COMPILED AND CIRCULATED BY DR. POULAMI ADHIKARY MUKHERJEE, ASSISTANT PROFESSOR, DEPARTMENT OF ZOOLOGY, NARAJOLE RAJ COLLEGE

UREA CYCLE

BY

DR. POULAMI ADHIKARY MUKHERJEE
ASSISTANT PROFESSOR
DEPARTMENT OF ZOOLOGY
NARAJOLE RAJ COLLEGE

ZOOLOGY: SEM- III, PAPER- C7T: FUNDAMENTALS OF BIOCHEMISTRY, UNIT 3: PROTEINS

Definition of Urea Cycle:

The urea cycle (also known as the ornithine cycle) is a cycle of biochemical reactions that produces urea $(NH_2)_2CO$ from ammonia (NH_3). This cycle involves a cyclic sequence of metabolic reactions occurring mainly in the liver and to some extent in the kidney where ammonia is converted to urea for removal of the ammonia produced in the metabolism of amino acids.

Features of Urea Cycle:

- The urea cycle is the metabolic pathway that transforms nitrogen to urea for excretion from the body.

- It is a metabolic process by which ammonia derived from amino acids is converted into urea in the liver.

- Nitrogenous excretory products are removed from the body mainly in the urine.

- Ammonia, which is very toxic in humans, is converted to urea, which is nontoxic, very soluble, and readily excreted by the kidneys.

- The urea excreted each day by a healthy adult (about 30 g) accounts for about 90% of the nitrogenous excretory products.

- **Urea is formed in the urea cycle from NH_4^+, CO_2, and the nitrogen of aspartate.**

- **This cycle occurs in ureotelic organisms.**

- **The urea cycle converts highly toxic ammonia to urea for excretion.**

- This cycle was the first metabolic cycle, which was discovered by Hans Krebs and Kurt Henseleit, 1932, five years before the discovery of the TCA cycle.

- Later on, this cycle was described in more detail by Ratner and Cohen.

- The urea cycle takes place primarily in the liver and, to a lesser extent, in the kidneys.

Location of Urea Cycle:

Cytosol and mitochondria of hepatocytes.

- **Substrates: NH_3 (as derived from oxidative deamination of glutamate); CO_2; aspartate; three ATP.**
- **Products: Urea; fumarate; H_2O.**

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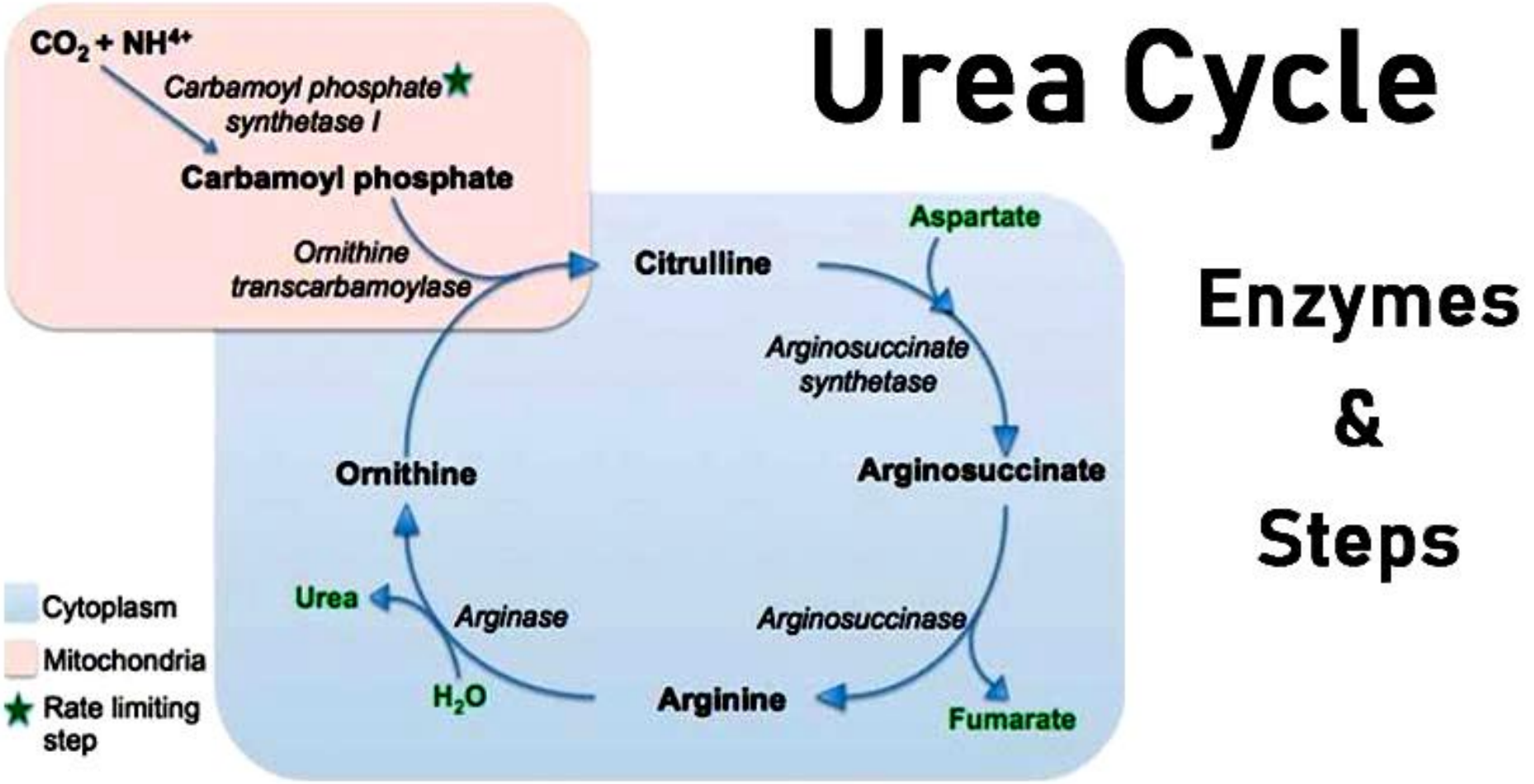

Steps in the Urea Cycle:

1. Transport of nitrogen to the liver

- Ammonia is very toxic, particularly to the central nervous system.

- The concentration of ammonia and ammonium ions in the blood is normally very low.

$$(NH_3 + H^+ \leftrightarrow NH_4^+)$$

- Ammonia travels to the liver from other tissues, mainly in the form of alanine and glutamine.

- It is released from amino acids in the liver by a series of transamination and deamination reactions.

- Ammonia is also produced by bacteria in the gut and travels to the liver via the hepatic portal vein.

2. Reactions of the urea cycle:

NH_4^+ and aspartate provide the nitrogen that is used to produce urea, and CO_2 provides the carbon. Ornithine serves as a carrier that is regenerated by the cycle.

- Carbamoyl phosphate is synthesized in the first reaction from NH_4^+, CO_2, and two ATP. Inorganic phosphate and two ADP are also produced.

- Enzyme: carbamoyl phosphate synthetase I, which is located in mitochondria and is activated by N-acetylglutamate.

- Ornithine reacts with carbamoyl phosphate to form citrulline. Inorganic phosphate is released.

- Enzyme: ornithine transcarbamoylase, which is found in mitochondria. The product, citrulline, is transported to the cytosol in exchange for cytoplasmic ornithine.

- Citrulline combines with aspartate to form argininosuccinate in a reaction that is driven by the hydrolysis of ATP to AMP and inorganic pyrophosphate.

- Enzyme: Argininosuccinate synthetase

- Argininosuccinate is cleaved to form arginine and fumarate.

- Enzyme: argininosuccinate lyase. This reaction occurs in the cytosol.

- The carbons of fumarate, which are derived from the aspartate added in reaction 3, can be converted to malate.

- In the fasting state in the liver, malate can be converted to glucose or to oxaloacetate, which is transaminated to regenerate the aspartate required for reaction 3.

- Arginine is cleaved to form urea and regenerate ornithine.

- Enzyme: arginase, which is located primarily in the liver and is inhibited by ornithine.

- Urea passes into the blood and is excreted by the kidneys.

- Ornithine is transported back into the mitochondrion (in exchange for citrulline) where it can be used for another round of the cycle.

- When the cell requires additional ornithine, it is synthesized from glucose via glutamate.

- Arginine is a nonessential amino acid in adults. It is synthesized from glucose via ornithine and the first four reactions of the urea cycle.

Steps and Reactions of Urea Cycle:

1. Formation of Carbamoyl Phosphate

2. Synthesis of Citrulline

3. Synthesis of Argininosuccinate

4. Cleavage of Argininosuccinate

5. Cleavage of Arginine

The entire process converts two amino groups, one from NH_4^+ and one from Aspartate, and a carbon atom

from HCO^{-3}, to the relatively nontoxic excretion product urea at the cost of four "high-energy" phosphate bonds (3 ATP hydrolyzed to 2 ADP and one AMP). The conversion from ammonia to urea happens in five main steps. The first is needed for ammonia to enter the cycle and the following four are all a part of the cycle itself. To enter the cycle, ammonia is converted to carbamoyl phosphate. The urea cycle consists of four enzymatic reactions: one mitochondrial and three cytosolic.

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Reactions of the urea cycle

Step	Reactants	Products	Catalyzed by	Location
1	$NH_3 + HCO^-_3 + 2ATP$	carbamoyl phosphate + $2ADP + P_i$	CPS1	mitochondria
2	carbamoyl phosphate + ornithine	citrulline + P_i	OTC, zinc, biotin	mitochondria
3	citrulline + aspartate + ATP	argininosuccinate + AMP + PP_i	ASS	cytosol
4	argininosuccinate	arginine + fumarate	ASL	cytosol
5	arginine + H_2O	ornithine + urea	ARG1, manganese	cytosol

1. Formation of Carbamoyl Phosphate:

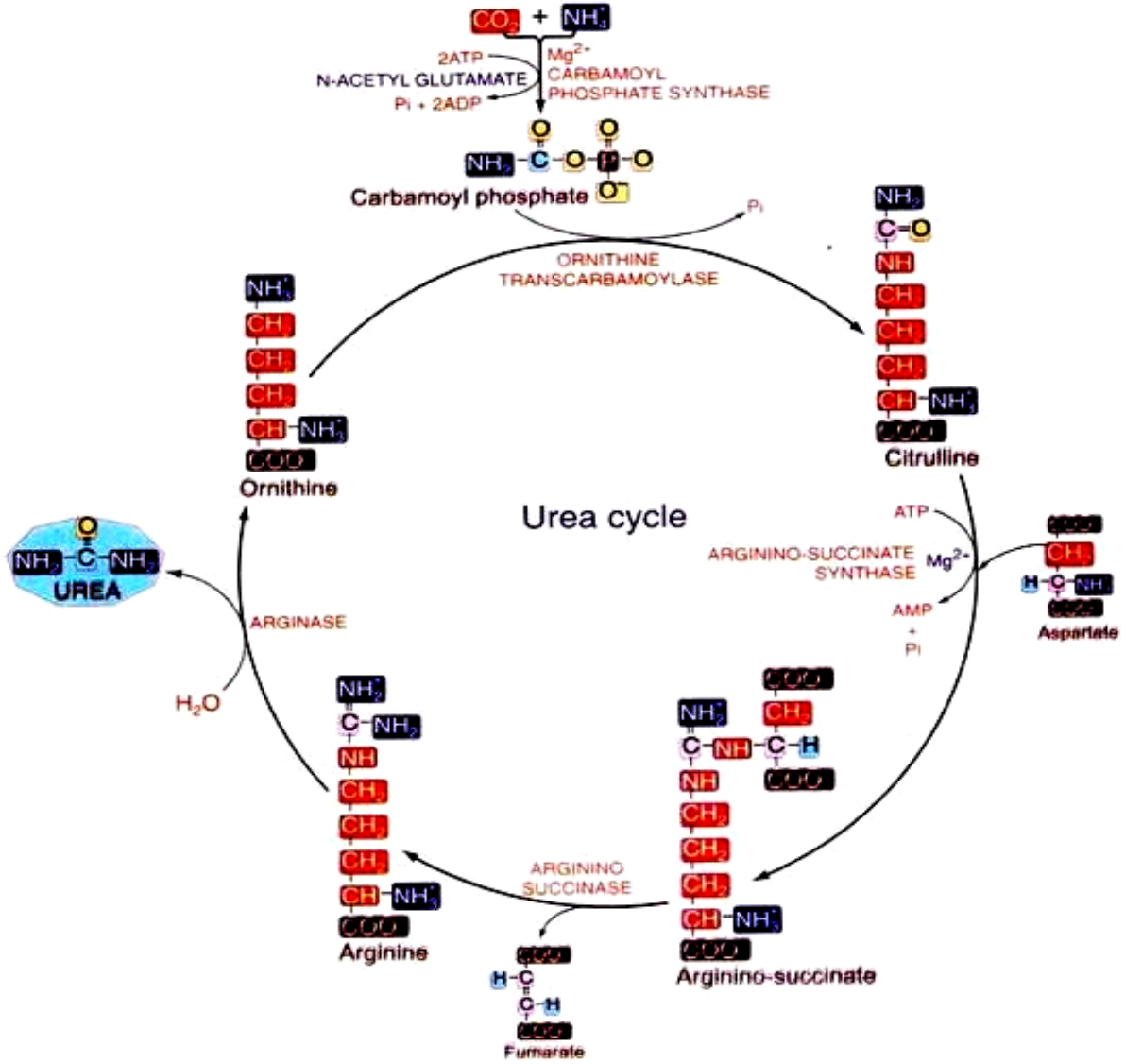
Condensation of ammonium ion with bicarbonate ion resulting in the formation of carbamoyl phosphate by the help of the enzyme carbamoyl phosphate synthase-I present in the liver mitochondria. It requires Mg^{2+} and a dicarboxylic acid i.e. N-acetyl glutamate. This step requires 2 ATPs.

2. Synthesis of Citrulline:

Carbamoyl phosphate formed in the first step combines with ornithine resulting in the synthesis of citrulline aided by the enzyme citrulline synthase or ornithine transcarbamoylase. Citrulline is easily permeable to the mitochondrial membrane and hence it diffuses into the cytosol.

3. Synthesis of Argininosuccinate:

In the cytosol, citrulline combines with the amino acid aspartate forming argininosuccinate catalysed by the enzyme argininosuccinate synthase. It requires ATP which is hydrolysed to AMP resulting in utilization of two high energy bonds. Mg^{2+} acts as cofactor.


2ATP
N-ACETYL GLUTAMATE
Pi + 2ADP
Mg2+
CARBAMOYL PHOSPHATE SYNTHASE
Carbamoyl phosphate
Pi
ORNITHINE TRANSCARBAMOYLASE
Ornithine
Citrulline
Urea cycle
ATP
ARGININO-SUCCINATE SYNTHASE
Mg2+
AMP + Pi
Aspartate
UREA
ARGINASE
H2O
Arginine
Arginino-succinate
ARGININO SUCCINASE
Fumarate

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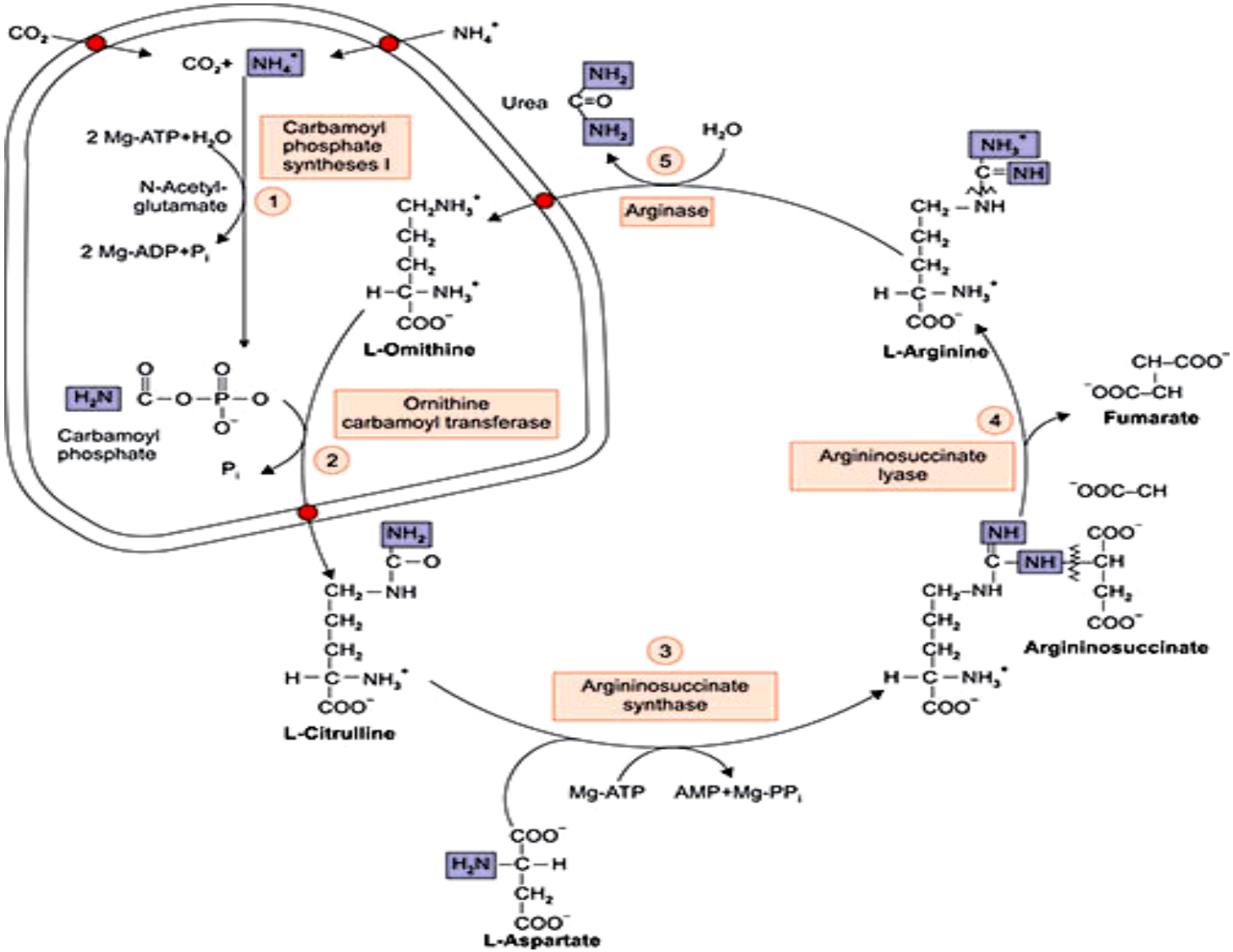

4. Cleavage of Argininosuccinate:

The enzyme argininosuccinase acts reversibly to cleave argininosuccinate into Arginine and fumarate. Fumarate enters the TCA cycle (the linkage between TCA and urea cycle is known as Krebs bi-cycle).

5. Cleavage of Arginine:

Arginine is lysed into ornithine and urea under the influence of the enzyme arginase. Hence arginine is known as semi-essential amino acid i.e. though it is synthesized in the body it is not available for protein synthesis. Ornithine is regenerated in this step and the urea cycle completes by the formation of urea. Ornithine and lysine are potent inhibitors of the enzyme arginase.

Arginase is also present in testis, renal tubules, mammary gland and skin in minute quantities. The intermediate amino acids formed in the urea cycle i.e. ornithine, citrulline and argininosuccinate are known as non-protein amino acids.

The overall equation of urea formation is:

$$NH_4 + CO_2 + \text{Asparate} + 3ATP \rightarrow \text{Urea} + \text{Fumarate} + 2ADP + 2P_i + AMP + PP_i$$

The urea cycle brings two amino groups and HCO_3 together to form urea. Thus toxic, insoluble ammonia is converted into non-toxic, water soluble, excretable urea. Hence, urea cycle disposes two waste products i.e. NH_4 and HCO_3. This fact suggests that urea cycle participates in the regulation of blood pH, which depends on the HCO_3/H_2CO_3. Though 3 ATPs are utilized, the ultimate cost of making a molecule of urea is 4 ATPs (one ATP is converted into AMP). The rate limiting steps of urea cycle are 1, 2, & 5.

Overall reaction equation:

In the first reaction, $NH_4^+ + HCO^{-3}$ is equivalent to $NH_3 + CO_2 + H_2O$.

Thus, the overall equation of the urea cycle is:

$$NH_3 + CO_2 + \text{aspartate} + 3\ ATP + 3\ H_2O \rightarrow \text{urea} + \text{fumarate} + 2\ ADP + 2\ P_i + AMP + PP_i + H_2O$$

Since fumarate is obtained by removing NH_3 from aspartate (by means of reactions 3 and 4), and $PP_i + H_2O \rightarrow 2\ P_i$, the equation can be simplified as follows:

$$2\ NH_3 + CO_2 + 3\ ATP + 3\ H_2O \rightarrow urea + 2\ ADP + 4\ P_i + AMP$$

Note that reactions related to the urea cycle also cause the production of 2 NADH, so the overall reaction releases slightly more energy than it consumes. The NADH is produced in two ways:

- One NADH molecule is produced by the enzyme glutamate dehydrogenase in the conversion of glutamate to ammonium and α-ketoglutarate. Glutamate is the non-toxic carrier of amine groups. This provides the ammonium ion used in the initial synthesis of carbamoyl phosphate.

- The fumarate released in the cytosol is hydrated to malate by cytosolic fumarase. This malate is then oxidized

to oxaloacetate by cytosolic malate dehydrogenase, generating a reduced NADH in the cytosol. Oxaloacetate is one of the keto acids preferred by transaminases, and so will be recycled to aspartate, maintaining the flow of nitrogen into the urea cycle.

We can summarize this by combining the reactions:

$$CO_2 + \text{glutamate} + \text{aspartate} + 3\ ATP + 2\ NAD^+ + 3\ H_2O$$

$$\rightarrow \text{urea} + \alpha\text{-ketoglutarate} + \text{oxaloacetate} + 2\ ADP + 2\ P_i +$$

$$AMP + PP_i + 2\ NADH$$

The two NADH produced can provide energy for the formation of 5 ATP (cytosolic NADH provides 2.5 ATP with the malate-aspartate shuttle in human liver cell), a net production of two high-energy phosphate bond for the urea cycle. However, if gluconeogenesis is underway in the cytosol, the latter reducing equivalent is used to drive the reversal of the GAPDH step instead of generating ATP.

The fate of oxaloacetate is either to produce aspartate via transamination or to be converted to phosphoenolpyruvate, which is a substrate for gluconeogenesis.

Important enzymes in Urea Cycle:

- **Carbamoyl phosphate synthetase I:** Converts ammonium and bicarbonate into carbamoyl phosphate. This is the rate-limiting step in the urea cycle. This reaction requires two ATP and occurs in the mitochondria.

- **Ornithine transcarbamoylase:** Combines ornithine and carbamoyl phosphate to form citrulline. Located in mitochondria.

- **Argininosuccinate synthetase:** Condenses citrulline with aspartate to form arginosuccinate. This reaction occurs in the cytosol and requires one ATP.

- **Argininosuccinate lyase:** Splits argininosuccinate into arginine and fumarate. Occurs in the cytosol.

- **Arginase:** Cleaves arginine into one molecule of urea and ornithine in the cytosol. The ornithine is then transported back into the mitochondria for entry back into the cycle.

Regulation of Urea Cycle:

- Carbamoyl phosphate synthetase I catalyzes the rate-limiting step of the cycle and is stimulated by N - acetylglutamate.

- Although the liver normally has a great capacity for urea synthesis, the enzymes of the urea cycle are induced if a high-protein diet is consumed for 4 days or more.

Purpose of the Urea Cycle:

The urea cycle allows for the excretion of NH_4^+ by transforming ammonia into urea, which is then excreted by the kidneys.

Functions of Urea Cycle:

- Amino acid catabolism results in waste ammonia.
- All animals need a way to excrete this product.

- Most aquatic organisms, or ammonotelic organisms, excrete ammonia without converting it.
- Organisms that cannot easily and safely remove nitrogen as ammonia convert it to a less toxic substance such as urea via the urea cycle, which occurs mainly in the liver.
- Urea produced by the liver is then released into the bloodstream where it travels to the kidneys and is ultimately excreted in urine.

- The urea cycle is essential to these organisms, because if the nitrogen or ammonia are not eliminated from the organism it can be very detrimental.
- In species including birds and most insects, the ammonia is converted into uric acid or its urate salt, which is excreted in solid form.

Metabolic Disorders of Urea Cycle:

Since urea cycle converts toxic ammonia to urea, disorders of this cycle lead to ammonia intoxication. This ammonia intoxication is more when there is block at step 1 or 2. Common symptoms of the disorders of urea cycle are vomiting in infancy, avoidance of high protein diet, intermittent ataxia, irritability, lethargy and mental retardation. Hyperammonemia occurs when there is a deficiency in one of more of the urea cycle enzymes, causing insufficient removal

of NH_4^+. Ammonia intoxication leads to CNS deterioration in the form of mental retardation, seizure, coma, and death.

1. Hyperammonemia type-I:

Due to the deficiency of carbamoyl phosphate synthase-I. It is a familial disorder.

2. Hyperammonemia type-II:

Due to the deficiency of ornithine transcarbamoylase. It is X-linked. Clinical finding is, the elevation of glutamine in the blood, CSF and urine.

3. Citrullinemia:

More citrulline is excreted in the urine i.e. upto 1 to 2 gm/day, due to the defect in the enzyme argininosuccinate synthase.

4. Arginino-succinic aciduria:

It is a rare recessive disease. Higher level of arginino-succinic acid in plasma and CSF. Usually present in the early age. Feeding arginine and benzoate promotes nitrogen excretion in these patients. This is due to lack of the enzyme argininosuccinase.

5. Hyper-argininemia:

High level of arginine due to lack of arginase enzyme.

Glucogenic and Ketogenic Amino Acids:

Those amino acids which on oxidation give intermediate compounds, resembling those of carbohydrate metabolism and which may be converted to glucose are termed as glucogenic amino acids. Ex. Ala, Arg, Asp, Asn, Cys, Gly, Glu, Gin, His, Pro, Met, Ser, Tyr, Val, Lys.

Those amino acids which form acetate or acetoacetate intermediates found during fatty acid metabolism are termed as ketogenic amino acids i.e. they can give rise to ketone bodies. Ex. Leu.

The amino acids lie, Lys, Phe, Tyr and Trp can give rise to both glucose and ketone bodies hence they are both glucogenic and ketogenic amino acids.

Oxidation of Carbon Skeleton of Amino Acids:

Once ammonia is released from the amino acids the remnant carbon back bone undergoes various oxidative reactions to yield one or the other intermediates of citric acid cycle (TCA cycle) as shown below—

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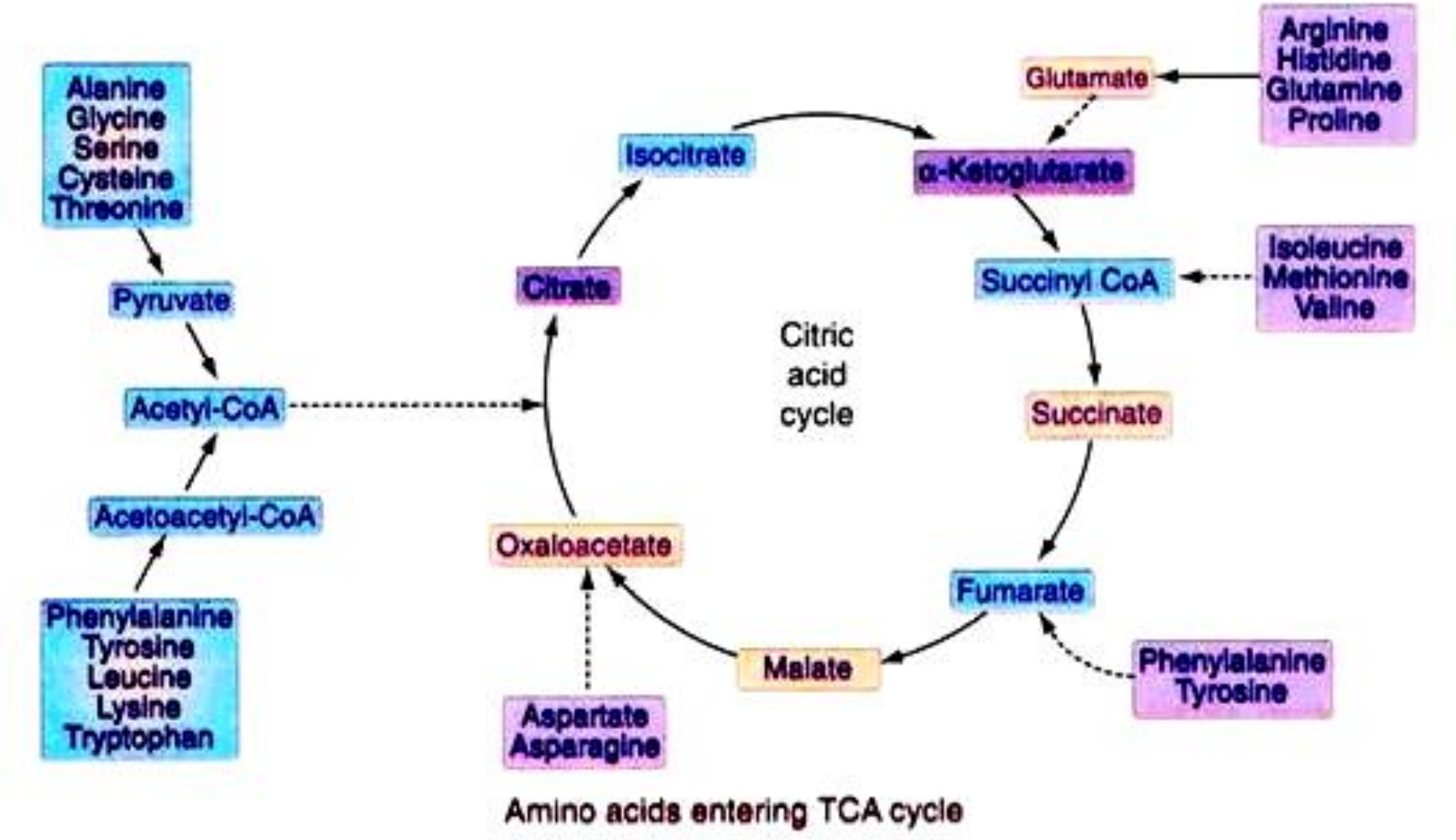

Amino acids entering TCA cycle

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THANK YOU

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