The Urea Cycle (Ornithine Cycle) — Introduction and Urea Cycle Disorders

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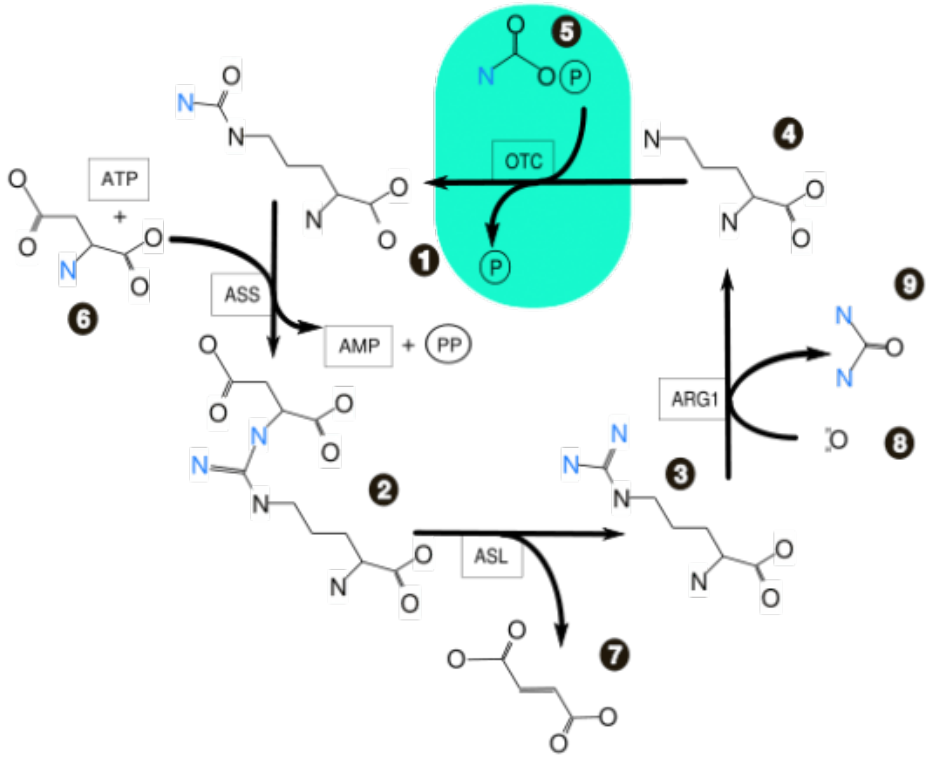
The daily protein turnover of a human being is approximately 300 g. Amino acids contain nitrogen atoms, which need to be eliminated by the body without developing too much toxic ammonia. Here, urea comes into play as an appropriate end product of protein degradation. The steps of synthesis in the urea 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.



Degradation of Proteins

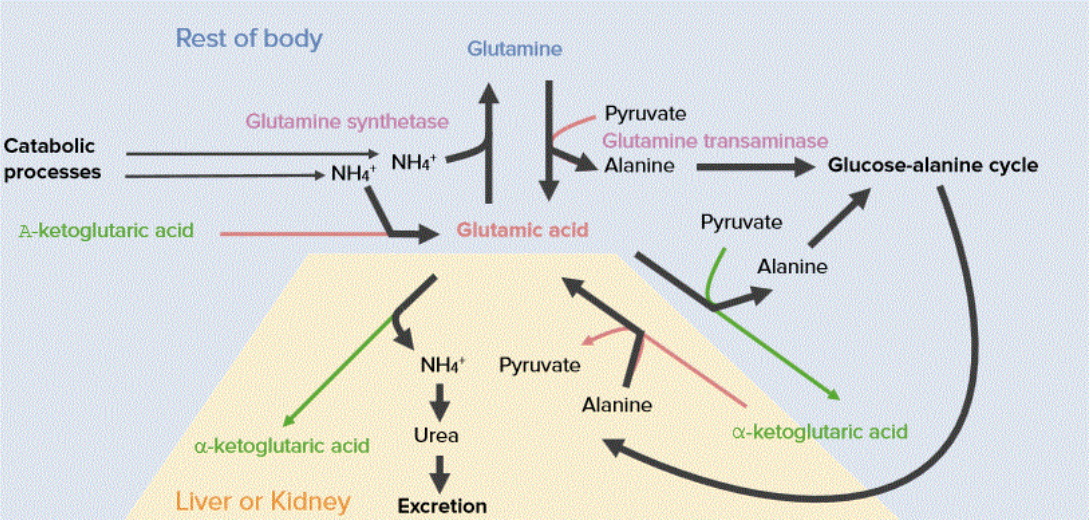
In the context of constant synthesis and breaking down of structural proteins in the body, free amino acids constantly occur which the body can either destroy or reuse for the synthesis of new proteins. The splitting of the amino group from the respective amino acid can occur in two ways. More frequent is the transfer of the amino group to an α-keto acid, which then becomes an amino acid. This process is referred to as **transamination**. Considerably rarer is **deamination**, the liberation of the amino group as ammonia (NH_3). Each transamination needs **pyridoxal phosphate (PLP)** as a cofactor (coenzyme). It is

the active form of **vitamin B6,** which often plays a role in the reactions of amino acid metabolism.

Transport of Nitrogen in the Blood

Via the aforementioned process of **transamination**, nitrogen atoms are bound to amino acids in the form of amino groups in the periphery, mostly cells of the skeletal musculature. They can then be delivered into the bloodstream and reach the liver. Here, amino acids can also be synthesized from intermediate byproducts of major metabolic pathways, like the citric acid cycle for instance.

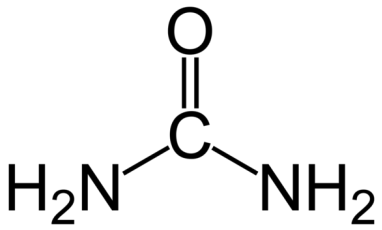
Glutamate is an amino acid central to nitrogen transport. It forms in the peripheral cells of the body via the transfer of an amino group to an α-ketoglutarate. It can serve as a substrate for transamination or it can pick up another amino group and is then delivered into the bloodstream as **glutamine**.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



"The Central Role of Glutamate" Image created by Lecturio

Another amino acid used for nitrogen transport is **alanine**. It represents the most important transport mechanism of nitrogen from the skeletal musculature to the liver. In the peripheral cells, alanine forms when glutamate transfers its amino group onto pyruvate. In the liver cell, alanine is then used for the formation of aspartate from oxaloacetate. After the amino group has been delivered, pyruvate again remains, which is then metabolized in the mitochondria or delivered to the process of gluconeogenesis. **A cycle forms in the latter case if glucose is delivered to the musculature again and oxidized into pyruvate there**. This cycle is also referred to as the **alanine-cycle.**

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 two nitrogen atoms. **Aside from glutamine, it is the most important molecule of the nitrogen transport in the blood.**



Excretion of nitrogen

- Amino acids through transamination make nitrogen mobile
- Toxicity of ammonia means nitrogen balance is critical 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** takes place exclusively in the liver. Therefore, when nitrogen atoms reach the liver via the alanine or glutamate transport system, they can be bound to an end product as urea that is easily secreted. This is extremely important, as the formation of ammonia (NH_3), which is neurotoxic, can be avoided. The reactions of the urea cycle take place in the hepatocytes. The first two of these reactions occur within the mitochondrion, and the following ones in the cytosol. The following schematic image provides an overview of the individual reaction steps of the urea cycle.

Note: The urea cycle takes place exclusively in the liver.

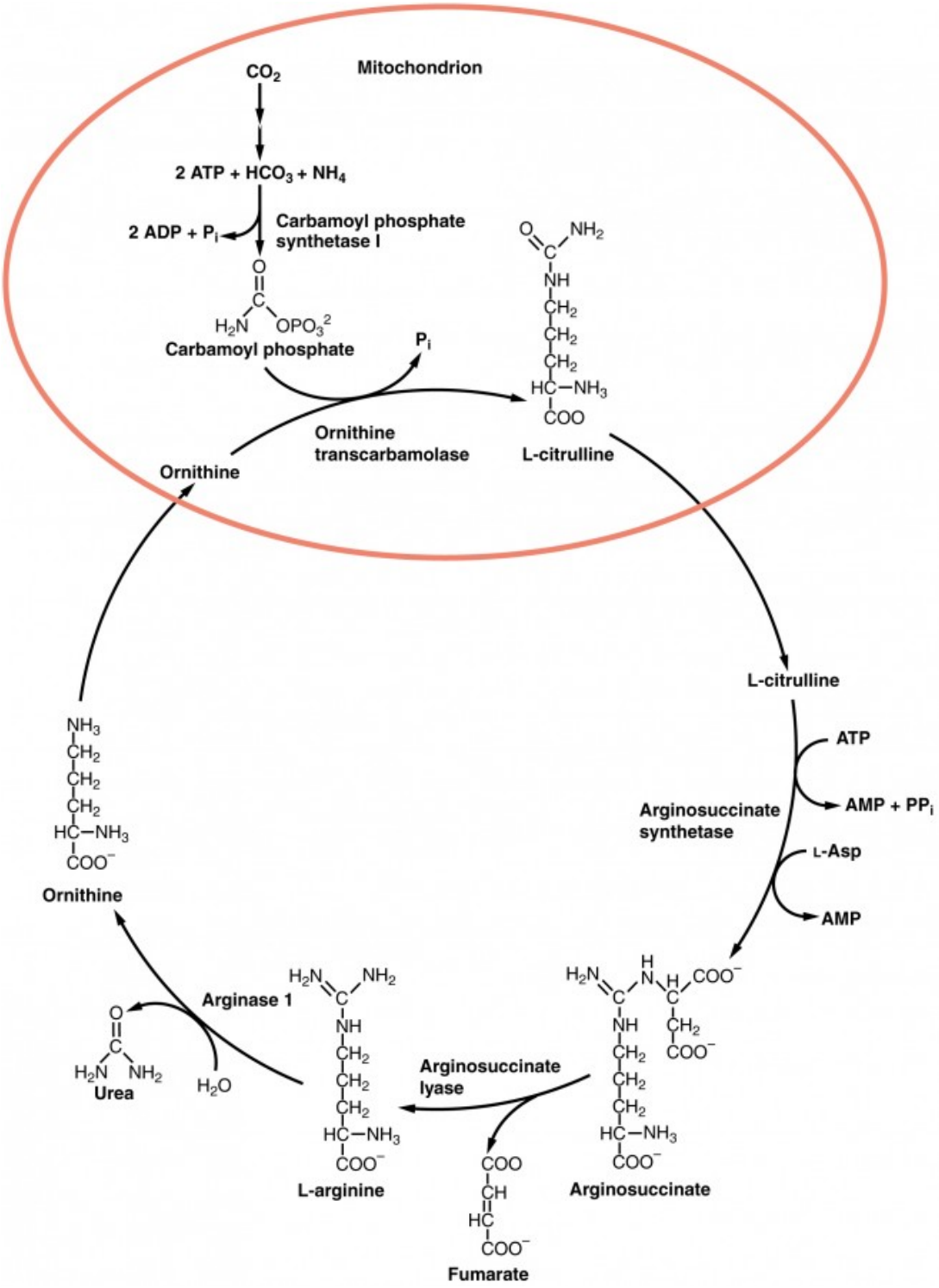


Image: “Urea Cycle” by Phil Schatz. License: CC BY 4.0

Reaction Steps of the Urea Cycle

1. Formation of Carbamoyl Phosphate (Mitochondrion)

Catalyzed by the enzyme **carbamoyl phosphate synthetase 1,** one molecule **carbamoyl phosphate** forms in the mitochondrial matrix from ammonia (NH_3) and CO2. Two molecules of ATP are consumed during this irreversible and committing step of the urea cycle. **Thus, the first nitrogen atom of the urea originates from ammonia from the degradation of amino acids or of purine bases.** The resulting carbamoyl phosphate is strongly polar so that it cannot pass the mitochondrial membrane.

2. Formation of Citrulline (Mitochondrion)

During the second reaction step, the carbamoyl residual of the carbamoyl phosphate is transferred to the amino acid ornithine. This amino acid is non-proteinogenic meaning that the body does not use it for the synthesis of proteins. **Citrulline** forms during this reaction, which is catalyzed by the **ornithine carbamoyl transferase**. Citrulline is non-proteinogenic as well and phosphate remains behind during this step. For the next reaction step, citrulline has to pass the mitochondrial membrane in order to reach the cytosol of the hepatocyte. This occurs via a translocator in the membrane, which exchanges citrulline with ornithine in the antiporter.

3. Formation of Argininosuccinate (Cytosol)

The second reaction occurs in the cytosol during which **argininosuccinate is synthesized** from citrulline and aspartate. Aspartate contains an amino group which binds to citrulline. Here, the second nitrogen atom enters the urea cycle. The responsible enzyme is **argininosuccinate synthetase.** This reaction requires energy, which originates from the hydrolysis of ATP to AMP. Thus, two bonds that are rich in energy are split here.

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

Catalyzed by **argininosuccinate lyase**, argininosuccinate is then split into the proteinogenic amino acid **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, the resulting arginine is hydrolyzed via **arginase** and the whole urea group is split off. During this process, both **urea** and the amino acid ornithine form which is transported back into the mitochondrial matrix via the mitochondrial membrane in exchange for citrulline. There, it is again available for the second reaction step so that the cycle closes here. Via special transport proteins in the cell membrane of the hepatocyte, the resulting urea molecule is delivered into the bloodstream.

Regeneration of Fumarate

During the course of the urea cycle, **fumarate** forms in the cytosol of the hepatocyte during the splitting of argininosuccinate. This is then transformed into **oxaloacetate** via the enzymes **fumarase** and **malate dehydrogenase** with malate as an intermediate product. These steps occur in the citric acid cycle, thus these reactions take place in the cytosol. Oxaloacetate can again be transaminated to aspartate so that another metabolic cycle forms. It is referred to as **aspartate cycle**. As fumarate is also an intermediate product of the **citric acid cycle**, it can be channeled into it as well.

Note: Part of the urea cycle takes place in the mitochondrial matrix, part in the cytosol of the hepatocyte.

Energy Balance of the Urea Cycle

The synthesis of urea is a comparably energy-costly process which is, however, accepted by the body in order to safely eliminate nitrogen. This means especially that the plasma levels of ammonia are to be kept as low as possible. There are two reaction steps in the urea cycle that require energy-rich bonds. **Carbamoyl phosphate synthetase 1** in the

mitochondrion requires two ATP molecules, which are hydrolyzed to ADP. In the cytosol, **arigininosuccinate synthetase** only requires one ATP molecule, which is, however, hydrolyzed twice to AMP and pyrophosphate. The pyrophosphate in the cytosol is very quickly transformed into two phosphate molecules. All in all, the urea cycle therefore requires three ATP molecules and the splitting of four energy-rich bonds.

Note: Even if only three ATP molecules occur, four energy-rich bonds are hydrolyzed in the urea cycle.

Regulation of the Urea Cycle

The pace-limiting reaction determining the synthesis of urea is the first reaction of the urea cycle, which is catalyzed by the **carbamoyl phosphate synthetase 1**. Allosteric binding of **N-acetylglutamate** activates the enzyme. This molecule proportionally increases to the concentrations of glutamate and acetyl-CoA. High concentrations of glutamate reflect a high volume of substrate and acetyl-CoA reflects sufficient amounts of energy-rich substances. If both requirements are present, the liver subsequently increases the synthesis of urea.

Note: The key enzyme of the urea cycle is carbamoyl phosphate synthetase 1.

Elimination of Urea

Approximately 30 g of urea are produced in the liver via the urea cycle and delivered into the blood stream every day. Depending on the amount of proteins 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 can be transported to the kidneys where it is excreted in the urine. Urea is the biggest portion of nitrogen-containing compounds in the urine.

Because urea is filtrated via the glomerular capillaries and partially re-absorbed, it lends itself as a laboratory monitoring parameter for kidney function. Urea concentration in the blood is one of the so-called **parameters of renal function** that – among others – are increased with impaired kidney function. An excess above normal values, along with a variety of clinical symptoms, is also referred to as **uremia**. However changes in daily protein intake must be taken into consideration, as they can render this parameter unreliable.

Urea Cycle Disorders

When the urea cycle in the liver is impaired, ammonia accumulates in the blood. This increased plasma concentration of ammonia (>250 µg/dl) or ammonium, respectively, the ionized form of ammonia, is called **hyperammonemia**. Depending on the severity and the age at manifestation, the neurotoxic ammonia inflicts its damaging effects on the brain and can lead to several neurological symptoms ending in irreversible brain damage and death. Pathophysiologically, a swelling of the astrocytes **due to increased glutamine levels** and the resulting development of brain edema are most likely the reason. The causes are mostly an inherited or acquired impairment of liver function.

Urea Cycle Defects

A possible cause of insufficient function of the urea cycle is a defect in one of the catalyzing enzymes. The following six enzymatic defects are described:

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

All of these metabolic diseases are inherited as an autosomal recessive trait with the exception of OTC deficiency, which is inherited as an X-linked chromosomal recessive trait. In the USA, the incidence rate is approximately 1:8,000. The age of manifestation can vary and may occur in any phase of life. In newborns especially acute courses are extremely life threatening. In advanced puberty and in adults, often diffuse neurological symptoms like headache, lack of concentration, flapping tremor of the hands, vomiting, and lethargy occur. Untreated, these enzymatic defects can lead to mental retardation and death.

The diagnosis can be made on the basis of increased ammonia plasma levels verified by laboratory examinations and substantiated 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. The only curative option remaining is a liver transplant.

Liver Cirrhosis

Another frequent cause is a progressive loss of function of the liver, i.e. due to liver cirrhosis. The lack of capacity for detoxification by the liver leads to increased ammonia levels. If neurological symptoms appear along with it, this is referred to as **hepatic encephalopathy.** Depending on the clinical degree, this disease is divided into stages one to four, where stage four corresponds with coma (hepatic coma). Ammonia concentration in the blood can be decreased with several methods but, in cases of progressive liver cirrhosis, the only curative option remains a liver transplant.

Nitrites

- Nitrite formed by ionization of nitrous acid (HNO_2) or reduction of nitrates
- Nitrite used to cure meats and prevent botulism
- Can be reduced to nitric oxide in hypoxic conditions
- In human diet 80—90 % from reduction of nitrates in vegetables
- Nitrates in vegetables from fertilizers or plant stresses
- Nitrite readily forms cancer-causing nitrosamines in stomach acid
- Nitrites oxidize hemoglobin´s iron from ferrous (II) to ferric (III) state — unable to carry oxygen — can be serious

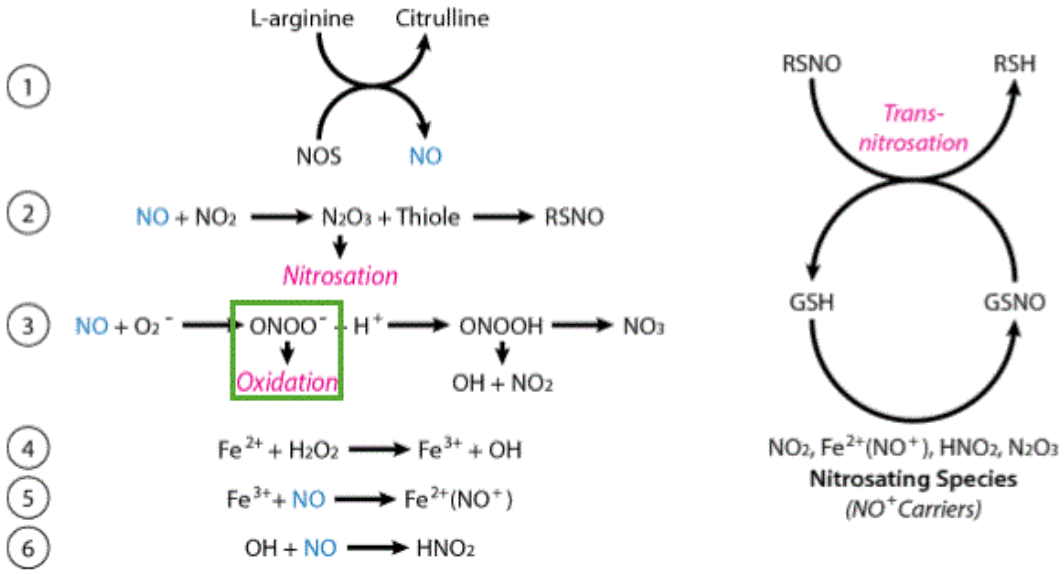
Nitrosamines

- Nitrosamines produced by reaction of nitrites and secondary amines, such as proline
- Strong acids (stomach) or high temperatures of frying favor production
- Found in processed meats, beer, cigarette smoke, chewing tobacco
- Formation inhibited by vitamin C
- Nitrosamines form DNA adducts and cause cancer in many animal species
- Likely carcinogens in humans
- Evidence for gastric and esophageal cancer risk

- Nitrosamines in tobacco form from nicotine
- NNK is nicotine derived and important in carcinogenesis
- NNK in tobacco and e-cigarettes
- NNK activation by P-450 activated signaling cascades & uncontrolled growth

Reactive Nitrogen Species

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



"Reactive nitrogen species can arise from any of the molecules described here" Image created by Lecturio

Review Questions

The answers can be found below the references.

1. Which statement is false concerning the urea cycle?

A. Ornithine is exchanged with citrulline in the antiporter via the mitochondrial membrane.
B. For the purpose of the synthesis of argininosuccinate, two energy-rich bonds are split.
C. The formation of citrulline occurs in the cytosol.
D. The enzyme arginase catalyzes a hydrolysis.
E. The formation of argininosuccinate occurs in the cytosol.

2. Which of the following enzymes determines the activity of the urea cycle?

A. Argininosuccinate synthetase
B. Arginase

C. Glutamate pyruvate transaminase
D. Malate dehydrogenase
E. Carbamoyl phosphate synthetase 1

3. Which is not a typical symptom of hyperammonemia?

A. Agitation
B. Sensitivity deficits in the lower extremity
C. Somnolence
D. Enormous tremor of the hands
E. Concentration weakness

References

Lowenthal, A., Mori, A., &Marescau, B. (1982). *Urea Cycle Diseases*. Boston, MA: Springer US.

Mönch, E. (2014). *Deficiencies of the urea cycle – clinical significance and therapy*. Bremen: UNI-MED-Verl.

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Correct answers: 1C 2E 3B

Notes