutamate by glutamate dehydrogenase (Fig. 85-3). Although this interpretation is commonly accepted, attempts to verify this hypothesis in respiring mitochondria have repeatedly shown that the vast portion of glutamate is not deaminated but rather transaminated,[16, 17] suggesting that glutamate may not be the principal precursor for citrulline biosynthesis. The virtual absence of experimental evidence supporting the hypothesis that oxidative deamination of glutamate via glutamate dehydrogenase is a source of ammonium for the biosynthesis of citrulline and urea has led some researchers to conclude that, "studies of glutamate dehydrogenase in liver have failed to yield any clear consensus of the role of this enzyme"[18] or "it is still not possible to define the role of this enzyme in animal tissues."[19] Krebs also has reviewed this subject.[20]

Fig. 85-3

Pathways of waste nitrogen synthesis from amino acids. Muscle, by virtue of its production of alanine and glutamine, is the major source of nitrogen destined for incorporation into urea. The role of the intestines, kidney, and liver are outlined as described in the text.

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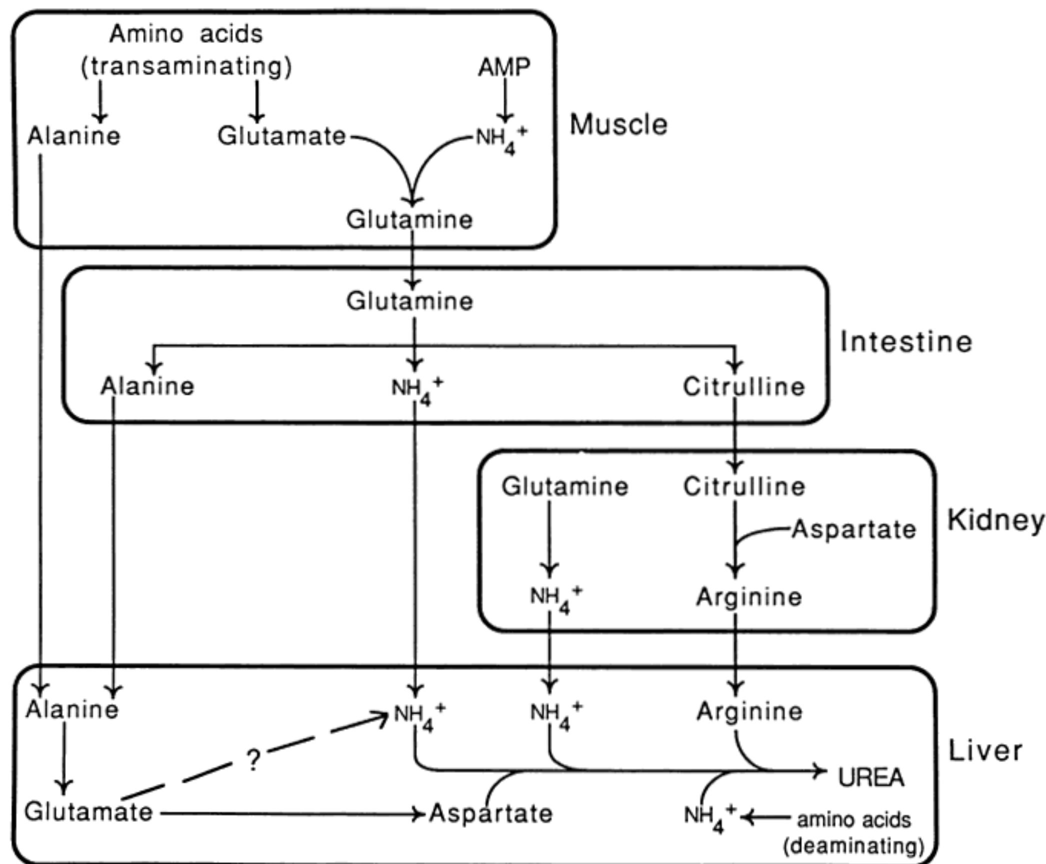

Jungermann[21] reviewed the role of metabolic zonation in the liver as it pertains to nitrogen homeostasis. It is proposed that periportal hepatocytes predominantly contain enzymes that catalyze transamination reactions and ureagenesis, whereas perivenous hepatocytes predominantly contain enzymes that catalyze the amidation or deamination of glutamate to glutamine or ammonium and ketoglutarate, respectively.

Cooper et al.[22] suggested that no more than 20 percent (and possible much less) of the α-amino moiety of liver glutamate is deaminated *in vivo*, but rather it is predominantly transaminated to aspartate and incorporated into urea. These studies were done in a series of three experiments in which each of ^{13}N-labeled glutamate, alanine, and glutamine (amide) was injected into the portal vein, after which the liver was freeze-clamped at intervals of 5 to 60 s and the distribution of the label described. Aspartate and urea were promptly labeled after ^{13}N-alanine and ^{13}N-glutamate were injected but not after ^{13}N-glutamine (amide). Ammonium and citrulline were not labeled, suggesting that little glutamate was deaminated. The absence of incorporation of glutamine nitrogen into the urea cycle in these *in vivo* experiments does not support the glutamine channeling hypothesis of Meijer.[23] The rapidity of nitrogen exchange among the linked transaminases in these and other studies[22] was striking—within 10 s of the injection of the labeled amino acids or ammonium. In previous studies[24] these investigators showed that intraportal vein injection of ^{13}N-ammonium resulted in labeling of citrulline.

From these studies it may be concluded that although glutamate may be deaminated, it may not be a major source of ammonium for the CPS reaction. As described below, extrahepatic glutamine metabolism provides the single most important source of ammonium for the CPS reaction. However, within the liver there are a number of other amino acids that are deaminated and may provide ammonium for ureagenesis (e.g., histidine, tryptophan, threonine, and lysine).

Extrahepatic Sources of Nitrogen for Ureagenesis

Intestine.

In a series of studies of the metabolism of the perfused rat intestine, Windmueller and Spaeth[25], [26] showed not only that glutamine carbon atoms were an important respiratory fuel but also that glutamine nitrogen was converted to the urea precursors ammonium, citrulline, and alanine, all of which were released into the portal circulation (Fig. 85-3). Whereas alanine and ammonium are taken up by the liver, citrulline apparently is not but rather is transported to the kidney, where it is converted to arginine.[27]

Kidney.

Rat kidney uptake of citrulline is approximately equivalent to its rate of intestinal release.[27], [28] This observation is compatible with the report that

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