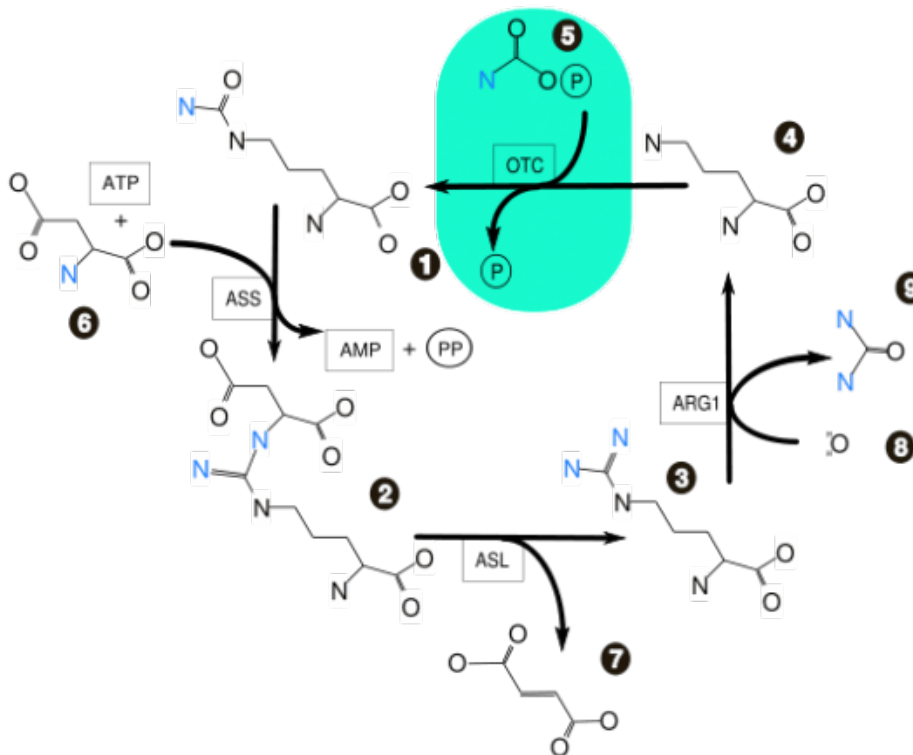


The Urea Cycle (Ornithine Cycle) and Urea Cycle Disorders

[See online here](#)

The daily protein turnover of a human being is approximately 300 g. Amino acids contain nitrogen atoms, which need to be eliminated by the body without developing too much toxic ammonia. Here, urea comes into play as an appropriate end product of protein degradation. The steps of synthesis in the urea cycle (also: ornithine cycle), which occurs in the liver, should be well understood. By the way, the urea cycle was first described in 1932 and is, therefore, the first described cyclic metabolic pathway.



Protein Degradation

The repeated synthesis and breakdown of structural proteins in the body release free amino acids constantly in the body, which can be destroyed or recycled for the synthesis of new proteins. The splitting of the amino group from the respective amino acid occurs in 2 ways. The 1st and the most frequent process is **transamination**, which entails the transfer of the amino group to an α -keto acid, which is then converted to an amino acid. The 2nd and relatively rare process known as **deamination** involves the release of the amino group as ammonia (NH_3). Each transamination reaction requires **pyridoxal**

phosphate (PLP) as a cofactor (coenzyme). It is the active form of **vitamin B6**, which often plays a role in amino acid metabolism.

Transport of Nitrogen in the Blood

During **transamination**, the nitrogen atoms are bound to amino acids in the form of peripheral amino groups, mostly in the cells of the skeletal musculature. The nitrogen atoms are then delivered into the bloodstream and reach the liver, where the amino acids are also synthesized from intermediates of major metabolic pathways, such as the citric acid cycle.

Glutamate is an amino acid central to nitrogen transport. It is synthesized in the peripheral cells of the body via the transfer of an amino group to an α -ketoglutarate. It serves as a substrate for transamination or binds with another amino group for delivery into the bloodstream as **glutamine**. In the human body, the plasma concentration of glutamine is the highest and primarily represents the transport of nitrogen to the kidney and the liver. In the kidney, glutamine is actually broken down to form ammonia. Ammonium, but not ammonia, is secreted in the proximal tubule, where it facilitates the neutralization of acids in the urine.

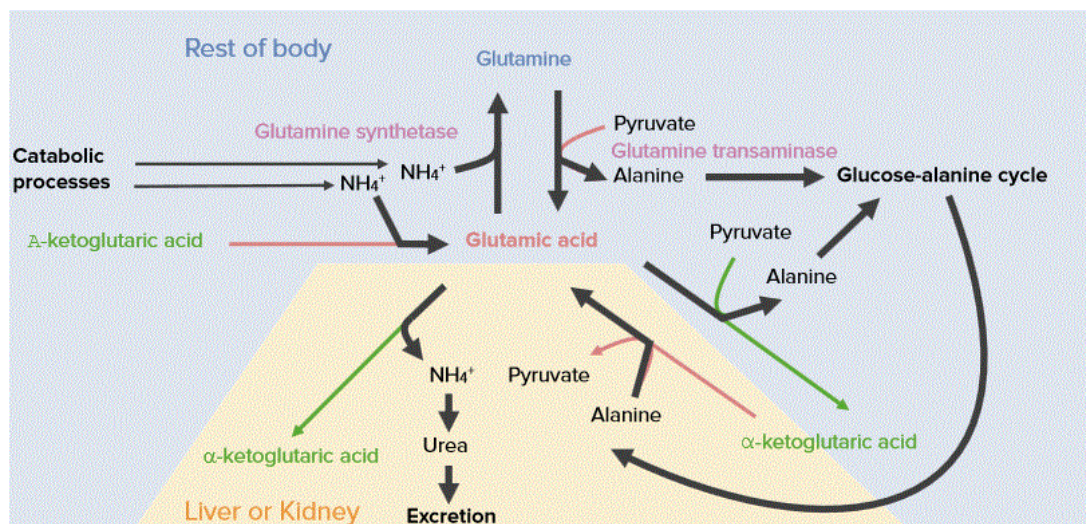
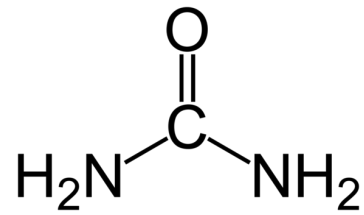


Image: The central role of glutamate. By Lecturio

Alanine is another amino acid used for nitrogen transport. It represents the most important transport mechanism of nitrogen from the skeletal musculature to the liver. In the peripheral cells, alanine is synthesized following the transfer of amino group from glutamate to pyruvate. In the liver cells, alanine is then used for the synthesis of aspartate from oxaloacetate. After the transfer of the amino group, pyruvate remains, which is then metabolized in the mitochondria or diverted to gluconeogenesis. **Delivery of glucose to the musculature and oxidation into pyruvate completes the cycle**, which is also referred to as the **alanine cycle**.

Image: Urea. By Lecturio



Urea is the product of the urea cycle and is delivered into the bloodstream by the liver. It is a water-soluble substance, which contains 2 nitrogen atoms. **In addition to glutamine, urea is the most important nitrogenous compound transported in the blood.**

Excretion of nitrogen

- Transamination of amino acids mobilizes nitrogen
- Toxicity of ammonia suggests the critical role of nitrogen balance in the body
- Excretion
 - Ammonotelic-excrete ammonia-fish
 - Uricotelic-excrete uric acid-birds
 - Ureotelic-excrete urea-most vertebrates, some invertebrates

Urea Cycle in the Liver

In the human body, the actual **urea cycle** occurs exclusively in the liver. Therefore, when nitrogen atoms reach the liver via the alanine or glutamate transport system, they are bound to an end product as urea that is easily excreted. The urea cycle is extremely important, as the formation of ammonia (NH₃), which is neurotoxic, can be avoided. The reactions of the urea cycle occur in the hepatocytes. The 1st 2 of these reactions occur within the mitochondrion and the subsequent steps in the cytosol. The following schematic diagram provides an overview of the individual reaction steps in the urea cycle.

N.B. The urea cycle occurs exclusively in the liver.

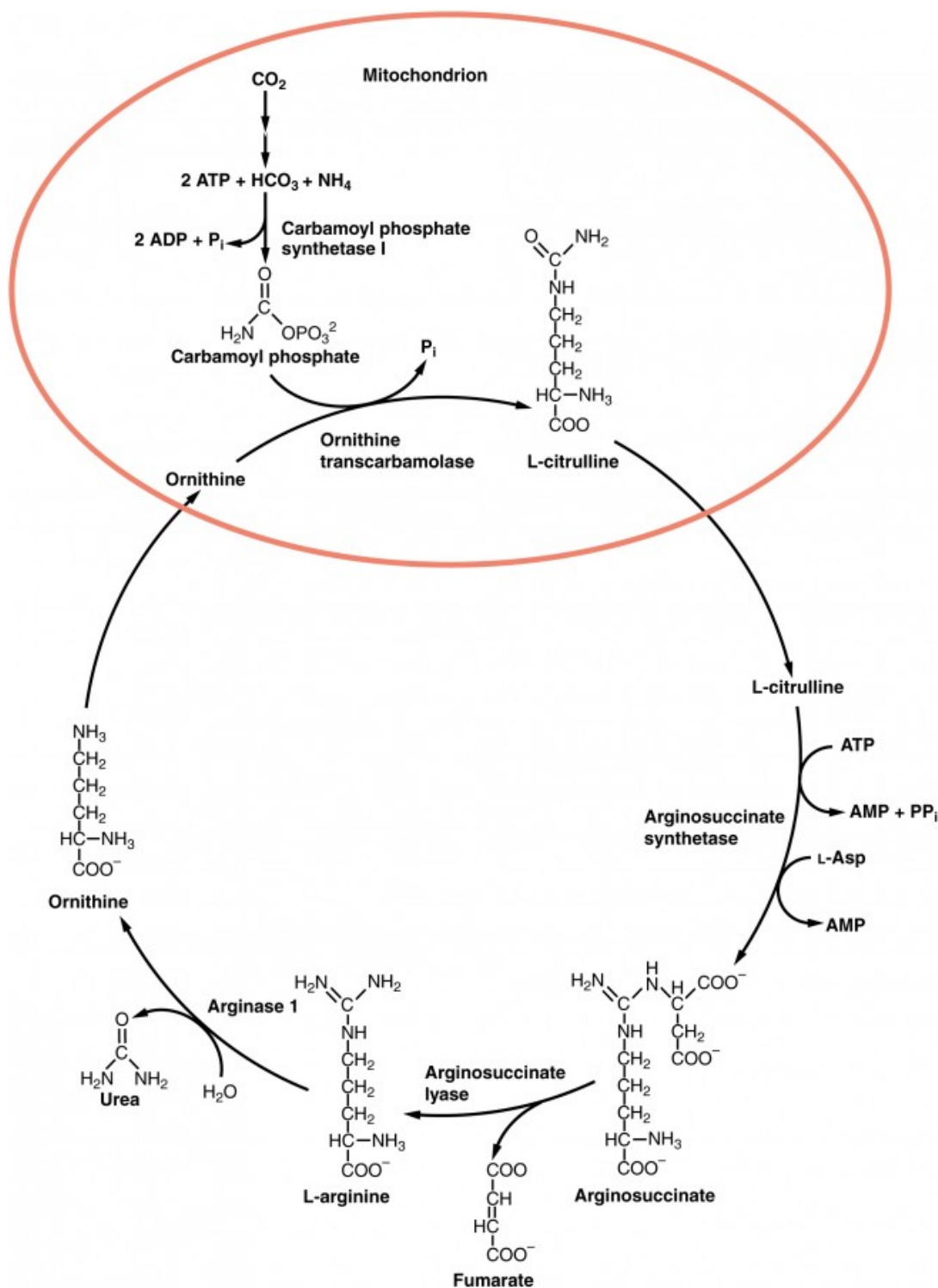


Image: Urea cycle. By Phil Schatz, License: [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)

Reaction Steps of the Urea Cycle

1. Formation of Carbamoyl Phosphate (Mitochondrion)

Catalyzed by the enzyme **carbamoyl phosphate synthetase 1**, a single molecule of **carbamoyl phosphate** forms in the mitochondrial matrix from ammonia (NH₃) and CO₂. Two molecules of ATP are consumed during this irreversible step of the urea cycle. **Thus, the 1st nitrogen atom in the urea originates from ammonia following the degradation of amino acids or purine bases.** The resulting carbamoyl phosphate is strongly polar, which prevents diffusion across the mitochondrial membrane.

2. Formation of Citrulline (Mitochondrion)

During the 2nd reaction step, the carbamoyl residue of the carbamoyl phosphate is transferred to the amino acid ornithine. Ornithine is not used for the synthesis of proteins.

Citrulline forms during this reaction, which is catalyzed by **ornithine carbamoyltransferase**. Citrulline is non-proteinogenic as well and the phosphate residue is left behind as a byproduct during this step. In the next reaction step, citrulline diffuses across the mitochondrial membrane in order to reach the cytosol of the hepatocyte. The diffusion occurs via a translocator in the membrane, which exchanges citrulline with ornithine in the antiporter.

3. Formation of Argininosuccinate (Cytosol)

The 2nd reaction occurs in the cytosol during which **argininosuccinate is synthesized** from citrulline and aspartate. Aspartate contains an amino group, which binds to citrulline. In this reaction catalyzed by **argininosuccinate synthetase**, the 2nd nitrogen atom enters the urea cycle. The reaction requires energy generated by the hydrolysis of ATP to AMP. Thus, 2 bonds that are rich in energy are split here.

4. Cleavage of Argininosuccinate to Arginine and Fumarate (Cytosol)

Catalyzed by **argininosuccinate lyase**, argininosuccinate is then cleaved into **arginine** and fumarate. The further use of the byproduct fumarate is explained later.

5. Hydrolysis of Arginine (Cytosol)

During the last reaction of the urea cycle, arginine is hydrolyzed via **arginase**, and the whole urea group is released. During this hydrolysis, both **urea** and ornithine form, and ornithine is transported back into the mitochondrial matrix via the mitochondrial membrane in exchange for citrulline. In the mitochondrial matrix, it is again available for the 2nd reaction step, which completes the cycle. The resulting urea is delivered into the bloodstream via special transport proteins located in the hepatocyte cell membrane.

Regeneration of Fumarate

During the course of the urea cycle, **fumarate** is generated in the cytosol of the hepatocyte during the cleavage of argininosuccinate. Fumarate is then transformed into **oxaloacetate** via the enzymes **fumarase** and **malate dehydrogenase**, releasing malate as an intermediate product. These steps occur during the citric acid cycle in the cytosol. Oxaloacetate is again transaminated to aspartate to restart another metabolic cycle, referred to as **the aspartate cycle**. As fumarate is also an intermediate product of the **citric acid cycle**, it can be channeled into it as well.

N.B. The urea cycle occurs partly in the mitochondrial matrix, and partly in the cytosol of the hepatocyte.

Energy Balance in Urea Cycle

The synthesis of urea is a comparably energy-intensive process, which is used by the body to safely eliminate nitrogen and maintain the plasma levels of ammonia as low as possible. The 2 reaction steps in the urea cycle that require energy-rich bonds are catalyzed by 2 different enzymes. **Carbamoyl phosphate synthetase 1** in the mitochondrion requires 2 ATP molecules, which are hydrolyzed to ADP. In the cytosol, **argininosuccinate synthetase** only requires a single ATP molecule, which is, however, hydrolyzed twice to AMP and pyrophosphate. The pyrophosphate in the cytosol is rapidly

converted into 2 phosphate molecules. Overall, the urea cycle requires three ATP molecules and the cleavage of 4 energy-rich bonds.

N.B. Even if only 3 ATP molecules are involved, 4 energy-rich bonds are hydrolyzed in the urea cycle.

Regulation of the Urea Cycle

The rate-limiting step in urea synthesis is the 1st reaction of the urea cycle, which is catalyzed by the **carbamoyl phosphate synthetase 1**. Allosteric binding of **N-acetylglutamate** activates the enzyme. The concentration of N-acetylglutamate increases proportionate to the concentrations of glutamate and acetyl-CoA. High concentrations of glutamate reflect a high volume of substrate, and acetyl-CoA levels reflect adequate amounts of energy-rich substances. If both requirements are met, the synthesis of urea in the liver is increased subsequently.

N.B. The key enzyme in the urea cycle is carbamoyl phosphate synthetase 1.

Elimination of Urea

Approx. 30 g of urea is generated in the liver via the urea cycle and delivered into the bloodstream every day. Depending on the amount of protein in the food, this value can be significantly greater or lower as the body only breaks down excess amino acids. Urea is soluble in water and transported to the kidneys where it is excreted in the urine. Urea constitutes the largest proportion of nitrogen-containing compounds in the urine.

Because urea is filtered via the glomerular capillaries and partially re-absorbed, it is a laboratory parameter used to monitor kidney function. Urea concentration in the blood is increased in impaired kidney function. An abnormally elevated level of urea, along with a variety of clinical symptoms, is also referred to as **uremia**. However, changes in daily protein intake must be considered, as they can render this parameter unreliable.

Urea Cycle Disorders

When the urea cycle in the liver is impaired, ammonia accumulates in the blood. The increased plasma concentration of ammonia (> 250 µg/dl) or ammonium, which is the ionized form of ammonia, is called **hyperammonemia**. Depending on the severity and the age at manifestation, the neurotoxic effects of ammonia trigger cerebral damage. The consequent manifestations of neurological symptoms result in irreversible brain damage and death. The pathophysiology involves swelling of the astrocytes **due to increased glutamine levels** resulting in brain edema. The etiology is mostly based on inherited or acquired impairment of liver function.

Urea Cycle Defects

The abnormal urea cycle is attributed to a defect involving 1 of the following 6 enzymes.

Affected Enzyme	Enzyme Defect
Carbamoyl phosphate synthetase 1	CPS deficiency
N-acetylglutamate synthetase	NAGS deficiency
Ornithine transcarbamylase	OTC deficiency
Argininosuccinate synthetase	ASS deficiency (citrullinemia type I)
Argininosuccinate lyase	ASL deficiency (argininosuccinic aciduria)

All of these metabolic diseases are inherited as autosomal recessive traits with the exception of OTC deficiency, which is inherited as an X-linked chromosomal recessive fashion. In the USA, the incidence rate is approx. 1:8,000. The age of manifestation varies and may occur in any phase of life. In newborns, especially the acute course is extremely life-threatening. Diffuse neurological symptoms such as headache, lack of concentration, flapping tremor of the hands, vomiting, and lethargy occur in late adolescence and in adults. Untreated, these enzymatic defects can lead to mental retardation and death.

The diagnosis is based on increased plasma levels of ammonia determined via laboratory examinations and validated by genetic testing. Acute treatment often consists of diuresis but in the long term, the patient must adhere to a diet low in proteins and nitrogen. Liver transplantation is the only curative option available.

Liver Cirrhosis

Liver cirrhosis is another frequent cause of progressive loss of hepatic function. The lack of hepatic detoxification leads to increased ammonia levels. If it is accompanied by neurological symptoms, it is referred to as **hepatic encephalopathy**. Depending on the degree of clinical severity, liver cirrhosis is divided into 4 stages, where stage 4 corresponds to coma (hepatic coma). Ammonia concentration in the blood can be decreased by several methods; however, in cases of progressive liver cirrhosis, a liver transplant is the only curative option.

Nitrites

- Nitrite is formed via ionization of nitrous acid (HNO_2) or reduction of nitrates.
- Nitrite is used to cure meats and prevent botulism.
- Nitrite is reduced to nitric oxide under hypoxic conditions.
- In the human diet 80–90% of nitrites are generated via the reduction of nitrates in vegetables.
- Nitrates in vegetables are derived from fertilizers or plant stresses.
- Nitrite readily forms cancer-causing nitrosamines in stomach acid.
- Nitrites oxidize iron in hemoglobin from ferrous (II) to ferric (III) state, which is unable to carry oxygen, with serious consequences.

Nitrosamines

- Nitrosamines are produced by the reaction of nitrites and secondary amines, such as proline.
- Strong acids (in the stomach) or high temperatures during frying favor nitrosamine synthesis.
- Nitrosamines are found in processed meats, beer, cigarette smoke, and chewing tobacco.
- Nitrosamine formation is inhibited by vitamin C.
- Nitrosamines form DNA adducts and cause cancer in many animal species.
- They are likely carcinogens in humans.
- Nitrosamines increase gastric and esophageal cancer risk.
- Nitrosamines in tobacco form from nicotine.
- NNK is nicotine derived and important in carcinogenesis.
- NNK occurs in tobacco and e-cigarettes.
- NNK is activated by P-450-induced signaling cascades and uncontrolled

growth.

Reactive Nitrogen Species

- Reactive nitrogen species are generated from any of the molecules described here.
- Peroxynitrite is the most potent reactive nitrogen species.
- Peroxynitrite is formed from nitric oxide and superoxide.
- Peroxynitrite reacts readily with DNA and protein, causing damage.
- Cysteine side chains are most easily oxidized.
- Tyrosine side chains of proteins are nitrosylated
- Transition metals, such as in hemoglobin, myoglobin, and cytochromes are oxidized.

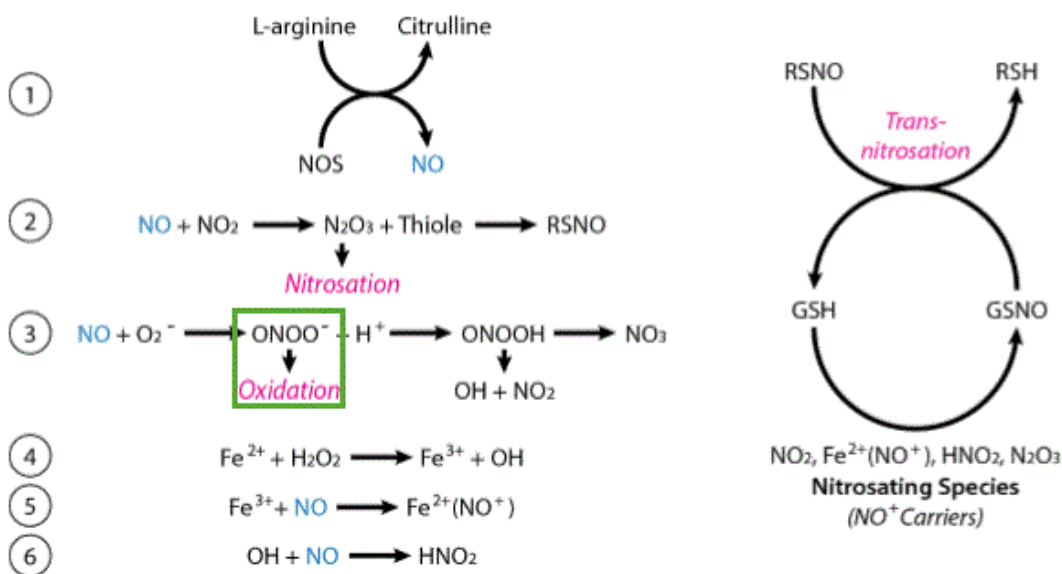


Image: Reactive nitrogen species can arise from any of the molecules described here. By Lecturio

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