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UREA CYCLE

Metabolic pathway

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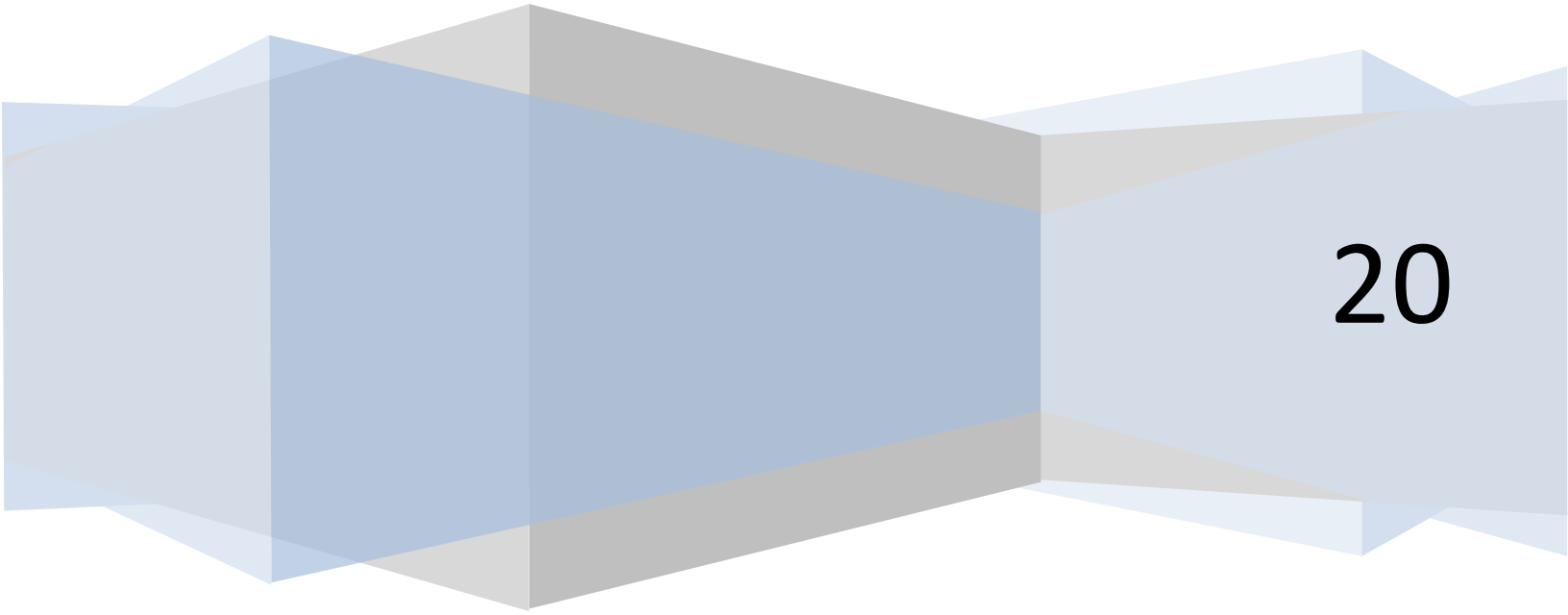

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Urea is the **end product of protein metabolism** (amino acid metabolism). The nitrogen of amino acids, converted to ammonia is toxic to the body. It is converted to urea and detoxified. As such, urea accounts for 80-90% of the nitrogen containing substances excreted in urine. Urea is **synthesized in liver** and transported to kidneys for excretion in urine. Urea cycle is the **first metabolic cycle** that was elucidated by Hans Krebs and Kurt Henseleit (1932), hence it is known as **Krebs-Henseleit cycle**.

Urea has **two amino (NH_2) groups**, one derived **from NH_3** and the other **from aspartate.** Carbon atom is supplied by CO_2. Urea synthesis is a five-step cyclic process, with five distinct enzymes. The first **two enzymes** are present in **mitochondria** while the **rest** are localized in **cytosol**. The details of urea cycle are described (**Figures 1& 2)**.

1. **Synthesis of carbamoyl phosphate**

Carbamoyl phosphate synthase I (CPS I) of mitochondria catalyses the condensation of NH_4^+ ions with CO_2 to form carbamoyl phosphate. This step consumes two ATP and is **irreversible**, and **rate-limiting**. CPS I requires **N-acetylglutamate** for its activity. Another enzyme, carbamoyl phosphate synthase II (CPS II) involved in pyrimidine synthesis is present in cytosol. It accepts amino group from glutamine and does not require N-acetylglutamate for its activity.

2. **Formation of citrulline**

Citrulline is synthesized from carbamoyl phosphate and ornithine by ornithine transcarbamoylase. Ornithine is regenerated and used in urea cycle. Therefore, its role is comparable to that of oxaloacetate in citric acid cycle. Ornithine and citrulline are basic amino acids. (They are never found in protein structure due to lack of codons). Citrulline produced in this reaction is transported to cytosol by a transporter system.

3. **Synthesis of arginosuccinate**

Arginosuccinate synthase condenses citrulline with aspartate to produce arginosuccinate. The second amino group of urea is incorporated in this reaction. This step requires ATP which is cleaved to AMP and pyrophosphate (PPi). The latter is immediately broken down to inorganic phosphate (Pi).

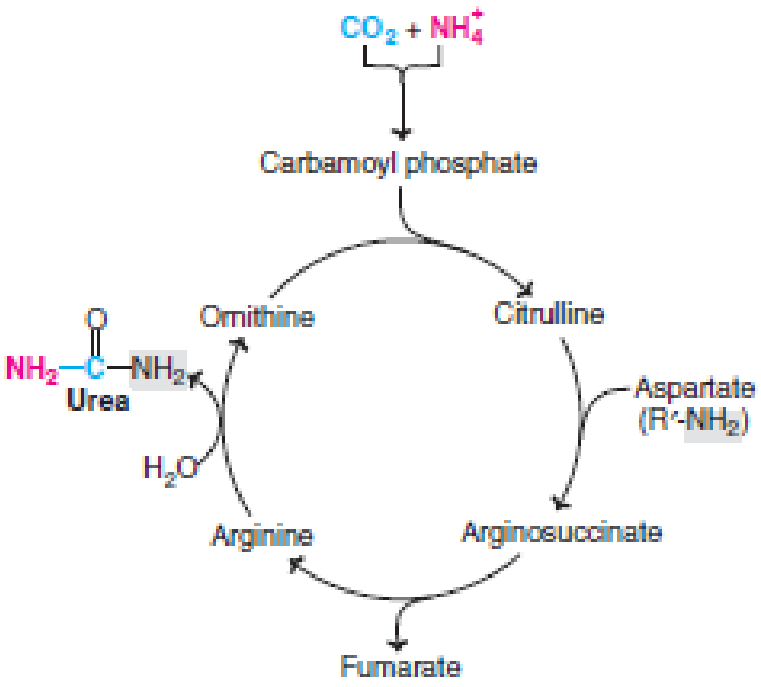

Figure 1: Outline of urea cycle.

4. **Cleavage of arginosuccinate**

Arginosuccinase cleaves arginosuccinate to give arginine and fumarate. Arginine is the immediate precursor for urea. Fumarate liberated here provides a connecting link with TCA cycle, gluconeogenesis etc.

5. **Formation of urea**

Arginase is the fifth and final enzyme that cleaves arginine to yield urea and ornithine. Ornithine, so regenerated, enters mitochondria for its reuse in the urea cycle. Arginase is activated by Co_2^+ and Mn_2^+. **Arginase** is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues. For this reason, arginine synthesis may occur to varying degrees in many tissues. But only the liver can ultimately produce urea.

Overall reaction and energetics

The urea cycle is irreversible and consumes 4 ATP. Two ATP are utilized for the synthesis of carbamoyl phosphate. One ATP is converted to AMP and PPi to produce arginosuccinate which equals to 2 ATP. Hence **4 ATP** are actually **consumed**.

$NH4^+$ + CO2 + Aspartate + 3ATP →Urea+ Fumarate + 2 ADP + 2 Pi + AMP + PPi

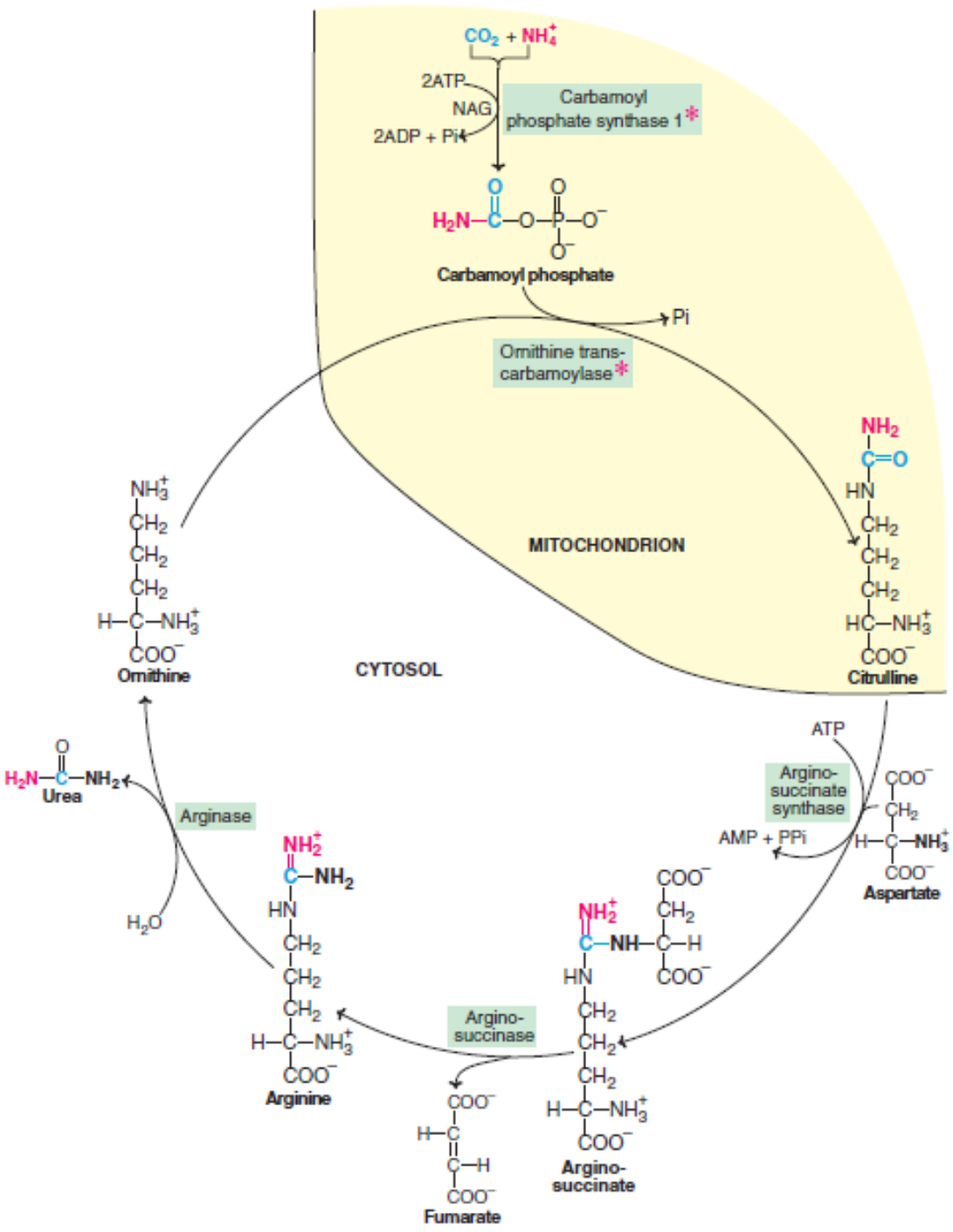

Figure 2: Reactions of urea cycle (NAG—N-acetylglutamate; in the formation of urea, one amino group is derived from free ammonium ion while the other is from aspartate; carbon is obtained from CO2; * mitochondrial enzymes, the rest of the enzymes are cytosomal).

Regulation of urea cycle

The first reaction catalysed by **carbamoyl phosphate synthase I** (CPS I) is **rate-limiting** reaction or committed step in urea synthesis. CPS I is allosterically activated by N-acetylglutamate (NAG). The rate of urea synthesis in liver is correlated with the concentration of N-acetylglutamate. High concentrations of arginine increase NAG. The consumption of a **protein-rich meal** increases the level of NAG in liver, leading to enhanced urea synthesis. Carbamoyl phosphate synthase I and glutamate dehydrogenase are localized in the mitochondria. They coordinate with each other in the formation of NH_3, and its utilization for the synthesis of carbamoyl phosphate. The remaining four enzymes of urea cycle are mostly controlled by the concentration of their respective substrates.

Integration between urea cycle and TCA cycle

Urea cycle is linked with TCA cycle in three different ways (**Figure 3**). This is regarded as **bicyclic integration** between the two cycles.

1. The production of **fumarate** in urea cycle is the most important integrating point with TCA cycle. Fumarate is converted to malate and then to oxaloacetate in TCA cycle. Oxaloacetate undergoes transamination to produce aspartate which enters urea cycle. Here, it combines with citrulline to produce arginosuccinate. Oxaloacetate is an important metabolite which can combine with acetyl CoA to form citrate and get finally oxidized. Oxaloacetate can also serve as a precursor for the synthesis of glucose (gluconeogenesis).

2. ATP (12) are generated in the TCA cycle while **ATP** (4) are utilized for urea synthesis.

3. Citric acid cycle is an important metabolic pathway for the complete oxidation of various metabolites to CO_2 and H_2O. The **CO_2** liberated in TCA cycle (in the mitochondria) can be utilized in urea cycle.

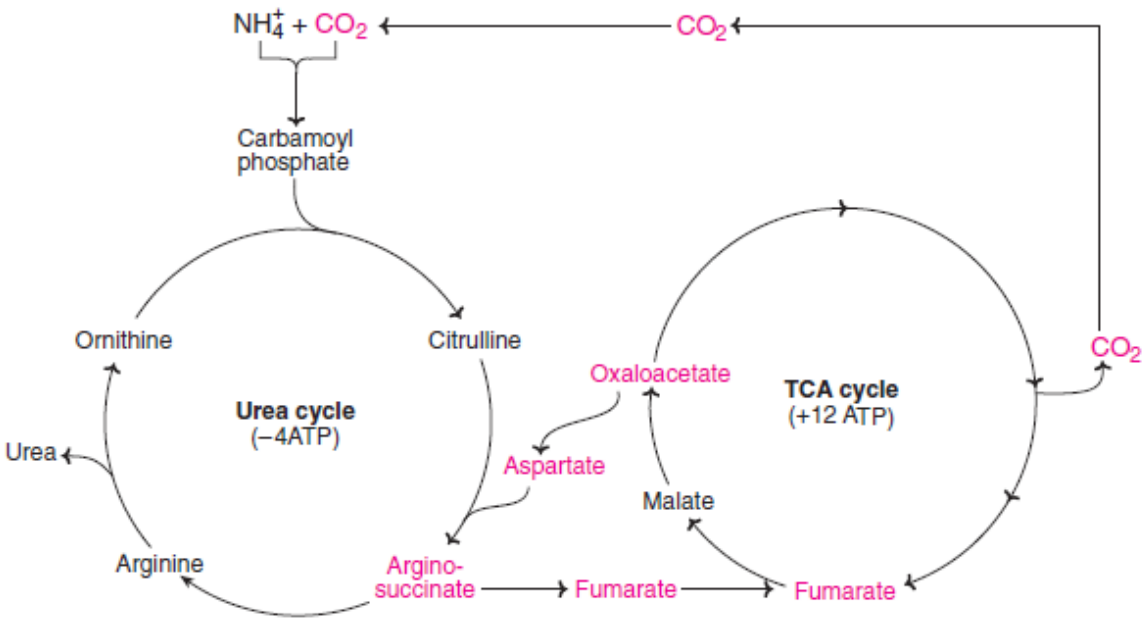

Figure 3: Interrelation between urea and tricarboxylic acid (TCA) cycle

Metabolic disorders of urea cycle

Metabolic defects associated with each of the five enzymes of urea cycle have been reported are given below. All the disorders invariably lead to a build-up in blood ammonia **(hyperammonemia)**, leading to toxicity. Other metabolites of urea cycle also accumulate which, however, depends on the specific enzyme defect. The clinical symptoms associated with defect in urea cycle enzymes include vomiting, lethargy, irritability, ataxia and mental re tardation.

Defect	*Enzyme involved*
Hyperammonemia type I	Carbamoyl phosphate synthase I
Hyperammonemia type II	Ornithine transcarbamoylase
Citrullinemia	Arginosuccinate synthase
Arginosuccinic aciduria	Arginosuccinase
Hyperargininemia	Arginase