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UREA CYCLE ENZYMES AND ITS DISORDERS

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Üre Döngüsü Enzimleri Ve Bozuklukları

ABSTRACT

The major pathway of nitrogen excretion in humans is in the form of urea synthesized in the liver. Then hepatic urea released into the blood and cleared by the kidneys.

Urea synthesis in the liver involves five enzymes.The diagnosis of metabolic disorders associated with deficiency of each of these enzymes have been reported in this presentation. Since the most important function of the urea cycle is to convert ammonia to the nontoxic compound urea, all disorders of urea synthesis leads to accumulation of ammonia. Thus, the clinical presentation of all urea cycle disorders are definitely related to acute or chronic ammonia intoxication.

Key Words : Urea Cycle, Enzymes, Disorders.

ÖZET

İnsanlarda azot atılımının başlıca yolu; karaciğerde üre formunda sentezlenmesidir. Daha sonra hepatik üre, kana salınır ve böbrekler vasıtasıyla temizlenir.

Karaciğerde üre sentezi 5 enzim gerektirir. Bu enzimlerin herbirinin yetersizlikleri ile ilgili metabolik bozukluklar ve tanıları, bu sunumda bildirilmiştir. Üre döngüsünün en önemli fonksiyonu amonyağı toksik olmayan üreye dönüştürmek olduğundan, üre senteziyle ilgili bütün bozukluklar kan ve diğer dokularda amonyağın birikimine yol açmaktadır. Bu nedenle, üre döngüsünün tüm bozukluklarının klinik görüntüsü, akut veya kronik amonyak intoksikasyonu ile kesin ilişkilidir.

Anahtar Kelimeler : Üre Döngüsü, Enzimler, Bozukluklar.

INTRODUCTION

Certain aquatic animals excrete ammonia directly into the environment as the major nitrogenous waste product. Birds, reptiles and insects channel waste nitrogen into the formation of relatively insoluble uric acid, an oxidized purine, as the predominant end product of nitrogen metabolism and are thus uricotelic (1). Uric acid is quite insoluble, it precipitates and can be excreted without a large water loss and without building up osmotic pressure. This is particulary important during each animal's lifetime that is spent in the egg. In man and other terrestrial vertebrates, the major excretory product of nitrogen metabolism is urea, and thus they are classified as ureotelic animals. This compound is highly soluble, being electrically neutral, does not affect the pH when it accumulates, as does ammonia (1,2).

Ammonia is produced by most cells and arises from catabolism of amino acids (see fig.1). In addition, a considerable quantity is produced by intestinal bacteria, from dietary protein and from urea present in fluids secreted into the gastrointestinal tract. This ammonia is absorbed into the portal venous blood and promptly removed by the liver. The synthesis of urea from ammonia in the liver is summarized in Figure 2. The pathway, which is cyclic, was discovered by Hans Krebs and Kurt Henseleit in 1932, five years before the other cycle for which Kreb's name is famous. Krebs and Henseleit were investigating the pathway of urea synthesis by adding possible precursors to liver slices and then measuring the amount of urea produced. When arginine was added, urea was produced in 30-fold molar excess over the amount of arginine administered.

Similar results were seen if either of two structurally related amino acids, ornithine or citrulline, was substituted for arginine (2).

The first step in the synthesis of urea is the formation of carbamoyl phosphate. Condensation of one molecule each of ammonia, carbon dioxide and phosphate form carbamoyl phosphate, the synthesis of which catalyzed by intramitochondrial carbamoyl phosphate synthetase (CPS I) (EC.6.3.4.16). In addition, this condensation requires Mg^{+2} and N-acetyl glutamate as allosteric activator. Carbamoyl-phosphate is also a precursor of pyrimidines and is synthesized in the cytosol by a distinct enzyme, carbamoyl-phosphate synthetase (CPS II) (3). The next step is the formation of citrulline through transfer of a carbamoyl moiety from carbamoyl-phosphate to ornithine. This reaction is catalyzed by another intramitochondrial enzyme, ornithine carbamoyl transferase (EC.2.1.3.3). The enzyme is expressed only in the liver and intestinal mucosa, where it is localized to the mitochondrial matrix. The enzyme is a trimer of identical subunits that have a molecular weight of 36000-39000. No allosteric modifiers have been identified. The rest of the steps in urea synthesis take place in the cytosol. Citrulline crosses the mitochondrial membrane to the cytosol, where it is linked with aspartate to form argininosuccinate synthetase (EC.6.3.4.5) in addition to Mg^{+2} and ATP. Cleavage of argininosuccinate to arginine and fumarate is catalyzed by argininosuccinate lyase (an other name : argininosuccinase) (EC. 4.3.2.1). Hydrolysis of arginine by arginase (EC.3.5.3.1) to urea and ornithine is the final step in the urea synthesis. The enzyme arginase is responsible for the cyclic nature of the urea biosynthetic pathway. Arginase activity is widespread in distribution in the animal kingdom; it is found not only in ureotelic but also in uricotelic animals. Arginase might provide a source of ornithine for polyamine synthesis, impliying it has a role in nonhepatic tissues. Since arginine is required for protein synthesis, the activity of arginase in cells must be regulated to assure availability of arginine. The enzyme from human liver has a molecular weight of 115000-120000. Estimates of the subunit size vary from 31000 to 35000, leading to some uncertainty about the oligomeric structure of the enzyme and fully activated human liver arginase contained 1.1 +/- 0.1 Mn^{+2}/subunit (3,4).

Ornithine enters the final step in urea synthesis. Ornithine enters the mitocondria and is recycled in the citrulline synthesis.

The Regulation of Urea Synthesis :

The regulation of urea synthesis in the liver is complex and several mechanisms are involved. It is well known that over several days the total activity of the enzymes involved varies with the protein intake (5). Corticosteroids and glucagon induce some of the urea cycle enzymes (6) and rapid changes in enzyme activity are mediated by N-acetyl glutamate, which is synthesized by N-acetyl glutamate synthetase (6,7). This enzyme is activated by arginine (7).

High doses of cortisone increased urea excretion and levels of urea cycle enzymes in postnatal rats. Hydrocortisone administration increased activity of CPS I, but not ornithine carbamoyl transferase in fetal rats. Hepatic arginase was induced by glucocorticoids. In contrast, testosterone stimulated arginase, but had no affect on hepatic arginase. Glucocorticoids act synergistically to increase activity of urea cycle enzymes and insulin suppresses these effects. The ratio of insulin to glucagon in vitro may participate in regulating the expression of urea cycle enzymes. Physiological doses of glucagon elevated the activity of all five urea cycle enzymes (8,9).

In our studies, it was observed that a 12-minute-incubation with adrenaline and progesterone decreased the arginase activities. At the end of this period tiroxine and insulin did not affect arginase activities. Insulin could decrease arginase activity by 10.3 % only at the 24'th hour (10). Arginase has been suggested to play an important role in cellular growth and development, particulary important to the fetus, by supplying L-ornithine for the synthesis of polyamines (11).

Many reports have been published on extrahepatic urea synthesis. Cohen et al. (12) noted that arginine is converted to urea in rat brain. Low activities of ornithine carbamoyl transferase and arginino succinase have been found in many tissues including skeletal muscle, heart, pancreas, testis and red blood cells. Normal fibroblasts grown in tissue culture convert citrulline to urea indicating that this tissue contains many enzymes in urea synthesis (13). In addition, kidney tissue appears to contain at least three of the urea synthesis enzymes (3). However, the very low enzymatic activities relative to those in hepatic cells suggest that the physiological contribution of extrahepatic urea synthesis is very small (3,13).

Plasma ammonia concentration :

Normally, the concentration of ammonia in blood remains low (less than 50 mM / l), as what is produced from catabolism of amino acids is rapidly metabolized by the liver, skeletal muscle and brain. Neonates may

have higher levels of plasma ammonia. Preterm and small-for-date infants appear to have even higher values (14). Several methods to estimate plasma ammonia have been described. Methods using strong alkali produced inaccurate results as some nitrogeneous compounds decomposed, liberating ammonia under the conditions used in the assay (15).

Current methods use ammonia selective electrode (16) ion exchange chromatography (17) and enzymatic reactions (18).

Blood for ammonia estimation needs to be collected correctly. Red cell ammonia is higher than that of plasma. hence, hemolysis invalidates the results. Plasma should be separated immediately as deamidization of amino acids artefactually increases the levels.

Clinical Features of Urea Cycle Defects :

Since 1985, many patients with inherited defects in urea synthesis have been described(19). The clinical presentation in these conditions vary in their severity and may present at any time during childhood. Quite often there is a family history of death of a neonate due to unknown cause. Each disorder may have a rapidly fatal form, or a milder form with a more chronic presentation. Hyperammonemia is thought to be the main factor causing encephalopathy, although depletion of 2-oxoglutarate due to formation of glutamine also appears to play a role (19,20). In the neonatal period, the presentation is usually acute within the first week. Poor feeding, vomiting, lethargy, irritability, tachypnea and / or convusions are the common presenting features. As encephalopathy progresses, loss of reflexes and apnea set in, followed by death. Pulmonary, gastrointestinal and intracranial hemorrhage may occur as terminal events. Some children do not present acutely, but present with progressive mental retardation or episodic vomiting, lethargy, and irritability, usually precipitated by high protein meals. Some may present with convulsions or even periodic attacks of coma. In some children these attacks are precipitated by infection, when there is a tendency for an increase in endogenous protein catabolism. In the acute cases there are no specific clinical features that differentiate one urea cycle defect from another. Even in chronic cases it is difficult to differentiate between them clinically. However, about 50% of patients with argininosuccinate lyase deficiency have characteristically brittle short hair (trichorrhexis nodosa), which has a typical microscopic appearance (21-23).

Diagnosis of Urea Cycle Disorders :

Although the primary defect in urea cycle

Tablo 1 : Causes of Hyperammonemia.

Inherited disorders of urea cycle	
organic acidemias	
Faty acid oxidation defects	
Transient neonatal hyperammonemia	
Other inherited disorders	
	-Lysinuric protein intolerance
	-Hyperornithinemia , hyperammonemia and homocitrullinuria
Hepatic disease	**-Advanced hepatic disease**
	-Reye's sendrome
Miscellaneous	**-Sodium valproate therapy**
	-Urinary tract infection
	-GI hemorrhage
	-Shock
	-IV feeding
	-Leukemia

disorders is an enzyme deficiency in urea formation, plasma urea is usually normal or only slightly reduced in most neonates. Hyperammonemia is a common feature in all urea cycle disorders, although this may be intermittent in subacute or late onset presentations. A block in the urea cycle leads to accumulation of ammonia causing toxic sings and symptomps. However, there are several other causes of hyperammonemia (see table 1), in addition to urea cycle defects (2, 24).

Hyperammonemia was observed in some of the organic acid disorders can confuse the picture since it is difficult to distinguish among them clinically (24,25). Plasma ammonia levels are not helpful in discriminating a urea cycle disorder from an organic acidemia . Although urea cycle disorders usually have plasma ammonia levels greater than 300 mmol/l, concentrations may be as low as 200 mmol/l depending upon the protein intake. Metabolic acidosis, ketonuria, and neutropenia point to an organic acidemia while low plasma urea and respiratory alkalosis favor the diagnosis of a urea cycle disorder (26) .

Ammonia itself is a respiratory stimulant, and therefore, respiratory alkalosis is a well known manifestation in a urea cycle disorder. In the case of an organic acid disorder, the respiratory alkalosis may be compensated by the metabolic acidosis. The plasma amino acid profile usually helps in the diagnosis. Elevated levels of glutamine are seen and abnormal amounts of citruline, argininosuccinic acid, or arginine may be observed, depending on the site of the block (see table 2). A presumptive diagnosis of carbamoyl phosphate synthetase deficiency and ornithine carbamoyl transferase deficiency is made if hyperammonemia and elevated glutamine levels are present in the absence of spesific amino acid abnormalities. In ornithine carbamoyl transferase deficiency, large amounts of orotic acid are excreted in the urine. Normal amounts of this metabolite are seen in carbamoyl-phosphate synthetase deficiency, while in other urea cycle disorders this is only slightly elevated (27,28). Confirmation of urea cycle disorders should be made by measurement of enzyme activity in suitable tissues (see table 3).

Tablo 2 : Biochemical Findings in Urea Cycle Disorders.

	AMMONIA	GLUTAMINE	AMINO ACIDS	URINE
CARB AMOYL PHOSPHATE SYNTHESTASE DEFICIENCY	↑	↑	⇒Citrulline	Normal orotic acid
ORNITHINE CARBAMOYL TRANSFERASE DEFICIENCY	↑	↑	⇒ Citrulline	↑↑ Orotic acid
ARGİNİNOSUCCINATE SYNTHETASE DEFICIENCY	↑	↑	↑↑ Citrulline	↑ Citrulline ↑ Orotic acid
ARGININOSUCCINATE LYASE DEFICIENCY	↑	↑	↑ Citrulline	↑↑ Argininosuccinic acid
ARGINASE DEFICIENCY	↑	↑	↑ Arginine	↑ Orotic acid ↑ Arginino succinic acid ↑ Dibasic amino acids

All urea cycle disorders, except ornithine carbamoyl transferase deficiency, which is X-linked, are inherited as autosomal recessive disorders. Heterozygotes have reduced levels of appropriate enzyme activities; however, the results have to be interpreted with caution as enzyme activitiy may depend on dietary protein intake. An intravenous alanine load test may help in detection of ornithine carbamoyl transferase deficiency carrier's (29,30).

Tablo 3 : Tissues in which urea cycle enzyme defects are expressed.

DISORDER	TISSUE
Carbamoyl phosphate synthetase deficiency	Liver
	Jejunal mucosa
Ornithine carbamoyl transferase deficiency	Liver
	Jejunal mucosa
Argininosuccinate synthetase deficiency	Liver
	Skin fibroblasts
Argininosuccinate lyase deficiency	Liver
	Red blood cells
	Skin fibroblasts
Arginase deficiency	Liver
	Red blood cells

Prenatal diagnosis of urea cycle enzyme defects is possible but not always straightforward. Carbamoly-phosphate synthetase deficiency and ornithine carbamoyl transferase deficiency can only be diagnosed by fetal liver biopsy. The other disorders can be diagnosed by amniotic fluid cell culture. Estimation of specific amino acid concentrations, e.g., citrulline, argininosuccinic acid in amniotic fluid may prove helpful in the appropriate enzyme defects. In case of arginase deficiency, fetal erythrocyte arginase level would provide helpful diagnostic information. Gene probes for ornithine carbamoyl transferase are now available and may be helpful in early prenatal diagnosis of this condition (31,32).

Argininosuccinic acid aciduria is probably the most frequent genetic disorder of urea cycle disorder.A neonatal form of the disease exhibits hiperammonemia and neurological symtoms. Patients with milder forms of the disease may later show episodes of hyperammonemia or chronic mental retardation and poor growth without acute episodes of metabolic disturbance.The diagnosis of argininosuccinic aciduria is based on the detection of substantial argininosuccinic acid and its anhydrides in plasma and urine where these compounds are barely detectable normally. Plasma levels of citrulline are elevated, but less than those in citrullinemia (33).

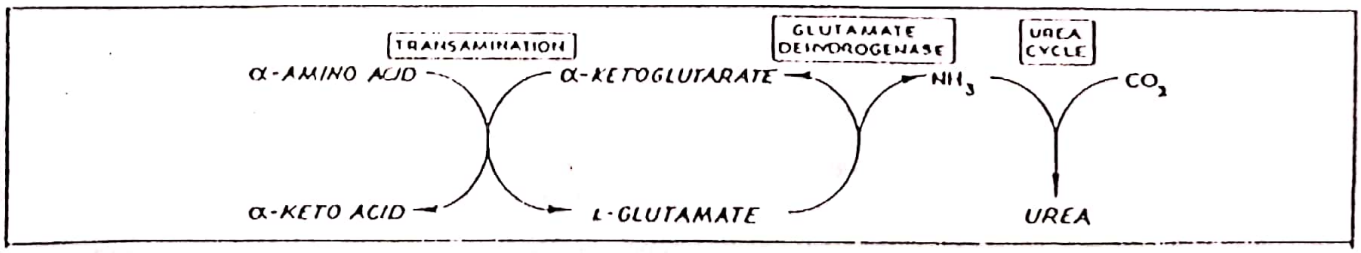

Fig 1 : Flow of nitrogen in amino acid catabolism ending in ammonia formation, which then enters the urea biosynthetic patway.

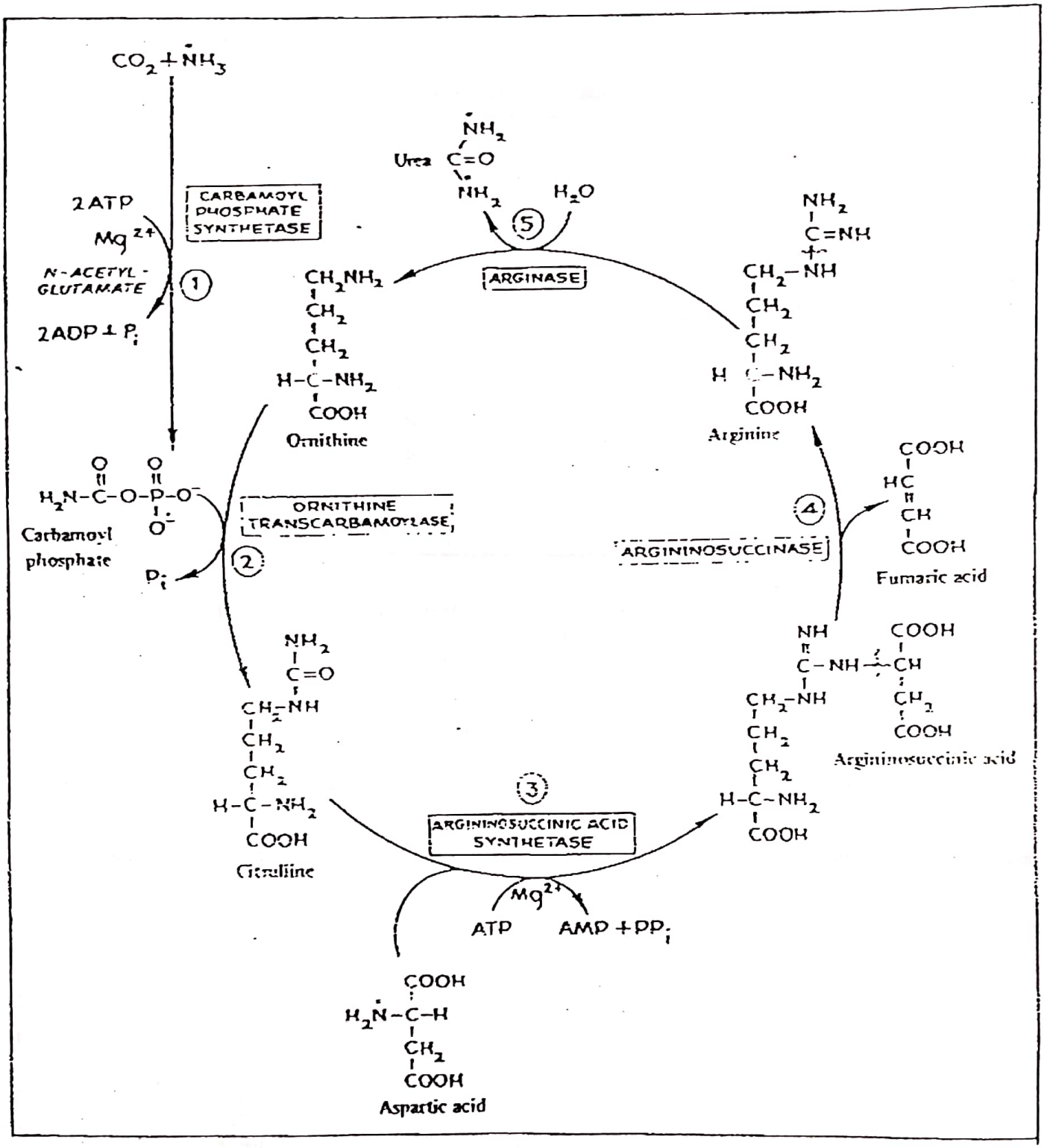

Fig 2 : Reactions and intermediate compounds of urea biosynthesis. The five enzymes whose deficiency may cause hyperammonemia are indicated by boxes.

Citrullinemia is diagnosed by the presence of elevated levels of plasma citrulline (>500 mM ; normal is 10 - 40 mM) with the absence of detectable argininosuccinic acid in the analysis. Prenatal diagnosis of citrullinemia is possible by combining measurements of citrulline in amniocytes, and incorporation of radioactive citrulline into protein by intact cultured amniocytes. Very low levels of enzyme activity have been observed in cultured fibroblasts from some heterozygotes, and caution is required to avoid errors in prenatal diagnosis (34).

The diagnosis of arginase enzyme disorder is based on the detection of five-to-ten fold elevations of plasma arginine. Arginase deficiency can be documented using erythrocytes, leukocytes or liver. The hepatic form of arginase is deficient in human argininemia (23). Prenatal diagnosis of argininemia is diffucult, since arginase cannot be reliably measured in cultured amniocytes. Fetal blood sampling or fetal liver biopsy might accurate prenatal diagnosis (35).

Therapy of Urea Cycle Disorders :

The synthesis of urea in ureotelic animals occurs in the liver by a series of enzymatic reactions, the urea cycle. I consider the five classical urea cycle enzymes in detail, including carbamoyl phosphate synthetase I, ornithine carbamoyl transferase, argininosuccinate sentetase, arginino succinate lyase and arginase. In addition to the five enzymes involved directly in urea synthesis, ancillary functions play critical roles. In this paper I presented a detailed review of the clinical and biochemical aspects of human urea cycle disorders. The complexities of differential diagnosis of hyperammonemia and in addition to urea cycle disorders were recently rewiewed in humans.These disorders include carbamoyl phosphate synthetase deficiency, the syndrome of hyper ammonemia, hiper argininemia and hypercitrulli-nemia (28,34), ornithine carbamoyl transferase deficiency, the syndrome of severe hyperammonemia and rapid death without treatment (25,32), argininosuccinate synthetase deficiency the syndrome of citrullinemia characterized by hyperammo-nemia and coma (33), and chronic mental retardation during infancy, argininosucci-nate lyase deficiency, the syndrome of argininosuccinic aciduria (13), and arginase deficiency, the syndrome of hyperargininemia, mental retardation, spastic diplegia, hypertonicity and hyperreflexia(22,35).

The starting point for treatment of these disorders is based on dietary management that attempts to provide a protein intake sufficient for growth, but that also minimizes the requirement for elimination of excess nitrogen. In practice, this strategy alone was not sufficient for the survival of most patients with severe urea cycle disorders. More severe protein restriction combined with the administration of keto acid analogues of essential amino acids improved the outlook for these patients. Arginine supplementation in the diet and subsequent administration of high doses of arginine in citrullinemia and argininosuccinic aciduria were used to promote the conversion of excess nitrogen to citrulline and argininosuccinic acid, both of which are less toxic than ammonemia and are excreted at modest rates (36). Citruline administration is used in carbamoyl phosphate synthetase I deficiency and ornithine carbamoyl transferase deficiency to avoid arginine insufficiency. Dietary therapy for argininemia is significantly

different, since neurological injuriy appers correlated with the elevated blood levels of arginine, and may be less attributable to hyperammo-nemia. Dietary therapy of argininemia consists of an extremely low-protein-diet supplemented with essential amino acids other than arginine (22).

More recently, two drugs have been used to provide an alternative pathway for nitrogen excretion. The administration of sodium benzoate results in the conjugation of benzoate to glycine to form hippuric acid, which is rapidly cleared by the kidney. Similary, the administration of phenylacetate results in conjugation with glutamine and excretion of phenylacetylglutamine. The combined use of these drugs has resulted in substantially improved survival for patients with inborn errors of urea cycle. Minor ilnesses with associated protein catabolism can lead to life- threatening episodes of hyperammonemia for these patients, despite these therapeutic modalities (3,36).

Human urea cycle disorders might be candidates for somatic gene replacement therapy.The cloned genes are becoming available for all of these enzymes, and expression of recombinant human ornithine carbamoyl transferase and human arginine succinate synthetase in tissue culture cells has been reported. Current strategies for somatic gene replacement therapy focus on the introduction of cloned cDNAs intro murine retroviral vectors. Expression can be directed from a foreign promoter, or the natural promoter for the gene can be used. One strategy introduces the recombinant retrovirus into bone marrow stem cells and repopulates the individual with autologous infected cells. For citrullinemia, argininosuccinic aciduria and argininemia metabolic data from patients suggest that expression of high levels of enzyme activity in other tissues such as bone marrow might provide therapeutic benefit. Alternative strategies for introduction of foreign DNA sequences into hepatocytes would be particularly relevant to urea cycle disorders. For carbamoyl phosphate synthetase I and ornithine carbamoyl transferase deficiency, where the enzyme is located in the mitochondria, specialized mitochodrial functions would probably require that gene replacement therapy be directed at hepatocytes (3,36).

The biochemical and clinical evidence indicates strongly that liver trans- plantation would adequately treat urea cycle patients. It is likely to be attempted with more advanced methods for organ transplantation (36).

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