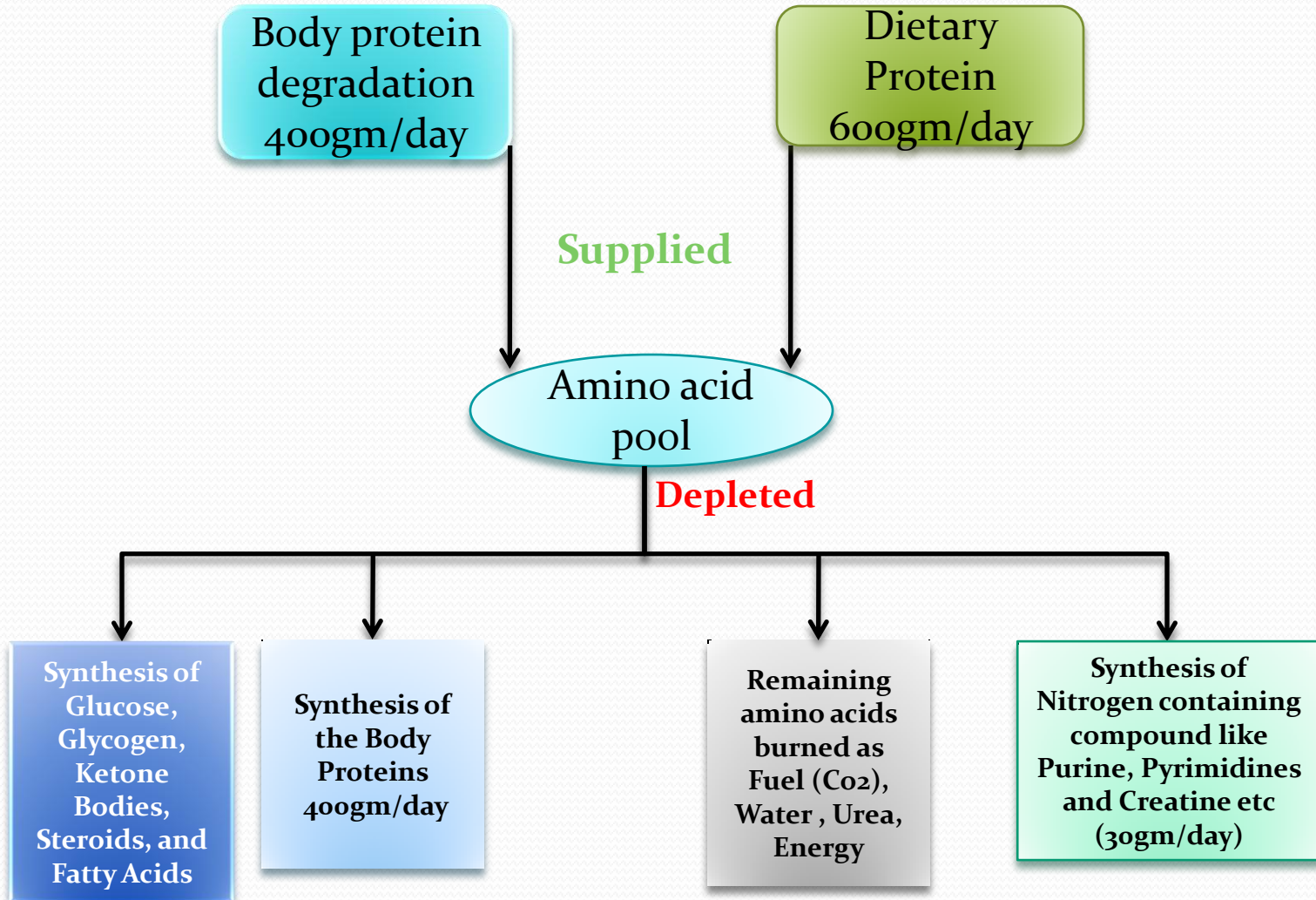


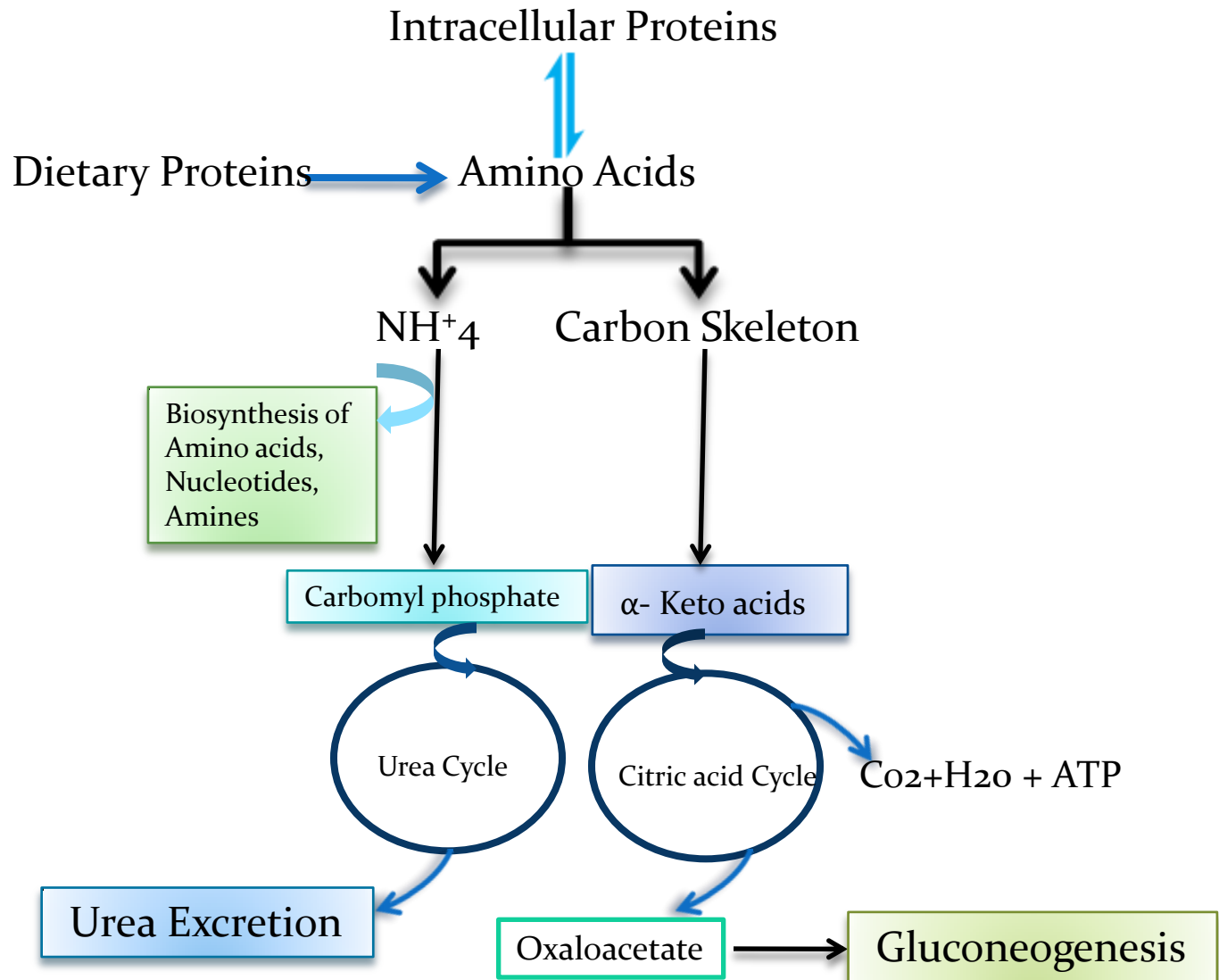
Important facts of the Protein Metabolism

- Proteins metabolism referred as synthesis of protein from Amino acids (**Anabolism**) and breakdown of proteins (**Catabolism**).
- In the Unit II, we discussed the synthesis (**Anabolism**) of various form of the proteins from different amino acids.
- Here we mainly focused on the **Catabolism** of the protein, which is integral part of metabolism of the **nitrogen-containing molecules**.
- Nitrogen enters in to the body in various form of compounds that is present in food, the most important is **amino acids (as dietary food)**.
- During Protein/amino acid metabolism (**Catabolism**), Nitrogen leaves the body as urea, ammonia, and in form of other derivatives.
- The **Catabolism** of the protein depends on **the amino acid pool and protein turnover (the rate of protein synthesis and degradation is equal)**.

- The amino acids present in through out of the body (cells, blood, and the extracellular fluids etc) is called **amino acid pools**. It is maintained by following factors as represented in below diagram:



Fate of Amino acids Catabolism in Mammals



Catabolism Steps of the Amino acids

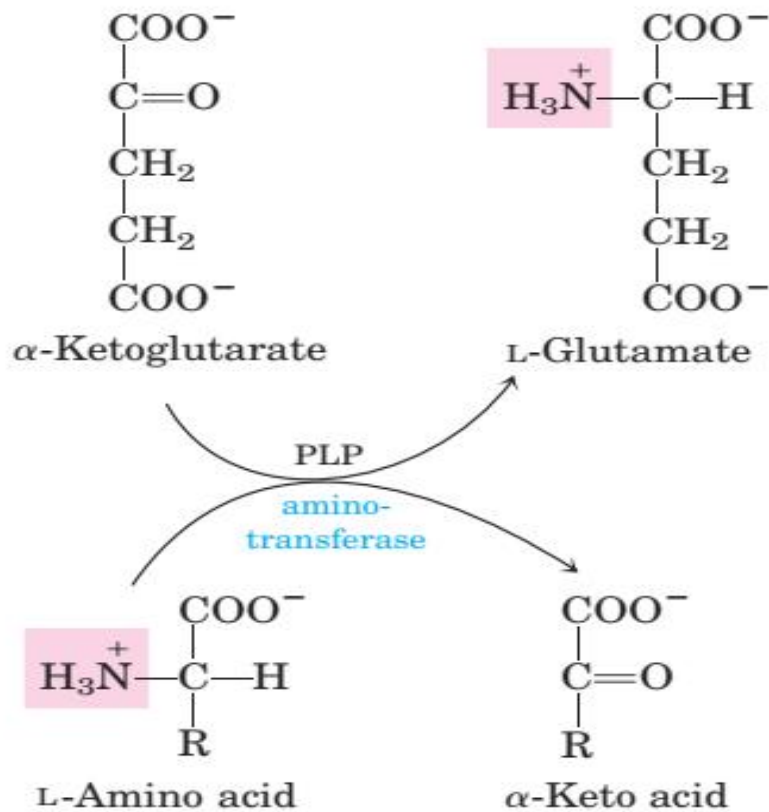
Step1: Transport of amino acids in to the cells: The transport of amino acids occurs from the extracellular fluids in to the cells by **active transport systems, driven by the hydrolysis of ATP**. There are seven different transport systems are known for amino acids in the cells .

Step 2: Transamination: It is a very crucial steps of the catabolism, removing of the α -amino group is essential for producing energy from any amino acid after that removal of remaining carbon skeletons is being metabolized .

Mechanism:

A. The first step in the catabolism of most amino acids is the transfer of their **α -amino group** to **α -ketoglutarate** .

B. The products are an **α -keto acid** (derived from the original amino acid) and **glutamate**.



Note:

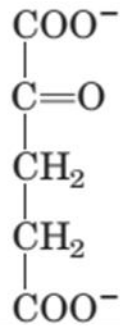
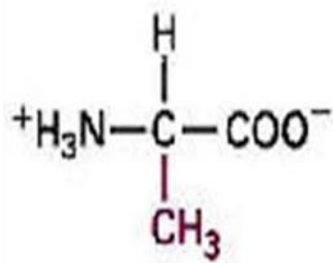
PLP (Pyridoxal Phosphate) acts as a cofactor. Pyridoxal phosphate functions as an intermediate carrier of amino groups at the active site of aminotransferases.

Aminotransferases: It is an enzyme of transamination reaction that requires a prosthetic group PLP (the coenzyme form of pyridoxine, or vitamin B6).

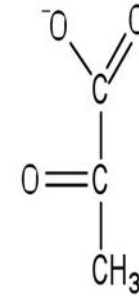
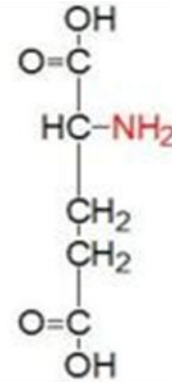
Fig. Enzyme Catalyzed Transamination reaction

Important facts about Aminotransferases

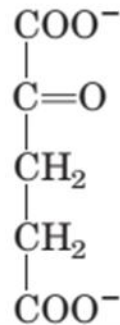
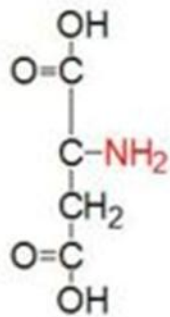
- There are two most important aminotransferases: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using substrate alanine and aspartate respectively that catalyzed **transamination** reactions.
- **Alanine aminotransferase (ALT)** is earlier called **glutamate-pyruvate transaminase**, present in many tissue. It transfer amino group of alanine to α -ketoglutarate, resulting in the formation of pyruvate and glutamate. ALT is normally found inside **liver** cells .
- **Aspartate aminotransferase (AST)** is earlier called **glutamate-oxaloacetate transaminase**, AST transfers amino groups from aspartate to glutamate , forming to oxaloacetate. Glutamate is used as important source of nitrogen in the urea cycle. AST is found in the **liver**, heart, skeletal muscle, kidneys, brain, and red blood cells
- Both ALT and AST have lots of clinical significance . The high level of plasma concentration indicates liver dysfunction, myocardial infarction and muscle disorders. Moreover, ALT is more specific than AST for liver disease because normally found inside **liver** cells.



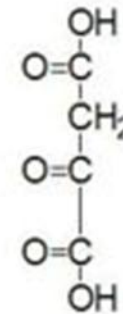
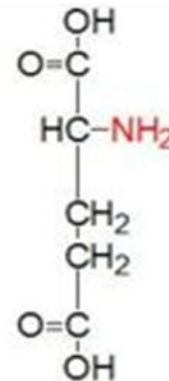
ALT



Alanine + α -Ketoglutarate \rightleftharpoons Glutamate + Pyruvate



AST



Aspartate + α -Ketoglutarate \rightleftharpoons Glutamate + Oxaloacetate

Step3. Oxidative deamination:

- After the transamination reactions that transfer amino groups, **oxidative deamination** occurs by **glutamate dehydrogenase** results in the liberation of the amino group as free ammonia from Glutamate. This reaction is occurs in the liver.
- The α -keto acids enter the central pathway of energy metabolism and ammonia in urea synthesis, liberated after oxidative deamination.
- **Glutamate** is a unique amino acid that only undergoes rapid oxidative deamination, that is catalyzed by **glutamate dehydrogenase**.
- The glutamate dehydrogenase of mammalian liver has the unusual capacity to use either NAD^+ or NADP^+ as cofactor.

Mechanism of Oxidative deamination:

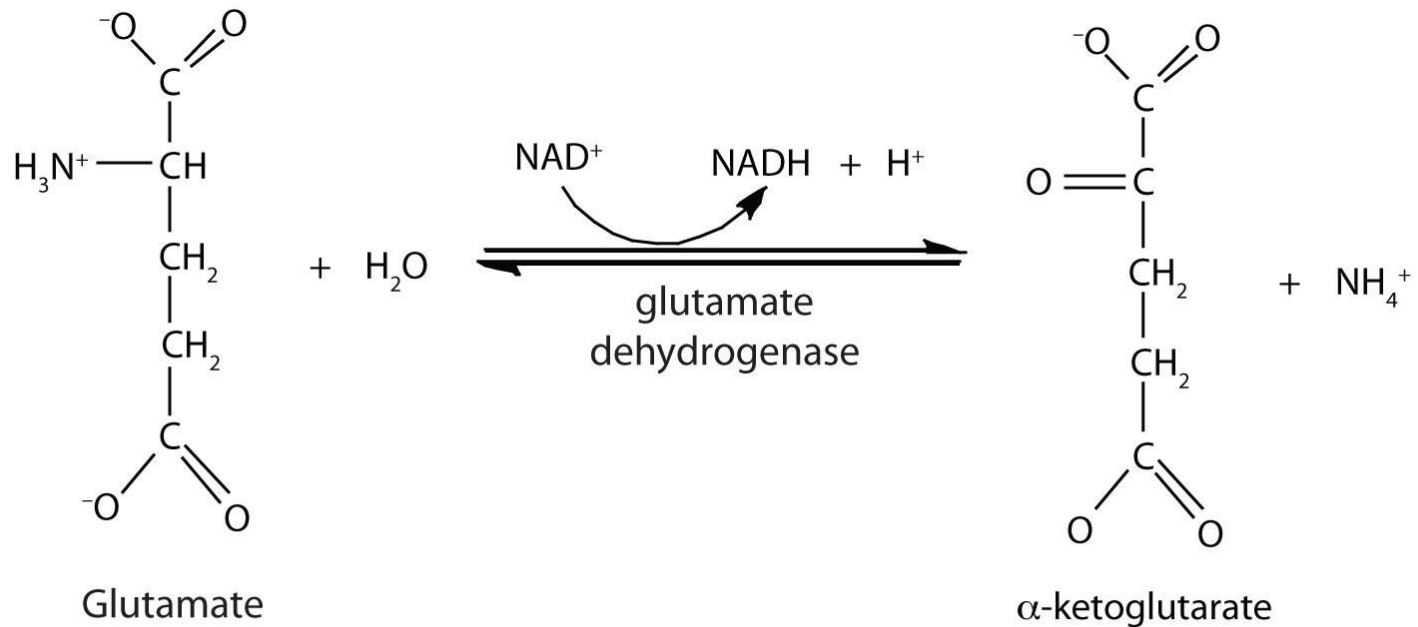


Fig. Reaction is catalyze by enzyme glutamate dehydrogenase

Note# D-Amino acid oxidase: In addition to the glutamate dehydrogenase, the D-Amino acid oxidase is an FAD-dependent peroxisomal enzyme that catalyzes the oxidative deamination of these amino acid isomers, which are found in plants. D-Amino acids are found in plants and in the cell walls of microorganisms.

Mechanism of Transport of ammonia to the Liver

- Since the ammonia is highly toxic in nature which is liberated through oxidative deamination, and can't transport directly to the liver alone.
- Therefore, there are two mechanisms available in humans for the transport of ammonia from peripheral tissues to the liver for conversion to urea.
- **The first:** it is found in most tissues, where uses glutamine synthetase to combine ammonia with glutamate to form **Glutamine** (a nontoxic transport form of ammonia).
- Then the glutamine is transported from blood to the liver, where it cleaved by **Glutaminase** to produce glutamate and free ammonia.
- **The second:** In form of **Alanine** in muscle, where pyruvate (the end product of aerobic glycolysis) involves transamination reaction to form alanine. The transport of ammonia in muscle pathway is called the **glucose-alanine cycle**.

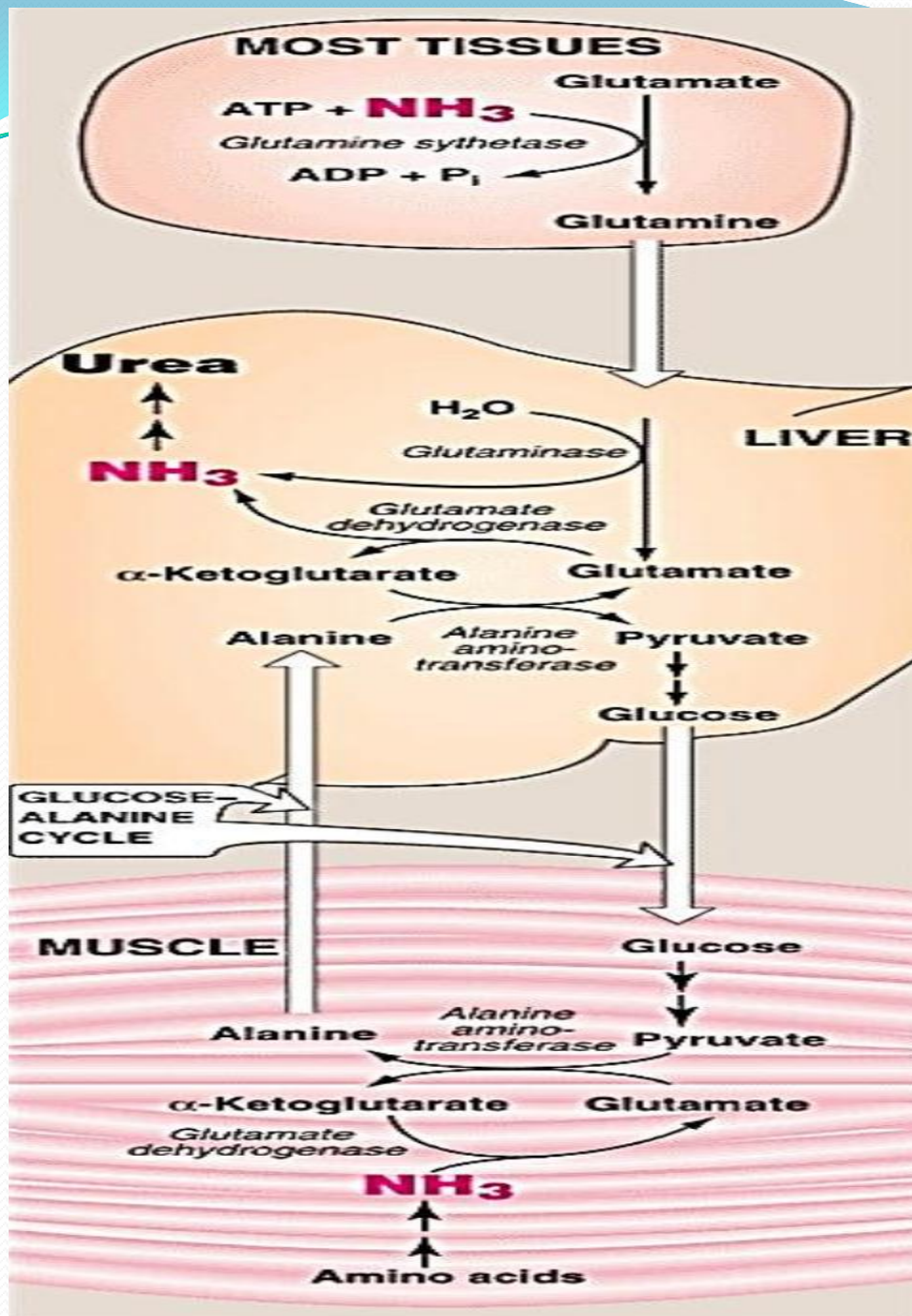


Fig: Transport of ammonia from peripheral tissues to the liver

Urea Cycle

There are different mode of nitrogen excretion in different organisms:

Ammonotelic: Excreting amino nitrogen as ammonia ex. Most aquatic species .

•**Uricotelic** : Excreting amino nitrogen in the form of uric acids in birds and reptiles .

•**Ureotelic** : Excreting amino nitrogen in the form of urea in terrestrial animals.

• So in ureotelic organisms, first ammonia deposited in the mitochondria of hepatocytes and get converted to urea is called **Urea cycle**.

• This pathway was discovered in 1932 by Hans Krebs and Kurt Henseleit.

• Urea production occurs almost exclusively in the liver and ultimately excreted through kidneys in form of urine.

There are five steps in the Urea Cycle:

- 1- Formation of carbamoyl phosphate
 - 2- Formation of citrulline
 - 3- Formation of argininosuccinate
 - 4- Formation of arginine and fumarate
 - 5- Formation of urea and ornithine
-
- Mitochondria of liver Cells
- Cytosol of liver Cells

Note#

• **Carbamoyl phosphate synthetase I (CPSI)**; Involves in the formation of carbamoyl phosphate that requires two molecules of ATP . It is rate limiting steps. Carbamoyl phosphate synthetase I requires **N-acetylglutamate (NAG)** as a positive allosteric activator.

• N-acetylglutamate is synthesized from acetyl coenzyme A and glutamate by **N-acetylglutamate synthase**. The level of NAG is controlled by protein-rich meal/less protein rich meal.

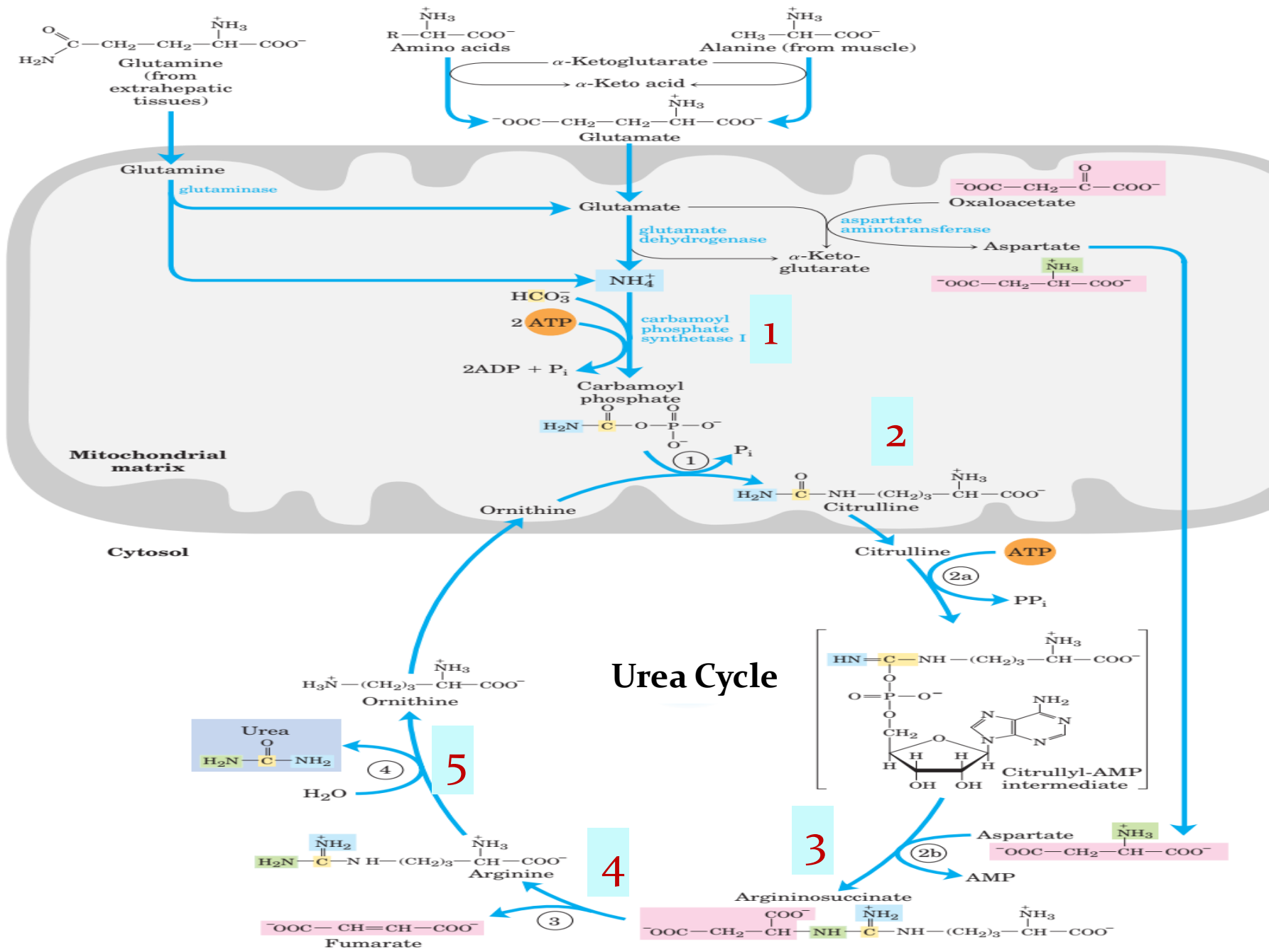


Fig. Urea cycle (Adopted from Lehninger (Principles of Biochemistry), page -666)

Important facts of the Urea Cycle

- Condensation of CO₂, ammonia, and ATP to form **carbamoyl phosphate** is catalyzed by mitochondrial **carbamoyl phosphate synthase I**.
- **Four high-energy phosphates (ATP)** are consumed in the synthesis of each molecule of urea: two ATP to restore two ADP to two ATP to restore AMP. Therefore, the synthesis of urea is irreversible, with a large, negative ΔG .
- The major metabolic role of **ornithine**, **citrulline**, and **argininosuccinate** in mammals is urea synthesis.
- One nitrogen of the urea molecule is supplied by free NH₃, and the other nitrogen by aspartate .
- Carbamoyl Phosphate Synthase I is the Pacemaker Enzyme of the Urea Cycle.
- Urea diffuses from the liver to blood to the kidneys, where it is filtered and excreted in the urine.

Important Disorders Related to Protein Metabolism

- **Hyperammonemia:** When the level of urea get elevated in blood than Normal level of ammonia ($5-50 \mu\text{mol/L}$), due genetic defects of the urea cycle, or liver disease Called **Hyperammonemia**. Its **emergency medical situation** because ammonia has a direct neurotoxic effect on the CNS. At high concentrations, ammonia can cause coma and death.
- **Neural Dysfunction:** High level of ammonia Impaired neural transmission process due to **increased formation of GABA**(gamma amino butyric acid) from glutamate.
- **Hyperargininemia:** In this defects elevated level of arginine is found in blood and cerebrospinal fluid .
- Clinical symptoms are common to all urea cycle disorders include vomiting, avoidance of high-protein foods, intermittent ataxia, irritability, lethargy, and mental retardation .