Urea cycle
Urea is the major end product of nitrogen catabolism in humans
Urea is the major disposal form of amino groups derived from amino acids, and accounts about 90% percent of the nitrogen-containing components of urine.

- One nitrogen of the urea molecule is supplied by free NH₃.
- the other nitrogen by aspartate.

[Glutamate is the immediate precursor of both ammonia (oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (transamination of oxaloacetate by aspartate aminotransferase).]

- The carbon and oxygen of urea are derived from CO₂.

Urea is produced by the liver, and then is transported in the blood to the kidneys for excretion in the urine.

A. Reactions of the cycle The first two reactions occur in the mitochondria, whereas the remaining cycle enzymes are located in the cytosol.
Urea syntesis

1-Formation of carbamoyl phosphate:
Condensation of CO2, ammonia, and 2ATP to form carbamoyl phosphate is catalyzed by mitochondrial carbamoyl phosphate synthetase I. Ammonia incorporated into carbamoyl phosphate is provided primarily by the oxidative deamination of glutamate by mitochondrial glutamate dehydrogenase - 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.** [Carbamoyl phosphate synthetase II participates in the biosynthesis of pyrimidines. It does not require N-acetylglutamate, and occurs in the cytosol.]

2. **Formation of citrulline:**
Ornithine and citrulline are basic amino acids. [They are not incorporated into cellular proteins, because there are no codons for these amino acids] .

**L-Ornithine transcarbamoylase** catalyzes transfer of the carbamoyl group of **carbamoyl phosphate** to **ornithine**, forming **citrulline** and **orthophosphate**. This reaction occurs in the mitochondrial matrix,
Both the formation of ornithine and the 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.

3. Synthesis of argininosuccinate: Argininosuccinate synthetase links aspartate and citrulline via the $\alpha$-amino group of aspartate. The $\alpha$-amino group of aspartate provides the second nitrogen that is ultimately incorporated into urea.
The formation of argininosuccinate is driven by the cleavage of ATP to AMP and pyrophosphate (PPi). This is the third and final molecule of ATP consumed in the formation of urea.

4-Cleavage of argininosuccinate: catalyzed by argininosuccinase 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. Malate can be transported into the mitochondria via the malate shuttle and reenter the TCA cycle.
cytosolic malate can be oxidized to oxaloacetate, which can be converted to aspartate or glucose.

5. Cleavage of arginine to ornithine and urea: Arginase cleaves arginine to ornithine and urea, and occurs almost exclusively in the liver. Thus whereas other tissues, such as the kidney, can synthesize arginine by these reactions, only the liver can cleave arginine and, thereby, synthesize urea. The other product, ornithine, reenters liver mitochondria and participates in additional rounds of urea synthesis. Ornithine and lysine are potent inhibitors of arginase, and compete with arginine. Arginine also serves as the precursor of the potent muscle relaxant nitric oxide (NO) in a Ca2+ -dependent reaction catalyzed by NO synthase.
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 CO2 and NH3 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\textsuperscript{1} reduces the number of intestinal bacteria responsible for this NH\textsubscript{3} production.

B. Overall stoichiometry of the urea cycle
Aspartate + NH\textsubscript{3} + CO\textsubscript{2} + 3ATP$\rightarrow$ Urea + fumarate + 2 ADP + AMP + 2Pi + PPi + 3H\textsubscript{2}O

C. Regulation of the urea cycle
Carbamoyl phosphate synthetase I is the pacemaker enzyme of the urea cycle.
N-Acetylglutamate is an essential activator for carbamoyl phosphatesynthetase I.
N-Acetylglutamate is synthesized from **acetyl CoA** and **glutamate** by N-acetylglutamate synthase. **Arginine** is an activator.

Therefore, the intrahepatic concentration of N-acetylglutamate increases after ingestion of a protein-rich meal, which provides both the substrate (glutamate) and the regulator of N-acetylglutamate synthesis. This leads to an increased rate of urea synthesis.
METABOLISM OF 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.

The level of ammonia in the blood must be kept very low (10-20 g/dL), 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 low levels of circulating ammonia must be maintained.
• **Transport of ammonia to liver**(glucose-alanine cycle)

• **Sources of ammonia:**
  1. Liver **(Transdeamination)**
  2. Renal/Intestinal **(glutaminase)**
  3. Bacterial urease
  4. Amines **(hormones/neurotransmitters)**
  5. Purines/Pyrimidines

• **Transport of ammonia in circulation** *(urea)(glutamine)*
The glutaminase reaction (the *amide* nitrogen, not the -amino nitrogen, is removed).
Transport of ammonia in the circulation

Ammonia is present at very low levels in blood. This is due:

1. **Urea**: rapid removal of blood ammonia by the liver. Formation of urea in the liver is the most important disposal route for ammonia. Urea travels in the blood from the liver to the kidneys, where it passes into the glomerular filtrate.

2. **Glutamine**: the fact that many tissues, particularly muscle, release amino acid nitrogen in the form of glutamine or alanine, rather than as free ammonia. This amide of glutamic acid provides a nontoxic storage and transport form of ammonia.
The ATP-requiring formation of glutamine from glutamate and ammonia by *glutamine synthetase* occurs primarily in the muscle and liver, but is also important in the nervous system, where it is the major mechanism for the removal of ammonia in the brain. Glutamine is found in plasma at concentrations higher than other amino acids a finding consistent with its transport function. Circulating glutamine is removed by the kidneys and deaminated by *glutaminase*. 
Acquired hyperammonemia: Liver disease is a common cause of hyperammonemia in adults. It may be a result of viral hepatitis, ischemia, or hepatotoxins. The detoxification of ammonia (that is, its conversion to urea) is, therefore, severely impaired, leading to elevated levels of circulating ammonia.

2. Hereditary hyperammonemia: Genetic deficiencies of each of the five enzymes of the urea cycle.
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.
All defects in urea synthesis result in ammonia intoxication. Intoxication is more severe when the metabolic block occurs at reactions 1 or 2. Clinical symptoms common to all urea cycle disorders include vomiting, avoidance of high-protein foods, intermittent ataxia, irritability, lethargy, and mental retardation.

Significant improvement and minimization of brain damage accompany a low-protein diet ingested as frequent small meals to avoid sudden increases in blood ammonia levels.
Hyperammonemia Type 1. A consequence of carbamoyl phosphate synthase I deficiency

Hyperammonemia Type 2. A deficiency of ornithine transcarbamoylase which is X-linked, affecting males, although female carriers have been clinically affected.

All of the other urea cycle disorders follow an autosomal recessive inheritance pattern.

Citrullinemia -- argininosuccinate synthase
Argininosuccinicaciduria - argininosuccinase
Hyperargininemia --- arginase

Gene Therapy Offers Promise for Correcting Defects in Urea Biosynthesis