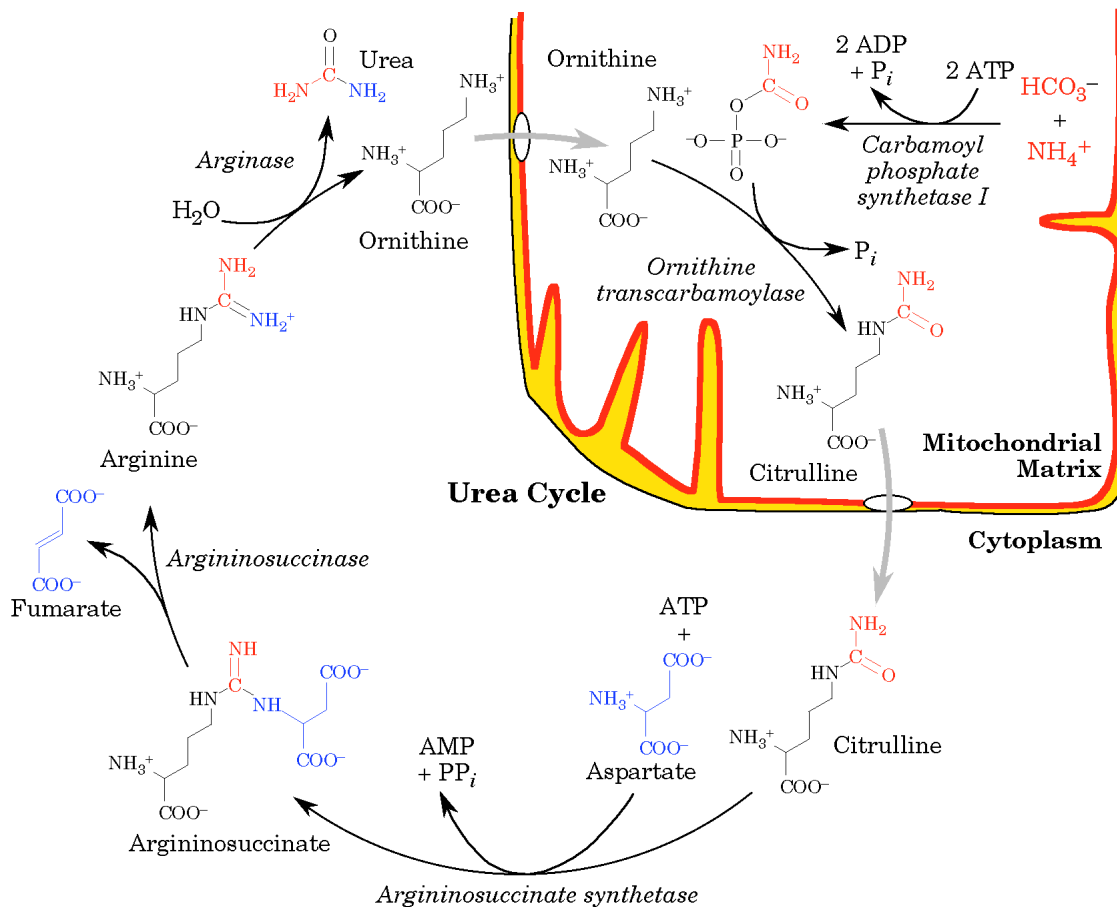


## The Urea Cycle

As has been mentioned, ammonium is toxic, and even small amounts will damage the nervous system. Genetic disorders in ammonium metabolism result in avoidance of high-protein foods and in mental retardation. Ammonium intoxication (e.g., as a result of decreased liver function) can be lethal. As a result, animals must control the amounts of free ammonium that are present, and often use a form of organic nitrogen as a waste product. In humans and most other terrestrial mammals, **urea** is the major nitrogen excretion product. Urea has the advantages of being relatively inexpensive to produce, being soluble in water, and being non-toxic.

Although most tissues can synthesize urea, most urea is produced in the **liver**. Because urea is uncharged, urea excretion does not involve the loss of any electrolytes as counter ions. Excretion of urea is, however, associated with considerable loss of water due to osmotic pressure.

Urea is produced as part of the series of reactions that comprise the urea cycle. The urea cycle is the first of the two major metabolic cycles discovered by Hans Krebs. In fact, the urea cycle was the first biological cycle to be discovered, and helped establish the concept for the discovery of the TCA cycle.



The urea cycle is smaller than the TCA cycle, and has fewer intermediates. Interestingly, all of the four intermediates are  $\alpha$ -amino acids, although three, ornithine, citrulline, and argininosuccinate are not found in proteins.

The urea cycle requires five reactions (of which four are part of the actual cycle). The first reaction is the primary regulated step. **Carbamoyl phosphate synthetase I**<sup>5</sup> is the mitochondrial enzyme that catalyzes the formation of carbamoyl phosphate from inorganic ammonium and carbonate. This enzyme is thus another enzyme capable of fixing ammonium. The usual fate of the ammonium fixed by carbamoyl phosphate synthetase I is excretion in the form of urea, and therefore this enzyme is usually considered separately from glutamine synthetase and glutamate dehydrogenase, which fix ammonium for use in metabolism.

In eukaryotic organisms, a different carbamoyl phosphate synthetase forms carbamoyl phosphate in the cytoplasm as the first step in pyrimidine biosynthesis. Unlike carbamoyl phosphate synthetase I, however, carbamoyl phosphate synthetase II uses glutamine as the ammonium donor instead of free ammonium.

Carbamoyl phosphate synthetase I requires the presence of the allosteric activator N-acetylglutamate (the product of the first step in ornithine biosynthesis) for activity. This regulation means that **carbamoyl phosphate synthetase I is the rate-limiting enzyme of the urea cycle.**

The other four enzymes are part of the actual cycle. The cycle begins with the addition of carbamoyl phosphate to ornithine by **ornithine transcarbamoylase** to produce citrulline. Citrulline then leaves the mitochondria using a specific transporter, because the remaining reactions occur in the cytoplasm. Once in the cytoplasm, citrulline is combined with aspartate by **argininosuccinate synthetase** to form argininosuccinate, in a reaction that requires ATP, and produces AMP and pyrophosphate. The next enzyme, **argininosuccinase**, performs a cleavage reaction that releases the TCA cycle intermediate fumarate and the amino acid arginine. Note that the arginine contains nitrogens derived from ornithine, from the free ammonium, and from the aspartate. Arginine is then cleaved by **arginase** to release urea and to regenerate ornithine. Ornithine also has a specific transporter that allows the ornithine to re-enter the mitochondria, completing the cycle.

As with the TCA cycle, the urea cycle is controlled by two factors: regulated enzymes and substrate availability. For the urea cycle the regulated enzyme is carbamoyl phosphate synthetase I. For the urea cycle, the availability of cycle intermediates and free ammonium also control the cycle. Thus, high levels of ornithine allow the cycle to proceed more rapidly.

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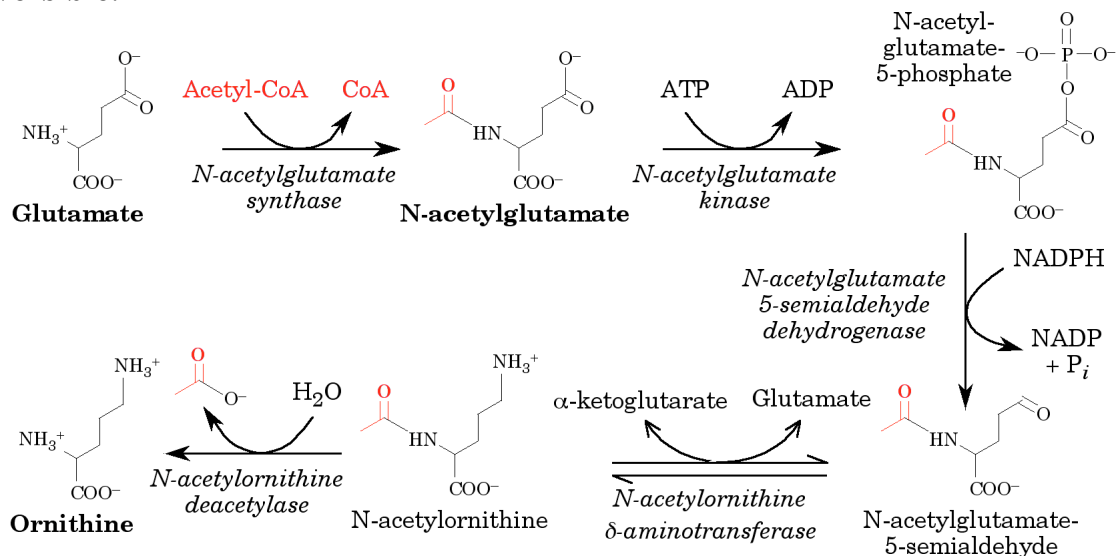
<sup>5</sup> Some textbooks call this enzyme carbamoyl phosphate “synthase” rather than “synthetase”. The strict nomenclature rule states that a “synthetase” is an enzyme that combines two molecules using ATP to provide the driving force, while a “synthase” combines two molecules without using ATP. For the purpose of this course, “synthase” and “synthetase” are effectively used interchangeably, although I am attempting to eliminate inconsistent usage.

In principle, the urea cycle can be used to synthesize or degrade arginine. Note, however that net synthesis of arginine requires input of one of the other urea cycle intermediates; net degradation of arginine requires net removal of one of these intermediates. As described above, the urea cycle does not result in an alteration in the amount of arginine.

## Ornithine

Ornithine is the equivalent of the TCA cycle intermediate oxaloacetate; levels of ornithine tend to control the rate of the urea cycle. Ornithine can be produced in several ways. One method of increasing ornithine levels is to take up arginine from a source outside the cell (either from protein breakdown or from a dietary source). A second method is to synthesize ornithine directly. Ornithine synthesis normally begins with glutamate, although proline can also act as a source of ornithine synthesis.

One pathway for the conversion of glutamate to ornithine is similar to the pathway for proline synthesis. However, the first step in the ornithine synthesis pathway, the N-acetylation of glutamate by **N-acetylglutamate synthase** forces ornithine rather than proline production. The N-acetyl group acts as a protecting group; lack of a free primary amine prevents the non-enzymatic pyrroline ring formation by glutamate-5-semialdehyde. N-acetylglutamate synthase also acts to produce the N-acetylglutamate required for carbamoyl phosphate synthetase I activity. The next two reactions, the phosphorylation of N-acetylglutamate by ATP, and the NADPH-dependent dephosphorylation reaction use ATP and NADPH to drive the production of N-acetylglutamate-5-semialdehyde. The semialdehyde oxygen is then replaced with an amino group by N-acetylornithine  $\delta$ -aminotransferase, followed by deprotection of the product by **N-acetylornithine deacetylase**. As with the kinase reaction, the loss of the protecting acetyl group helps to make the pathway irreversible.

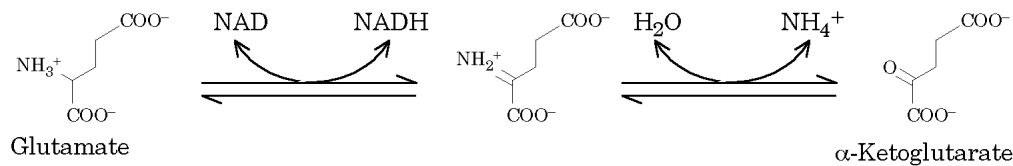


Ornithine can also be synthesized from unprotected glutamate-5-semialdehyde by ornithine  $\delta$ -aminotransferase in a reverse of the ornithine breakdown pathway. The

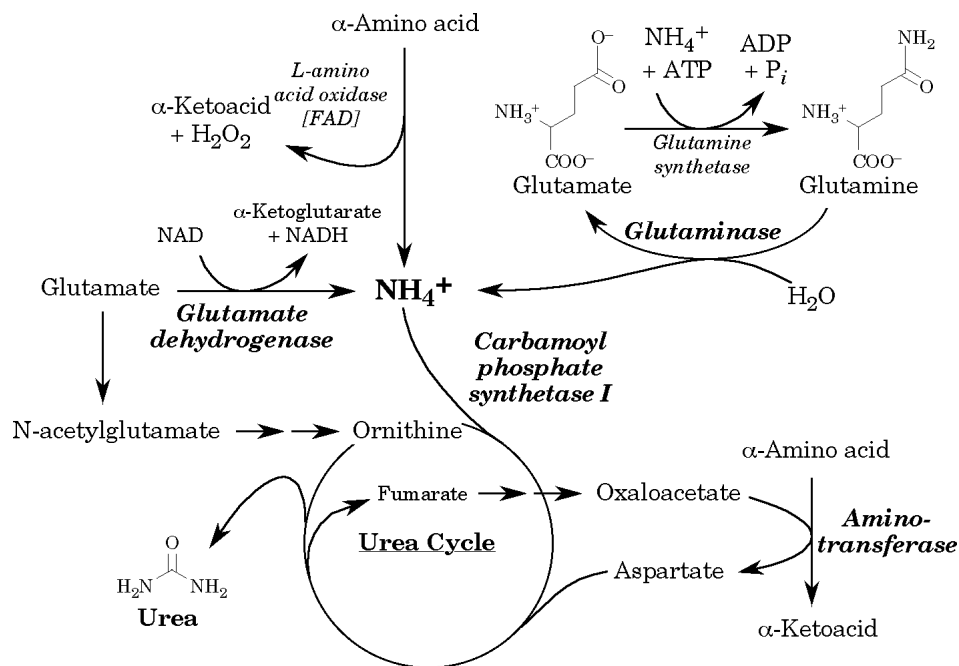
substrate glutamate-5-semialdehyde can be produced from either glutamate or proline.

### Regulation of ammonium metabolism

In humans, the most important reaction for releasing ammonium from amino acids is catalyzed by **glutamate dehydrogenase**. Since glutamate obtains the ammonium via aminotransferase reactions, glutamate dehydrogenase allows the release of ammonium from essentially any amino acid via glutamate. The glutamate dehydrogenase reaction (shown below) is an oxidative deamination: the enzyme forms a Schiff base in the dehydrogenase step, followed by hydrolysis of the Schiff base.



Because it is critical in releasing the toxic free ammonium, glutamate dehydrogenase is a regulated enzyme. Several allosteric effectors regulate glutamate dehydrogenase; GTP and NADH inhibit its activity, while ADP stimulates the enzyme. The glutamate dehydrogenase reaction has a large positive  $\Delta G^\circ$  (about 30 kJ/mol), and therefore favors retention of ammonium in the glutamate. This assists in maintaining a low ammonium concentration, as long as glutamate levels are not excessively high.



Ammonium can also be released from glutamine by the action of **glutaminase**, which releases the amide nitrogen. Glutaminase activity appears to be regulated primarily by the fact that the location of the enzyme is limited to the liver and

kidney.

Another pathway for release of ammonium from amino acids is the action of **L-amino acid oxidase**, a liver enzyme that directly deaminates amino acids. This enzyme is normally present in low levels, and is a relatively minor contributor to the pool of free ammonium. As with glutamate dehydrogenase, L-amino acid oxidase catalyzes an oxidative deamination. However, L-amino acid oxidase uses FAD as its electron acceptor. Regenerating the oxidized flavin requires generation of hydrogen peroxide because the enzyme cannot donate electrons to the electron transport pathway.

As mentioned previously, the major enzyme used for incorporating free ammonium into organic compounds, **glutamine synthetase** is also regulated. Glutamine synthetase activity is stimulated by  $\alpha$ -ketoglutarate. This allows glutamine synthetase to some extent counter the effects of glutamate dehydrogenase.

Finally, the urea cycle, the main process used in humans for the excretion of excess ammonium, is regulated largely by the availability of glutamate. Glutamate acts as the source of the free ammonium via glutamate dehydrogenase. Glutamate also frequently acts as an  $\alpha$ -amino donor for the aminotransferase reaction that supplies aspartate with the nitrogen it donates to urea. Glutamate acts as the source of N-acetylglutamate, the stimulator of the urea cycle limiting enzyme carbamoyl phosphate synthetase I. And lastly, glutamate also acts as a substrate for ornithine synthesis.

In animals, while the enzymes responsible for fixing and releasing ammonium are important, other factors are actually more important in controlling nitrogen metabolism. The protein content of the diet is one factor, because a high protein diet will result in increased amino acid breakdown. The other factor is the ratio of the rate of protein synthesis to the rate protein breakdown. If the animal is undergoing net protein breakdown, especially as a result of limited food availability, amino acid breakdown will increase.

### **Nitrogen transport**

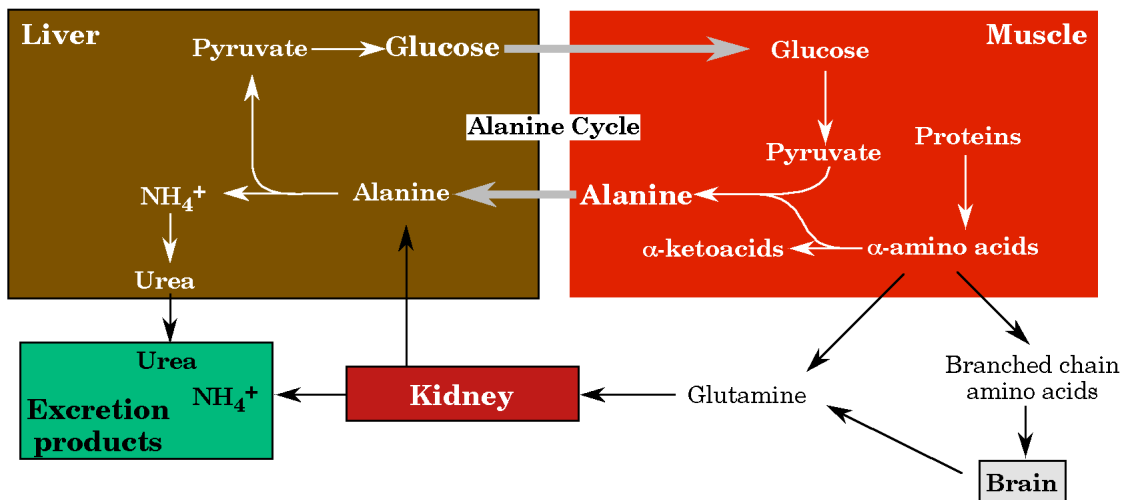
Small amounts of all of the amino acids are usually present in the blood to act as substrates for protein synthesis, or as the result of release following protein breakdown. Between meals (and especially during fasting), the muscle and liver are important in maintaining circulating levels of free amino acids. The liver is the site of the majority of amino acid synthesis, while the muscle breaks down protein and releases free amino acids into circulation to act as energy sources for the body, and to act as substrates for gluconeogenesis in the liver.

As has been mentioned, the toxicity of free ammonium means that, whenever possible, ammonium is maintained in an organic form. In most tissues, excess ammonium is either fixed by the glutamine synthetase reaction, or in the case of ammonium already present in an amino acid, is transferred to  $\alpha$ -ketoglutarate by an aminotransferase reaction. These processes result in accumulation of glutamine, which is then released into the blood stream.

Although glutamine is a useful, non-toxic ammonium carrier molecule, release of glutamine depletes the tissue of glutamate. This is particularly important in the brain, because glutamate is a neurotransmitter, and is the precursor of  $\gamma$ -aminobutyric acid (GABA), another neurotransmitter. In addition, synthesis of glutamate requires  $\alpha$ -ketoglutarate, and therefore release of glutamine depletes the tissue of TCA cycle intermediates, unless the tissue is well supplied with replacement intermediates.

Because of its use as an ammonium carrier, glutamine levels in circulation are usually higher than those of most amino acids. During protein breakdown that occurs in early fasting, levels of alanine and branched-chain amino acids are also higher than those of other amino acids. The levels of the branched-chain amino acids are elevated because the muscle and liver tend not to breakdown these compounds. Instead, the branched-chain amino acids act as fuel for the brain and other tissues. The levels of alanine are elevated because of a process called the alanine cycle.

The **alanine cycle** is the method the muscle uses to transfer nitrogen to the liver for conversion to urea. When the muscle degrades protein, it uses an aminotransferase to remove the nitrogen from most amino acids; the aminotransferase uses pyruvate as the ammonium acceptor, forming alanine. The alanine is released into the blood stream, to be taken up by the liver. Liver aminotransferases begin the process of diverting the alanine nitrogen to urea production, and the pyruvate produced from deaminated alanine is used as a gluconeogenic substrate. The glucose thus produced is then released into the bloodstream. The muscle can take up glucose for energy and in order to generate more pyruvate for additional nitrogen transport. Like the Cori cycle for lactate, the alanine cycle allows the muscle to “borrow” the liver mitochondria for energy production, and allows the muscle to transfer nitrogen to the liver while using amino acid breakdown for energy.



### Nitrogen excretion

Normal individuals excrete most (80-90%) of nitrogen in urea. However, under some conditions, it may be necessary to excrete protons (for example, during conditions of

high ketone body synthesis). Release of free ammonium into the urine allows excretion of protons, and assists in maintaining plasma pH (note that ammonium excretion requires the loss of counter ions such as chloride).

Excretion of ammonium requires large amounts of water. In fish, this is not a problem; many fish excrete free ammonium, which becomes diluted in their environment.

Urea excretion also requires significant amounts of water. Birds cannot carry large amounts of excess water because of weight considerations. Desert animals do not have large amounts of excess water, due to the difficulty of obtaining water in an arid environment. These animals therefore normally release nitrogen in the form of uric acid and other purines. These molecules are relatively insoluble in water, and are excreted in feces rather than urine. Purine production saves water but is energetically expensive compared to urea.

In humans, overproduction of purine waste products results in a painful accumulation of insoluble uric acid crystals, especially in the joints. This will be discussed later, during discussion of purine metabolism.

**Side note: Clinical measurement of urea**

A frequently performed blood test measures “blood urea nitrogen” (BUN). Blood urea levels can be elevated as a result of a high protein diet, but increases in blood urea are frequently the result of renal disorders. The urea acts as a marker: the urea is not a cause of the problems, because urea is non-toxic in physiologically achievable concentrations. However, significantly elevated urea levels are only present in the bloodstream as a result of kidney dysfunction.

## Summary

The urea cycle is a series of reactions that converts toxic ammonium into the non-toxic nitrogen excretion product urea. The urea cycle requires ornithine as a carbon backbone, and aspartate and free ammonium as nitrogen donors.

The free ammonium used in the urea cycle is largely released from glutamate by glutamate dehydrogenase. This reaction occurs in the mitochondria. The carbamoyl phosphate synthetase I and ornithine transcarbamoylase reactions also occur in the mitochondria, allowing the ammonium to be handled under controlled conditions. The remaining reactions of the urea cycle, however, occur in the cytoplasm.

The urea cycle is regulated by substrate availability and by the enzyme carbamoyl phosphate synthetase I, which is regulated by N-acetylglutamate. Both N-acetylglutamate and ornithine are synthesized from glutamate.

Nitrogen is normally transported around the body in the form of amino acids. The amino acids glutamine and alanine are especially important nitrogen transport molecules. Many tissues incorporate excess nitrogen into glutamine for transport to the liver and kidney. The muscle prefers to use alanine; the alanine cycle allows the muscle to obtain rapidly accessible energy from the liver in the form of glucose while exporting excess nitrogen.

Urea is the major nitrogen excretion product in humans. Free ammonium is also used to excrete nitrogen, under conditions where proton excretion is also necessary. Excretion of both ammonium and urea requires large amounts of water; individuals on high protein diets are at risk for potentially serious dehydration unless they drink large amounts of fluids.