

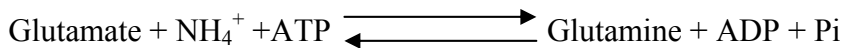
# The Urea Cycle

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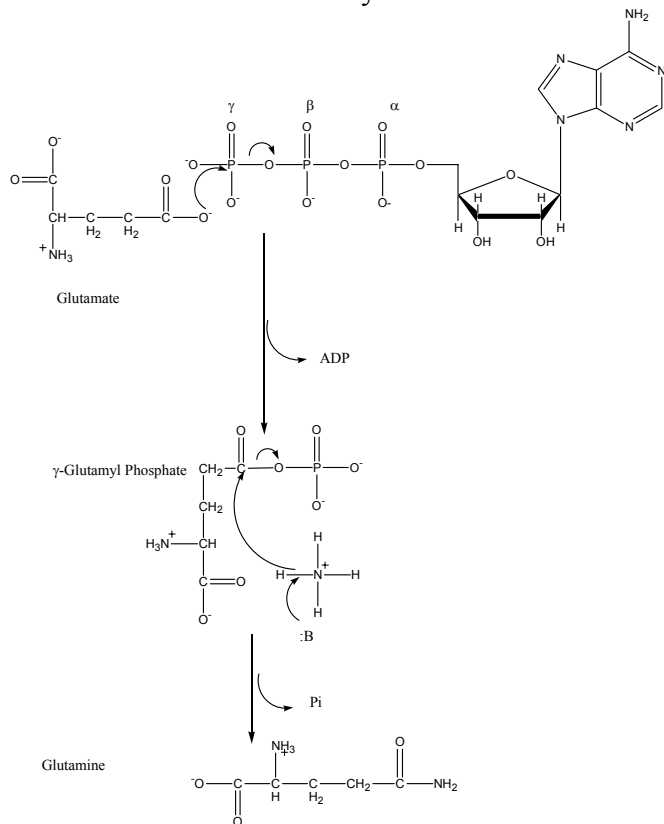
## I. Ammonia Toxicity

Every amino acid contains at least one amino group. Therefore every amino acid degradation pathway has a key step where the amino group is removed. In short amino acid catabolism generates ammonia. Brain tissue is very sensitive to ammonia. Ammonia intoxication produces a comatose state. Cells get rid of excess ammonia by the reductive amination of  $\alpha$ -ketoglutarate to form glutamate by glutamate dehydrogenase and the conversion of glutamate into glutamine by glutamine synthetase. Both of these enzymes are found in high concentrations in the brain. A high concentration of ammonia shifts the equilibrium of these two reactions towards glutamine. Glutamine synthetase use ATP to activate glutamate for amination to form glutamine. The result is high concentrations of ammonia deplete cells of ATP. ATP is essential for brain function.

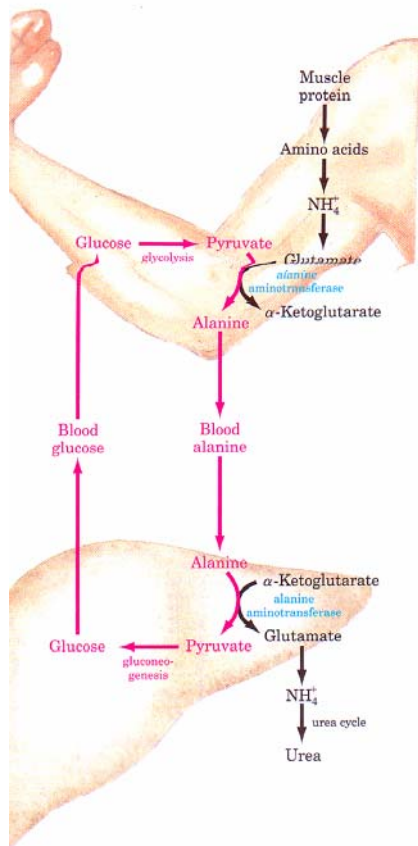


Glutamate is a neurotransmitter. Glutamate is also the precursor for  $\gamma$ -aminobutyrate (GABA) which is another important neurotransmitter. High concentrations of ammonia deplete the concentration of glutamate which produces a similar decrease of GABA which impairs brain function.

## Mechanism of Glutamine Synthetase



Most amino acids are catabolized in the liver. Some of the ammonia produced in amino acid catabolism in the liver is used to synthesize nitrogenous biomolecules such as nucleotides. The excess ammonia is converted into urea for excretion.



Only the liver and kidneys can convert ammonia into urea. Other tissues, such as muscle tissue, catabolize proteins for energy. The ammonia produced is quite toxic to a variety of tissues. Excess ammonia must be converted into a nontoxic compound that can function as an amino group carrier in the blood to transport ammonia from the tissues to the liver where the ammonia will be converted into urea. There are two primary carriers of amino group groups in the blood, glutamine and alanine.

Muscle tissues catabolize amino acids for a source of energy. In the muscle tissue, excess ammonia is transferred to pyruvate to form alanine by alanine aminotransferase. Alanine is released into the blood stream. The liver absorbs the alanine, deaminates it to reform pyruvate.

Other tissues such as brain tissue take excess ammonia, react it with glutamate to form glutamine by the enzymatic action of glutamine synthetase. Glutamine is then released into the blood stream and absorbed by the liver or the kidneys. In the liver and kidneys there is a glutaminase enzyme that hydrolyzes the glutamine into glutamate. Under normal conditions the kidneys extract little glutamine out of the blood stream. But in metabolic acidosis the kidney increases glutamine absorption and hydrolysis. The ammonia produced in the kidneys under these conditions is not converted into urea but excreted as ammonium salts.

## II. The Urea Cycle

Excess amino groups are channeled into a single excretory end product.

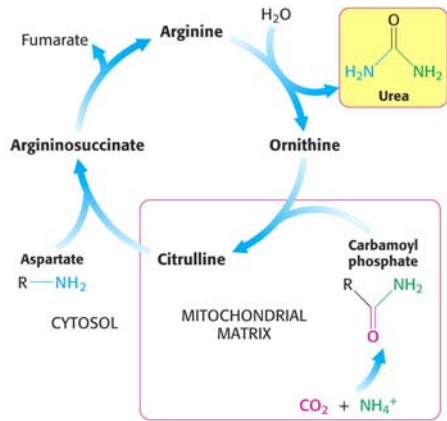
Bony fish excrete ammonia and are thus called **ammonotelic animals**. The liver of these bony fish releases ammonia into the blood stream where it is carried to the gills where it is rapidly cleared.

Birds and reptiles excrete uric acid and thus are called uricotelic animals. Excretion of urea requires lots of water. If birds secreted urea, they would be weighed down by the large volume of water required and would be unable to fly. Instead they secrete uric acid in their feces.

Terrestrial animals such as ourselves excrete urea and are called ureotelic animals.

In ureotelic organisms, excess ammonia is channeled to the mitochondria of liver cells and converted into carbamoyl phosphate. Carbamoyl phosphate enters the nitrogen into the urea cycle. The urea cycle was discovered by none other than Hans Krebs. What a biochemical pioneer he was. The urea produced in the liver is released into the blood stream. The kidneys filter out the urea. The urea is ultimately excreted in the urine.

The urea cycle consists of 4 reactions. The first reaction occurs in the matrix of the mitochondria. The subsequent reactions occur in the cytosol. This is a pathway that spans two cellular compartments. The first reaction is catalyzed by ornithine transcarbamoylase which transfers a carbamoyl group from carbamoyl phosphate to ornithine to form citrulline.

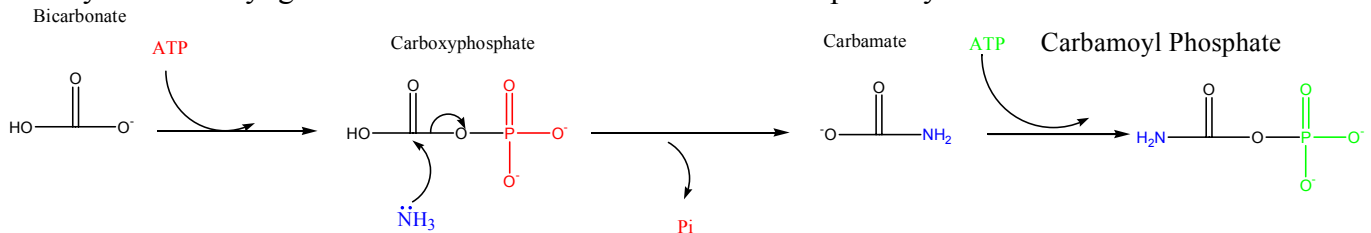


The second reaction is catalyzed by argininosuccinate synthetase. This enzyme uses ATP to activate citrulline by forming a citrullyl-AMP intermediate. This intermediate is attacked by the amino group of an aspartate residue to form argininosuccinate.

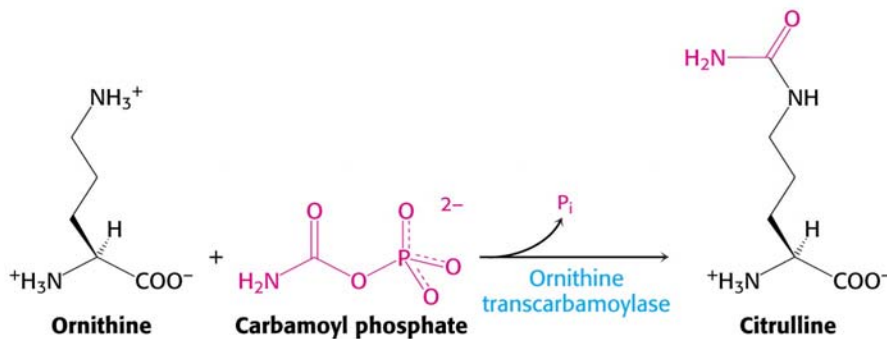
The third step is catalyzed by argininosuccinate lyase which cleaves argininosuccinate into fumarate and arginine.

The last step is catalyzed by arginase which cleaves arginine to produce urea and ornithine completing the cycle.

The first step of the urea cycle begins in the mitochondrial matrix. Ammonia is produced in the mitochondrial matrix by the enzymatic activities of glutaminase and glutamate dehydrogenase. Carbamoyl phosphate synthetase I is the enzyme that takes the ammonia, bicarbonate and 2 molecules of ATP to produce carbamoyl phosphate. This enzyme activates bicarbonate by the same method used by biotin containing enzymes. In fact carbamoyl phosphate synthetase is homologous to the biotin family of enzymes. Carbamoyl phosphate synthetase I activates bicarbonate by phosphorylation with ATP to form carboxyphosphate. Ammonia then reacts with carboxyphosphate to form a carbamate intermediate. A second molecule of ATP is used to phosphorylate the carbamate intermediate to form carbamoyl phosphate. Carbamoyl phosphate synthetase I is the first committed step of the urea cycle. As one would expect this enzyme is allosterically regulated. This enzyme is allosterically activated by N-acetyl glutamate. N-acetylglutamate is a precursor for ornithine biosynthesis and is an essential activator for the urea cycle. N-acetyl glutamate also serves to coordinate related pathways.

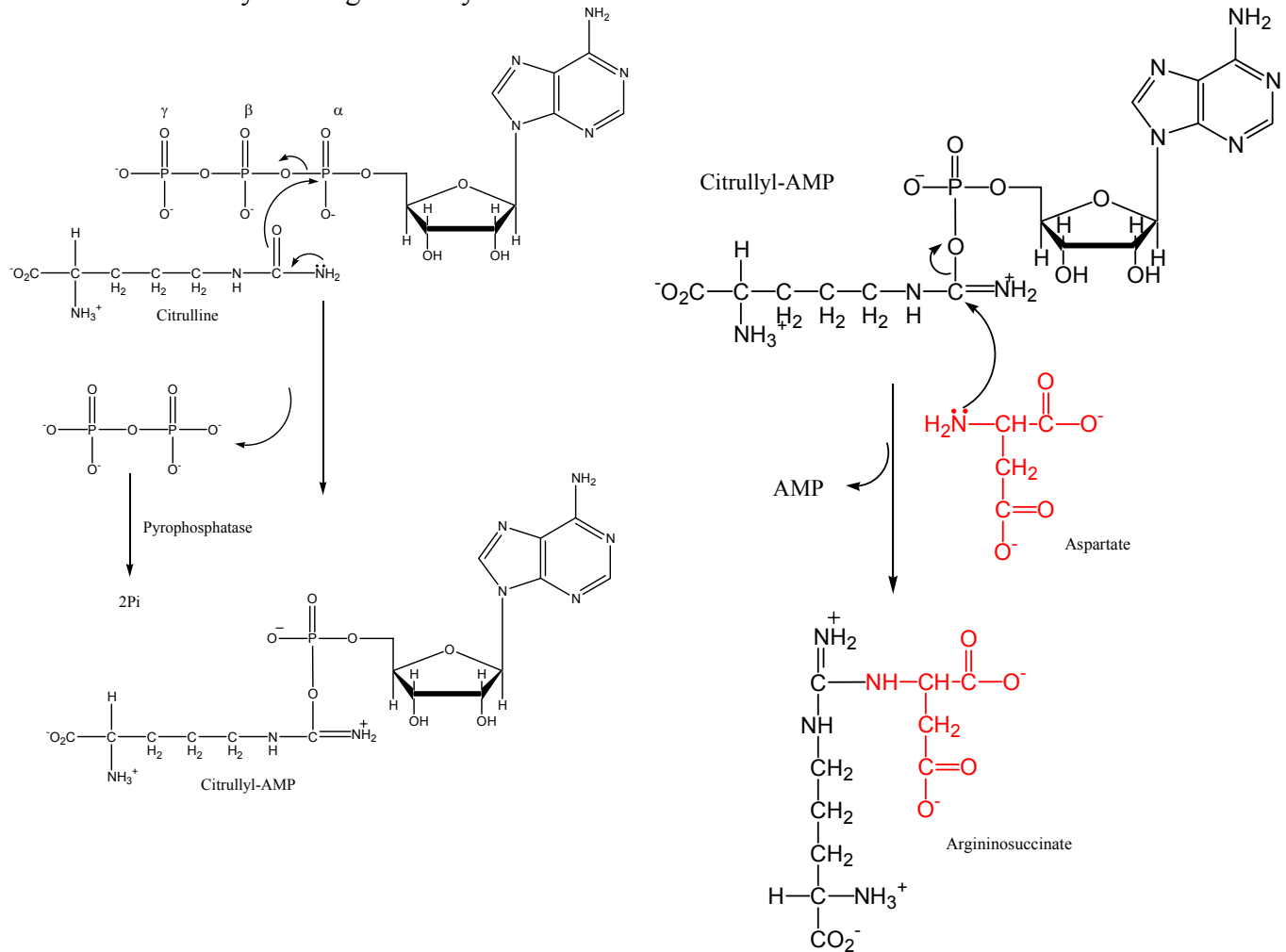


The carbamoyl phosphate formed by this reaction enters into the urea cycle as a substrate for ornithine transcarbamoylase which catalyzes the following reaction.



There is a citrulline transporter that transports citrulline from the matrix of the mitochondria to the cytosol. There is also an ornithine transporter that transport ornithine from the cytosol into the matrix of the mitochondria. The rest of the reactions of the urea cycle occur in the cytosol.

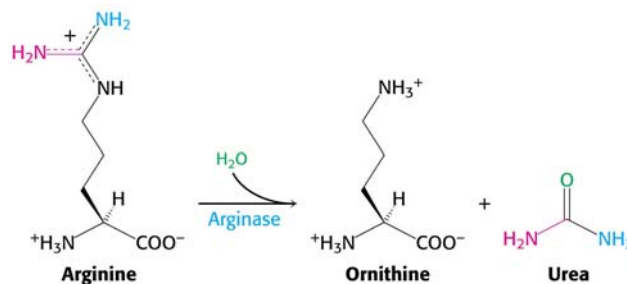
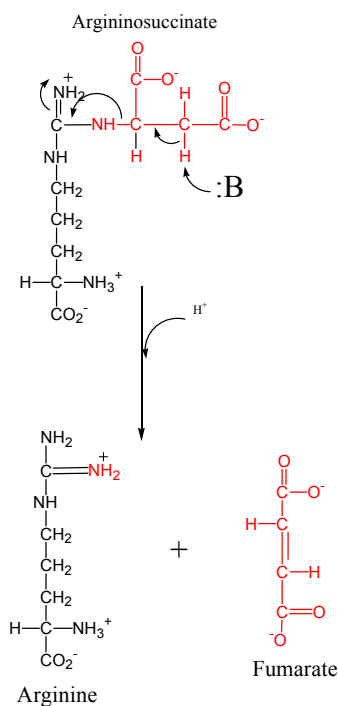
The next step of the urea cycle is catalyzed by argininosuccinate synthetase. This enzyme uses ATP to activate citrulline by forming a citrullyl-AMP intermediate.

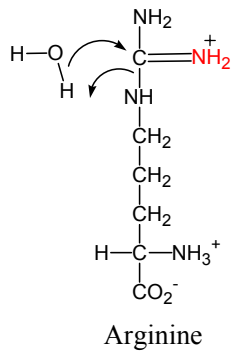


In the second half of the argininosuccinate synthetase reaction, the  $\alpha$ -amino group of aspartate attacks the imino carbon releasing AMP and producing argininosuccinate.

The third step of the urea cycle is catalyzed by argininosuccinate lyase which catalyzes the reaction shown on the left: The products of this reaction are arginine and fumarate. The fumarate product is an important link between the urea cycle and the citric acid cycle.

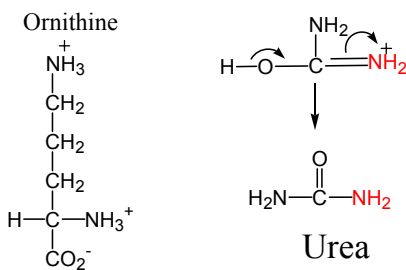
The last reaction of the urea cycle is catalyzed by arginase which catalyzes the hydrolysis of guanidino group to produce urea and ornithine. Ornithine is then transported back in the matrix of the mitochondria and the cycle is complete.





There are two nitrogens in urea. One came from the ammonia produced in the matrix in the mitochondria captured in the form of carbamoyl phosphate. The second nitrogen was the  $\alpha$ -amino group of the aspartate substrate in the argininosuccinate synthetase reaction. The sole carbon of urea was originally the carbon of the bicarbonate.

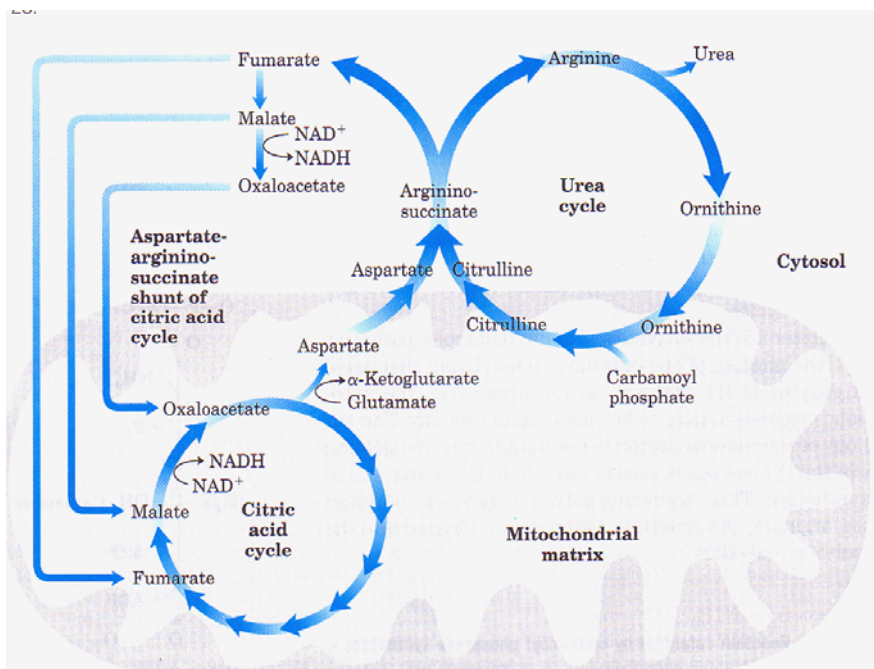
The fumarate formed in the argininosuccinate lyase reaction is an intermediate of the citric acid cycle. There are a variety of ways this fumarate produced in the cytosol can be transported across the inner mitochondrial membrane into the matrix. One is the direct transportation of fumarate across the membrane. There is a cytosolic fumarase enzyme which converts fumarate into malate. Malate can be transported across the inner mitochondrial membrane. There is also a cytosolic malate dehydrogenase which will convert malate into oxaloacetate. Oxaloacetate can also be transported into the matrix of the mitochondria. Oxaloacetate produced this way in the cytosol is a substrate for PEP carboxylase and proceed on through the gluconeogenic pathway.



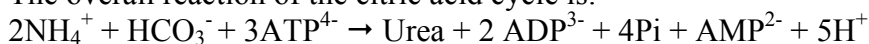
In the mitochondria oxaloacetate is transaminated by glutamate by aspartate amino transferase to produce the aspartate which is then

transported into the cytosol where it is required for the argininosuccinate synthetase reaction. These reactions together are called the aspartate-argininosuccinate shunt.

The summary of the links between the urea cycle and the citric acid cycle is shown below.



The overall reaction of the citric acid cycle is:

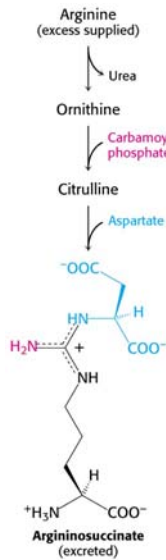


### III. Inherited Deficiencies in the Urea Cycle.

The synthesis of urea in the liver is the primary route of removing excess ammonia. A deficiency in any of the enzymes of the urea cycle has devastating consequences. Deficiencies in the activities of the

enzymes of the urea cycle lead to hyperammonemia. Genetic defects are manifested in hyperammonemia days after birth. The infant becomes lethargic and vomits periodically. Coma and brain death soon follow. A high concentration of ammonia in the infants brains leads to brain swelling. Strategies have been developed to overcome enzyme deficiencies.

### Argininosuccinate lyase deficiency.



For example a genetic defect caused by a deficiency of argininosuccinate lyase can be partially overcome by providing a surplus of arginine and a restricted protein diet. The surplus arginine is converted into urea and ornithine by arginase.

The ornithine produced reacts with carbamoyl phosphate to form citrulline. Citrulline is then condensed with aspartate to form argininosuccinate.

The concentration of argininosuccinate builds up and is excreted. In this way argininosuccinate substitutes for urea in carry nitrogen out of the body.

### Deficiencies in CPSI and Ornithine Transcarbamylase.

With a deficiency of CPSI or ornithine transcarbamylase the ability to synthesize citrulline is impaired. Under these conditions excess ammonia accumulates in the liver and kidneys as glycine and glutamine. The challenge is to get rid of these two amino acids. This is accomplished by supplementing a protein restricted diet with incredibly large amounts of benzoate and phenylacetate. Benzoate is converted into Benzoyl CoA which then reacts with glycine to form hippurate. Likewise phenylacetate is converted into phenylacetyl CoA which then reacts with glutamine to form phenylacetylglutamine. These two compounds can then be excreted to partially overcome the genetic defects.

