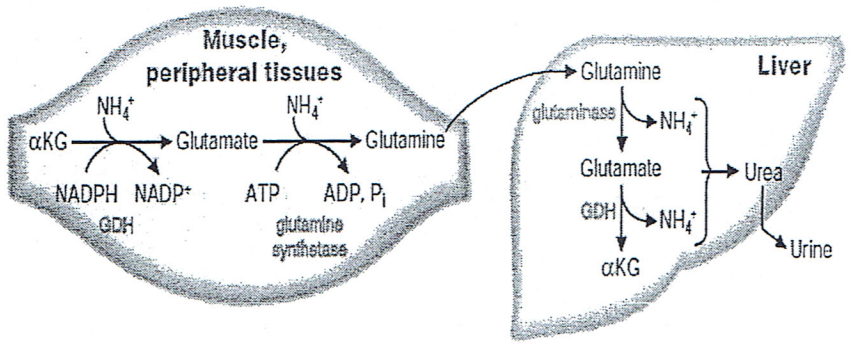

Synthesis of glutamine in peripheral tissues and its transport to the liver.

- The second transport mechanism, used primarily by **muscle**, involves transamination of pyruvate (the end product of aerobic glycolysis) to form alanine. Alanine is transported by the blood to the liver, where it is converted to pyruvate, again by transamination. In the liver, the pathway of gluconeogenesis can use the pyruvate to synthesize glucose, which can enter the blood and be used by muscle—a pathway called the glucose-alanine cycle.

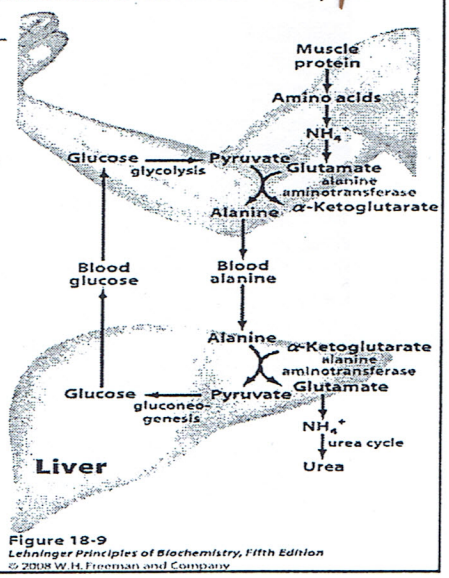

Figure 18-9
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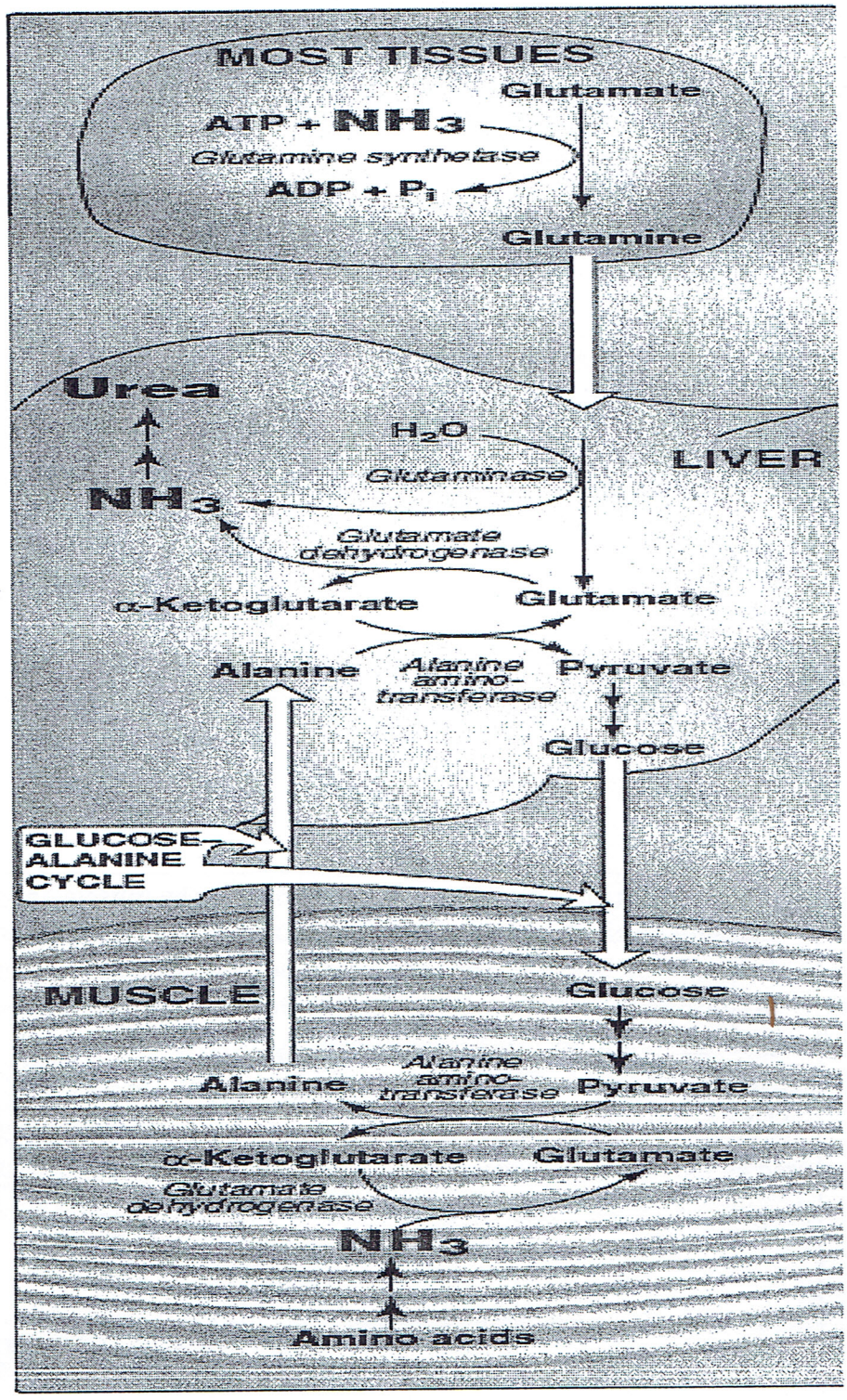
Glucose-alanine cycle. Alanine serves as a carrier of ammonia and of the carbon skeleton of pyruvate from skeletal muscle to liver. The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.



Transport of ammonia from peripheral tissues to the liver.

Sources of ammonia

Transdeamination—the linking of aminotransferase and glutamate dehydrogenase reactions—producing ammonia. However, substantial amounts of ammonia can be obtained from other sources.

1. From glutamine: The kidneys generate ammonia from glutamine by the actions of renal glutaminase and glutamate dehydrogenase.
2. From bacterial action in the intestine: Ammonia is formed from urea by the action of bacterial urease in the lumen of the intestine.
3. From amines: Amines obtained from the diet, and monoamines that serve as hormones or neurotransmitters, give rise to ammonia by the action of amine oxidase.
4. From purines and pyrimidines: In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as ammonia.

Ammonia is produced by all tissues during the metabolism of a variety of compounds, and it is disposed of primarily by formation of urea in the liver. However, the level of ammonia in the blood must be kept very low, because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system (CNS).

There must, therefore, be a metabolic mechanism by which nitrogen is moved from peripheral tissues to the liver for ultimate disposal as urea, while at the same time maintaining low levels of circulating ammonia.

AMMONIA TOXICITY

- Ammonia is toxic to the central nervous system.
- Ammonia may be toxic to the brain in part because it reacts with α-ketoglutarate to form glutamate. The resulting depleted levels of α-ketoglutarate then impair function of the tricarboxylic acid (TCA) cycle in neurons.
- **Symptoms of ammonia intoxication** include tremor, slurred speech, blurred vision, coma, and ultimately death.

D. UREA CYCLE

Urea is the major disposal form of amino groups derived from amino acids, and accounts for about 90% of the nitrogen-containing components of urine. One nitrogen of the urea molecule is supplied by free ammonia, and the other nitrogen by aspartate. [Note: Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of oxaloacetate by AST).] The carbon and oxygen of urea are derived from CO_2. Urea is produced by the **liver**, and then is transported in the blood to the kidneys for excretion in the urine.

- NH_3, CO_2, and the amide nitrogen of aspartate provide the atoms of urea.
- Fish excrete highly toxic NH_3 directly. Birds convert NH_3 to uric acid. Higher vertebrates convert NH_3 to urea.

Reactions of the cycle

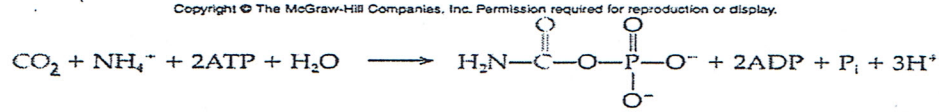
The first two reactions leading to the synthesis of urea occur in the mitochondria, whereas the remaining cycle enzymes are located in the cytosol. (Reactions **1** and **2** occur in the matrix of liver mitochondria and reactions **3** , **4** , and **5** in liver cytosol).

1. Formation of carbamoyl phosphate: Condensation of CO_2, ammonia, and ATP to form carbamoyl phosphate is catalyzed by mitochondrial carbamoyl phosphate synthetase I.
2. Formation of citrulline: The carbamoyl portion of carbamoyl phosphate is transferred to ornithine by ornithine transcarbamoylase (OTC) as the high-energy phosphate is released as Pi.
3. Synthesis of argininosuccinate from combines citrulline with aspartate by Argininosuccinate synthetase.
4. Cleavage of argininosuccinate by argininosuccinate lyase to yield arginine and fumarate.
5. Cleavage of arginine to ornithine and urea by Arginase.

1. Carbamoyl Phosphate Synthetase I Initiates Urea Biosynthesis

- Condensation of CO_2, ammonia, and ATP to form carbamoyl phosphate is catalyzed by mitochondrial carbamoyl phosphate synthetase I **(reaction 1)** .

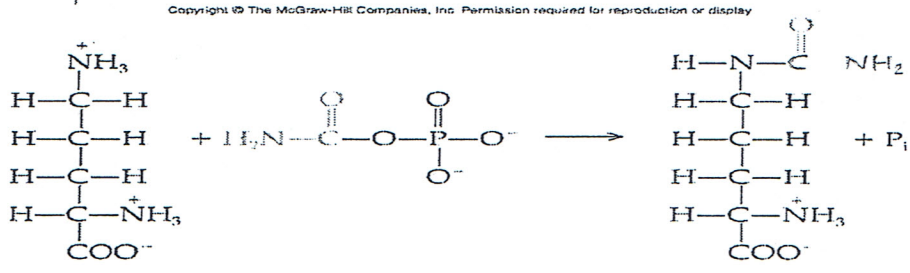
Formation of carbamoyl phosphate by carbamoyl phosphate synthetase I is driven by cleavage of two molecules of ATP. Ammonia incorporated into carbamoyl phosphate is provided primarily by the oxidative deamination of glutamate by mitochondrial glutamate dehydrogenase. Ultimately, the nitrogen atom derived from this ammonia becomes one of the nitrogens of urea. Carbamoyl phosphate synthetase I requires N-acetylglutamate as a positive allosteric activator. [**Note:** Carbamoyl phosphate synthetase II participates in the biosynthesis of pyrimidines. It does not require N-acetylglutamate, uses glutamine as the nitrogen source, and occurs in the cytosol.]



Carbamoyl phosphate

2. Carbamoyl Phosphate Plus Ornithine Forms Citrulline

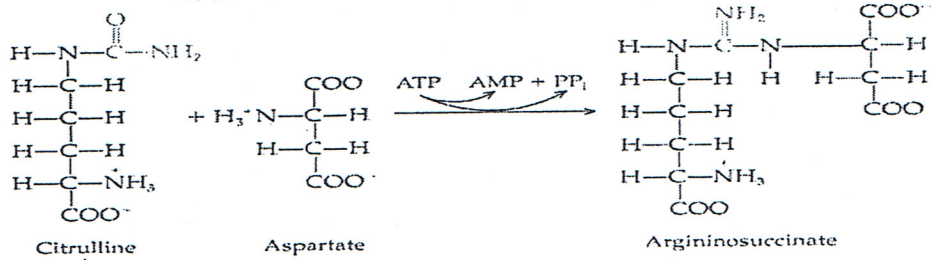
- **L-Ornithine transcarbamoylase** catalyzes transfer of the carbamoyl group of carbamoyl phosphate to ornithine, forming citrulline and orthophosphate **(reaction 2)**. While the reaction occurs in the mitochondrial matrix, both the formation of ornithine and the subsequent metabolism of citrulline take place in the cytosol. Entry of ornithine into mitochondria and exodus of citrulline from mitochondria therefore involve mitochondrial inner membrane transport systems.



Ornithine Carbamoyl phosphate Citrulline

3. Citrulline Plus Aspartate Forms Argininosuccinate

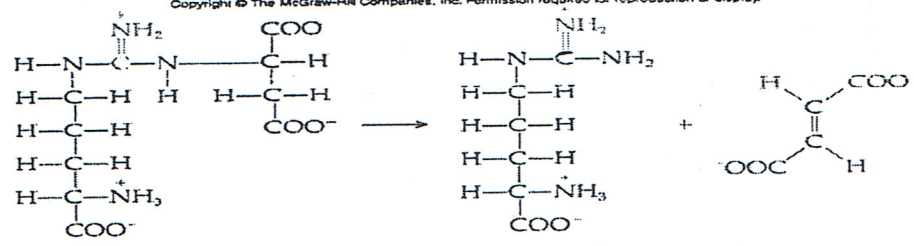
- **Argininosuccinate synthetase** links aspartate and citrulline via the amino group of aspartate **(reaction 3)** and provides the second nitrogen of urea. The reaction requires ATP.



Citrulline Aspartate Argininosuccinate

4. Cleavage of Argininosuccinate Forms Arginine & Fumarate

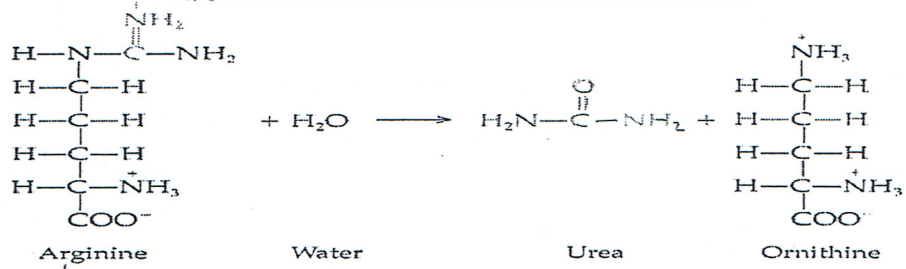
Argininosuccinate is cleaved by argininosuccinate lyase to yield arginine and fumarate. The **arginine** formed by this reaction serves as the immediate precursor of urea. **Fumarate** produced in the urea cycle is hydrated to malate, providing a link with several metabolic pathways. For example, the malate can be transported into the mitochondria via the malate shuttle, reenter the tricarboxylic acid cycle, and get oxidized to oxaloacetate (OAA), which can be used for gluconeogenesis. Alternatively, the OAA can be converted to aspartate via transamination, and can enter the urea cycle.



Argininosuccinate Arginine Fumarate

5. Cleavage of Arginine Releases Urea & Re-forms Ornithine

Hydrolytic cleavage of the guanidino group of arginine, catalyzed by liver arginase, releases urea **(reaction 5).** The other product, ornithine, reenters liver mitochondria for additional rounds of urea synthesis. Urea is then transported to the kidney and excreted in urine.



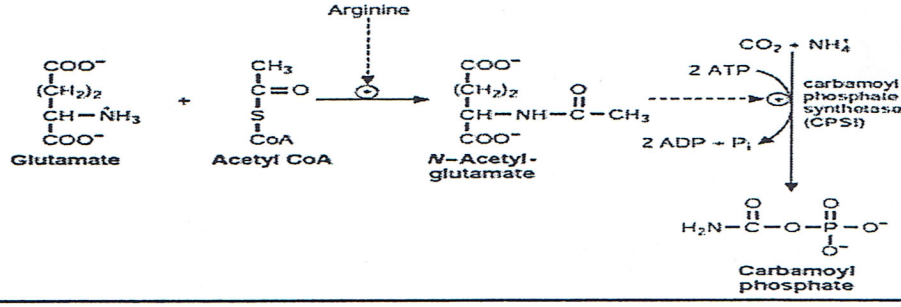
- **Net reaction**

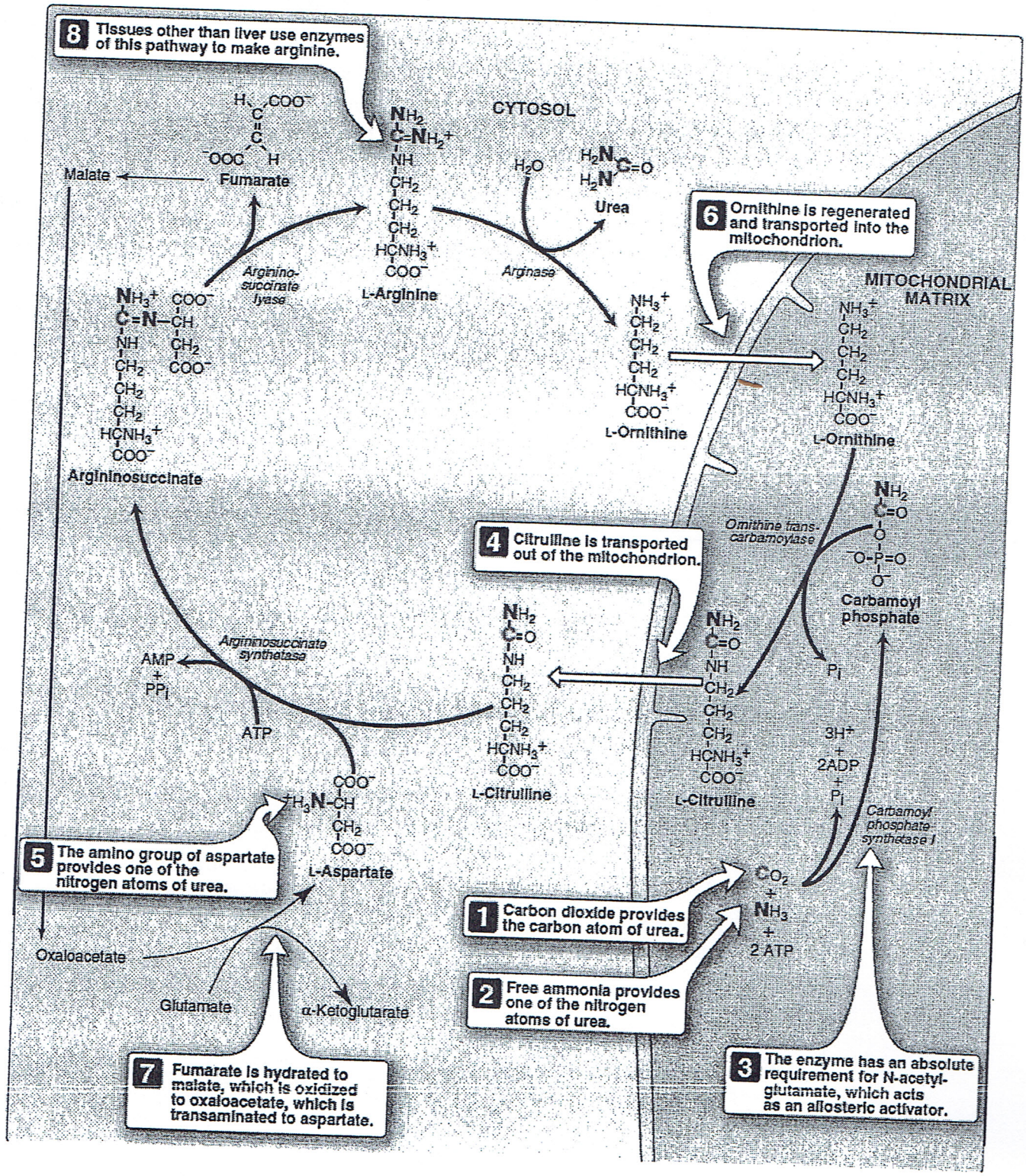
Aspartate + NH_3 + CO_2 + 3 ATP + H_2O ⟶

urea + fumarate + 2 ADP + AMP + 2 P_i + PP_i

Regulation of the urea cycle

N-Acetylglutamate is an essential activator for carbamoyl phosphate synthetase I—the rate-limiting step in the urea cycle. N-Acetylglutamate is synthesized from acetyl coenzyme A and glutamate by N-acetylglutamate synthase, in a reaction for which arginine is an activator. Therefore, the intrahepatic concentration of N-acetylglutamate increases after ingestion of a protein-rich meal, which provides both a substrate (glutamate) and the regulator of N-acetylglutamate synthesis. This leads to an increased rate of urea synthesis.

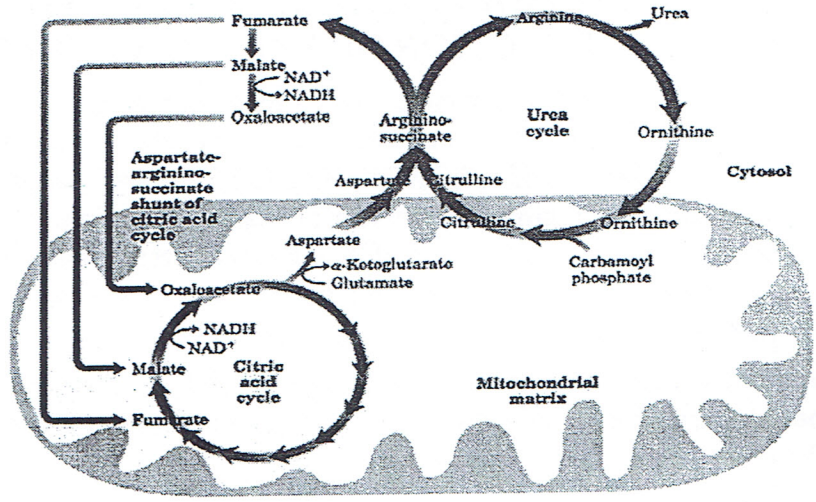




Reactions of the urea cycle.

Fate of urea:

Urea diffuses from the liver, and is transported in the blood to the kidneys, where it is filtered and excreted in the urine. A portion of the urea diffuses from the blood into the intestine, and is cleaved to CO_2 and NH_3 by bacterial urease. This ammonia is partly lost in the feces, and is partly reabsorbed into the blood. In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut. The intestinal action of urease on this urea becomes a clinically important source of ammonia, contributing to the hyperammonemia often seen in these patients. Oral administration of neomycin reduces the number of intestinal bacteria responsible for this NH_3 production.



Links between the urea cycle and citric acid cycle.

The interconnected cycles have been called the "Krebs bicycle." The pathways linking the citric acid and urea cycles are called the **aspartate-argininosuccinate shunt.**

Hyperammonemia:

1. Acquired Hyperammonemia: Liver disease is a common cause of hyperammonemia in adults, and may be due, for example, to viral hepatitis or to hepatotoxins such as alcohol.

2. Hereditary Hyperammonemia. e.g. genetic deficiencies of Urea cycle enzymes.

- Inherited Defects of the Urea Cycle Cause Hyperammonemia and Can Lead to Brain Damage.

The synthesis of urea in the liver is the major route of removal of NH_4^+. A blockage of carbamoyl phosphate synthesis or of any of the four steps of the urea cycle has devastating consequences because there is no alternative pathway for the synthesis of urea. All defects in the urea cycle lead to an elevated level of NH_4^+ in the blood (hyperammonemia).

METABOLIC DISORDERS ARE ASSOCIATED WITH EACH REACTION OF THE UREA CYCLE

- Urea cycle disorders are characterized by hyperammonemia, encephalopathy, and respiratory alkalosis. Four of the five metabolic diseases, deficiencies of carbamoyl phosphate synthetase, ornithine transcarbamylase, argininosuccinate synthetase, and argininosuccinate lyase, result in the accumulation of precursors of urea, principally ammonia and glutamine. Ammonia intoxication is most severe when the metabolic block occurs at reactions 1 or 2.

[Note: The hyperammonemia seen with arginase deficiency is less severe because arginine contains two waste nitrogens and can be excreted in the urine.

- Clinical symptoms common to all urea cycle disorders include vomiting, intermittent ataxia, irritability, lethargy, and severe mental retardation.

- Metabolic diseases are associated with defects in each enzyme of the urea cycle, of the membrane-associated ornithine transporter, and of N-acetylglutamate synthase.

- **N-Acetylglutamate Synthase:**

N-Acetylglutamate synthase (NAGS) catalyzes the formation from acetyl-CoA and glutamate of the N-acetylglutamate essential for carbamoyl phosphate synthetase I activity.

L-Glutamate + Acetyl-CoA → N-Acetyl-L-glutamate + CoASH

While the clinical and biochemical features of NAGS deficiency are indistinguishable from those arising from a defect in phosphate synthetase I, a deficiency in NAGS may respond to administered N-acetylglutamate.

- **The Ornithine Transporter:**

Hyperornithinemia,hyperammonemia, and homocitrullinuria syndrome **(HHH syndrome)** results from mutation of the ORNT1 gene that encodes the mitochondrial membrane ornithine transporter. The failure to import cytosolic ornithine into the mitochondrial matrix renders the urea cycle inoperable, with consequent hyperammonemia, and hyperornithinemia due to the accompanying accumulation of cytosolic ornithine. In the absence of its normal acceptor ornithine, mitochondrial carbamoyl phosphate carbamoylates lysine to homocitrulline, resulting in homocitrullinuria.

- **Hyperammonemia Type 1:** A consequence of carbamoyl phosphate synthetase I deficiency (reaction 1), this relatively infrequent condition (estimated frequency 1:62,000) probably is familial.

- **Hyperammonemia Type 2:** The X-chromosome linked deficiency termed "hyperammonemia type 2" reflects a defect in ornithine transcarbamoylase (reaction 2). The mothers also exhibit hyperammonemia and an aversion to high-protein foods. Levels of glutamine are elevated in blood, cerebrospinal fluid, and urine, probably as a result of enhanced glutamine synthesis in response to elevated levels of tissue ammonia.

When ornithine transcarbamoylase (OTC) is deficient, the carbamoyl phosphate that normally would enter the urea cycle accumulates and floods the pathway for pyrimidine biosynthesis. Under these conditions, excess orotic acid (orotate), an intermediate in pyrimidine biosynthesis, is excreted in the urine.

- **Citrullinemia:** (Argininosuccinate Synthetase deficiency; reaction 3) The inability to condense citrulline with aspartate results in accumulation of citrulline in blood and its excretion in urine (citrullinemia), (in this rare disorder, plasma and cerebrospinal fluid citrulline levels are elevated, and 1–2g of citrulline are excreted daily).

- **Argininosuccinicaciduria:** [Argininosuccinase; (Argininosuccinate Lyase) deficiency] A rare disease characterized by elevated levels of argininosuccinate in blood, cerebrospinal fluid. The metabolic defect is the absence of argininosuccinase (reaction 4). Diagnosis by measurement of erythrocyte argininosuccinase activity can be performed on umbilical cord blood or amniotic fluid cells.

- **Hyperargininemia:** (Arginase deficiency) This defect is characterized by elevated blood and cerebrospinal fluid arginine levels, low erythrocyte levels of arginase (reaction 5).

DISORDERS OF UREA CYCLE :

UCD	Enzyme deficiency
Hyperammonaemia type I	1
Hyperammonaemia type II	2
Citrullinemia	3
Arginino succinat aciduria	4
Hyperargininaemia	5

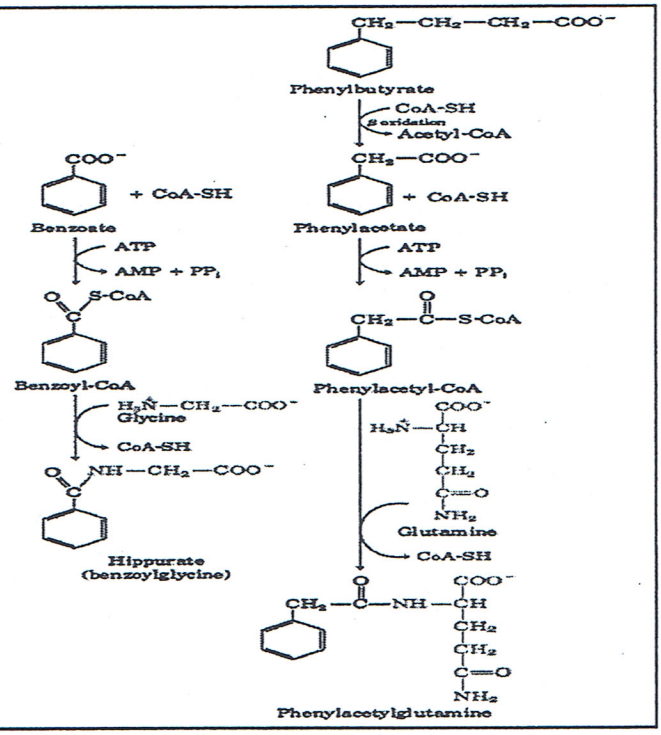
- **Hereditary deficiency** of any of the Urea Cycle enzymes leads to **hyperammonemia** - elevated [ammonia] in blood.
- Elevated ammonia is toxic, especially to the brain.
- If not treated immediately after birth, severe mental retardation results.

Treatment for deficiencies in urea cycle enzymes

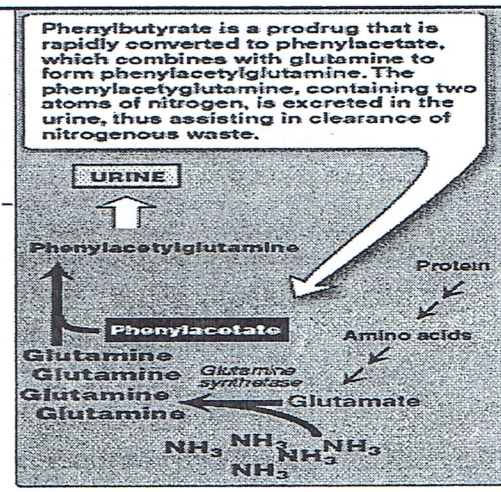
- A variety of treatments are available for individuals with urea cycle defects. Careful administration of the aromatic acids benzoate or phenylbutyrate in the diet can help lower the level of ammonia in the blood.
- Benzoate is converted to benzoyl-CoA, which combines with glycine to form **hippurate**. The glycine used up in this reaction must be regenerated, and ammonia is thus taken up in the glycine synthase reaction.
- Phenylbutyrate is converted to phenylacetate by oxidation. The phenylacetate is then converted to phenylacetyl-CoA, which combines with glutamine to form **phenylacetylglutamine**. The resulting removal of glutamine triggers its further synthesis by glutamine synthetase in a reaction that takes up ammonia.
- Both **hippurate** and **phenylacetylglutamine** are nontoxic compounds that are excreted in the urine.

Treatment for deficiencies in urea cycle enzymes.

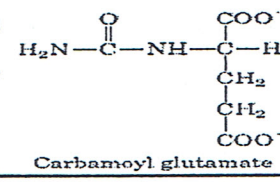
- The aromatic acids benzoate and phenylbutyrate, administered in the diet, are metabolized and combine with glycine and glutamine, respectively. The products are excreted in the urine. Subsequent synthesis of glycine and glutamine to replenish the pool of these intermediates removes ammonia from the bloodstream.



Treatment of patients with urea cycle defects by administration of phenylbutyrate to aid in excretion of ammonia.



Other therapies are more specific to a particular enzyme deficiency. Deficiency of N-acetylglutamate synthase results in the absence of the normal activator of carbamoyl phosphate synthetase I. This condition can be treated by administering **carbamoyl glutamate**, an analog of N-acetylglutamate that is effective in activating carbamoyl phosphate synthetase I.


Carbamoyl glutamate