UREA FORMATION (KREBS-HENSELEIT CYCLE)

The removal of excess of NH3 derived from amino acid catabolism in the tissues or from bacterial action in the gut is accomplished by the production of urea which is excreted in the urine. Steps of urea synthesis have been elucidated by **Krebs** and **Henseleit** (1932)

Characteristic Features • It is a ***cyclic process***, **five reactions** which involves ***ornithine, citrulline, arginine and aspartic acid.*** • ***Site of synthesis:*** urea formation takes place in liver in mammals and all of the enzymes involved have been isolated from Liver tissue **Note *Other Organs*** • **Kidneys:** Urea cycle operates in a limited extent. Kidney can form up to ariginine but cannot form urea, ***as enzyme arginase is absent in kidney*** tissues.

• **Brain:** Brain can synthesise urea from citrulline, but lacks the enzyme for forming citrulline from ornithine. ***Thus, neither the kidneys nor the brain can form urea in significant amounts.***

• ***Location of enzymes:*** It is partly mitochondrial and partly cytosolic. • Two mol. of NH_3 and one mol. of CO_2 are converted to one mol. of urea for each turn of the cycle and ***orinithine is regenerated at the end, which acts as a catalytic agent.*** • The overall process in each turn of cycle ***requires 3 mols of ATP. Evidences to show that the synthesis occurs only in Liver:***

1. If kidneys are removed in an experimental animal, there is sharp rise in blood urea level. **2.** The above can be prevented if Liver is also removed. **3.** The enzyme *Arginase* has only been isolated from Liver. The enzyme is absent in kidneys, Brain (activity is very low) and other tissues. **4.** In cirrhosis liver, functioning of liver is much below normal, blood urea levels decrease with a simultaneous increase in NH3. Similar results are seen where the liver is excluded from circulation by an anastomosis between portal vein and vena cava (portocaval shunt). ***Stages*** The reactions of urea cycle can be studied in **five** sequential enzymatic reactions. • ***Reaction 1:*** Synthesis of carbamoyl-phosphate • ***Reaction 2:*** Synthesis of citrulline • ***Reaction 3:*** Synthesis of argininosuccinate • ***Reaction 4:*** Cleavage of argininosuccinate • ***Reaction 5:*** Cleavage of arginine to form ornithine and urea

Reaction 1: Synthesis of Carbamoyl-P (Mitochondrial) In this reaction, HCO_3, NH^{+4} and phosphate derived from ATP reacts to form **carbamoyl-P** (also called **Carbamyl-P**). The reaction is catalysed by the mitochondrial-enzyme ***Carbamoyl phosphate synthetase 1.*** There are **2 types** of the enzyme:

• ***Carbamoyl synthetase I:*** Occurs in mitochondria of Liver cells. It is involved in urea synthesis. • ***Carbamoyl synthetase II:*** Present in cytosol of liver cells which is involved in pyrimidine synthesis (Refer to metabolic fate of carbamoyl-P). Mitochondrial carbamoyl phosphate synthetase I catalyses the ATP-dependant conversion of HCO^{-3} and NH^{+4} to the energy-rich, mixed anhydride carbamoyl phosphate.

• Nucleophilic attack of HCO– 3 on the γ-phosphoryl group of ATP produces the energy-rich intermediate carboxyl-P and releases ADP. • Nucleophilic addition of NH3 to the carbon of carboxyl phosphate, followed by elimination of Pi, yields carbamate.
• A second-phosphoryl group transfer from another ATP to the carboxylate oxygen of carbamate produces carbamoyl-P.

Role of N-acetyl Glutamate (AGA) Exact role of N-acetyl glutamate is not known. Its presence brings about some conformational changes in the enzyme molecule and ***affects the affinity of the enzyme for ATP.***

Reaction 2: Synthesis of Citrulline: (Mitochondrial) ***Ornithine transcarbamoylase*** enzyme, also called as ***ornithine carbamoyl transferase*** is found associated with carbamoylphosphate synthetase I in the mitochondrial matrix. • It catalyses the nucleophilic addition of ornithine to the carbonyl group of carbamoyl-P to produce Citrulline. • During this reaction, the δ-NH2 group of ornithine attaches to the carbonyl group

of carbamoyl-P and the phosphate group (Pi) is released. **Note 1.** Ornithine which is regenerated in cytosol in the 5th reaction, is transported into the mitochondrial matrix by a specific ***transport protein*** in the inner mitochondrial membrane. **2.** Similarly Citrulline which is produced in mitochondrial matrix is transported across the inner mitochondrial membrane to the cytosol by a specific ***transport protein***.

Reaction 3: Synthesis of Argininosuccinate: (cytosolic): • After citrulline has been transported to the cystosol, it condenses with Aspartate to form argininosuccinate in an ATP-dependant reaction catalysed by ***argininosuccinate synthetase.*** •

Reaction 4: Cleavage of Argininosuccinate: (Cytosolic) • In this reaction of urea cycle, the enzyme ***argininosuccinase*** also known as ***Argininosuccinate Lyase*** catalyses conversion of Argininosuccinate to arginine and fumarate. ***The urea cycle is linked to the TCA cycle through the production of fumarate. Amino acid catabolism, is therefore directly coupled to energy production.*** • *Argininosuccinase* is ***cold-labile enzyme*** of mammalian liver and kidney tissues. Loss of activity in the cold is associated with dissociation into two-protein components. This dissociation is prevented by Pi, arginine, and argininosuccinate.
Fate of fumarate: The fumarate is converted to oxaloacetate (OAA) via the *fumarase* and *malate dehydrogenase* reactions and then transaminated to regenerate aspartate to participate in the cycle.

Reaction 5: Cleavage of Arginine to Ornithine and Urea
The last reaction of the urea cycle completes the cycle. It is catalysed by the enzyme ***arginase,*** which is found only in the liver cells. Arginase catalyses hydrolysis of the guanidine group of arginine, ***releasing urea*** and ***regenerating ornithine.*** Ornithine now enters mitochondrion through inner mitochondrial membrane by a specific transport protein. Highly purified *arginase* from mammalian liver cells is activated by CO^{++} and Mn^{++}

1. Detoxification of NH_3: Major biological role of this pathway is the detoxication of NH3. Toxic ammonia is converted into a nontoxic substance urea and excreted in urine. 2. Biosynthesis of arginine: The urea cycle also serves for the biosynthesis of arginine from ornithine in liver, kidney and intestinal mucosa. Kidney and intestinal mucosa probably contribute most of the body arginine because they possess all the urea cycle enzymes except arginase. Hence they can form upto arginine and cannot form urea. The arginine is used for protein synthesis. **Source of C and N of Urea**
• One Nitrogen of NH2 group is derived from the NH+4 ion (Reaction 1).
• Other Nitrogen of NH2 group is provided by Aspartate (Reaction 3).
• Bicarbonate, HCO^- ion, provides the carbon atom of urea.

INHERITED DISORDERS ASSOCIATED WITH UREA CYCLE Inherited disorders due to inherited deficiency of enzymes of urea cycle have been described:
1. Hyperammonemia Type I: A familial disorder, enzyme deficiency: Carbamoyl-PSynthetase I, produces hyperammonemia and symptoms of ammonia toxicity.
2. Hyperammonemia Type II: (Also called Ornithinemia) • Inheritance: X-chromosome linked • Enzyme deficiency: Ornithine transcarbamoylase
• Produces hyperammonemia and symptoms of NH3 toxicity. Citrullinemia A rare disorder • Inheritance: Autosomal recessive • Enzyme deficiency: Argininosuccinate synthetase. • Two types of deficiency: (a) One type: Mutation in regulator gene: Enzyme is absent in Liver, normal Km for citrulline. (b) Other type: Mutation in structural gene: Amount of enzyme normal in liver, affects catalytic site and has abnormally high Km for citrulline. • Clinically: Presents with hyperammonemia and NH3 toxicity, and mental retardation

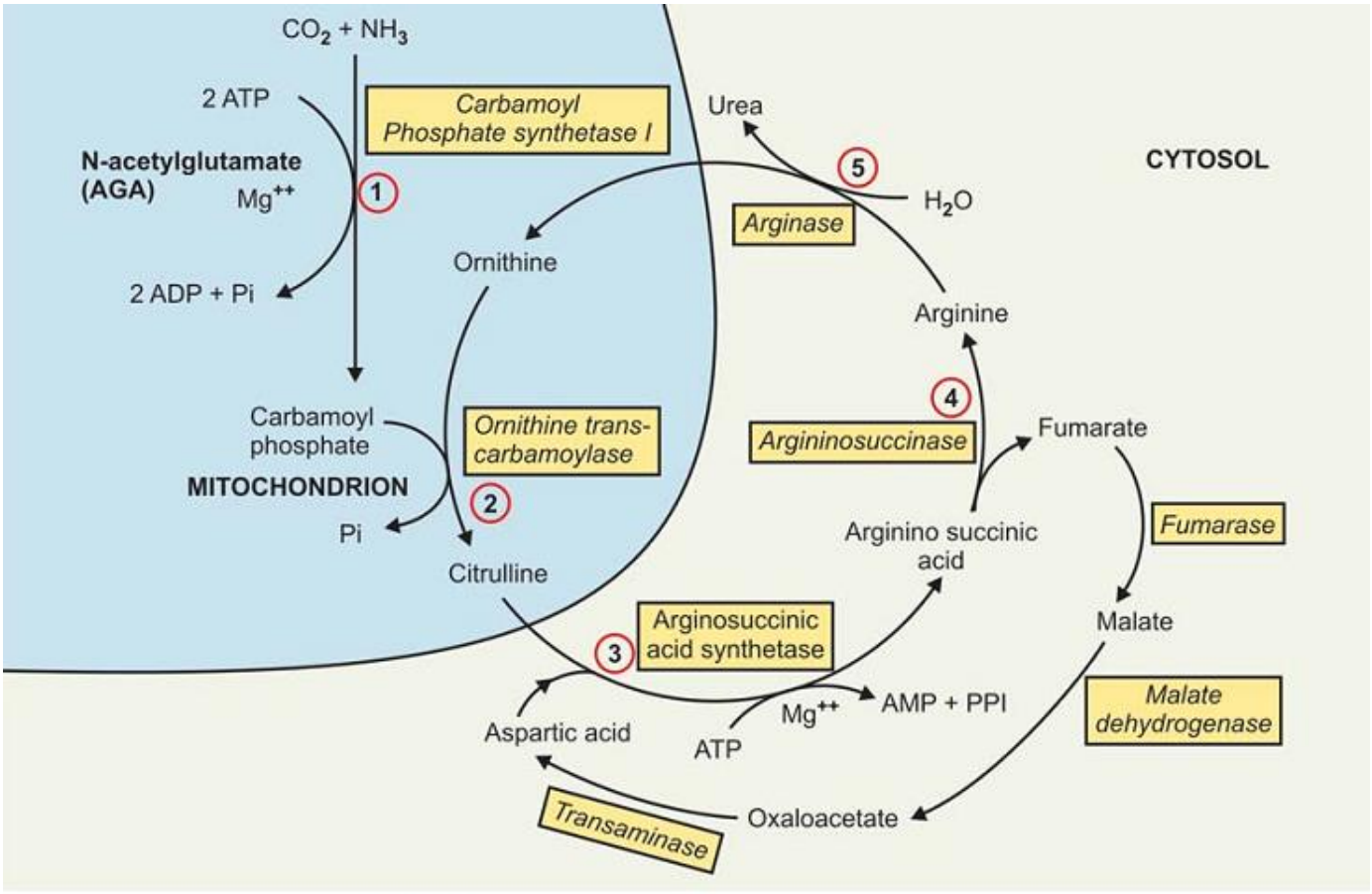

Fig. 27.4: Biosynthesis of urea or ornithine—urea cycle