METABOLISM OF PROTEIN

TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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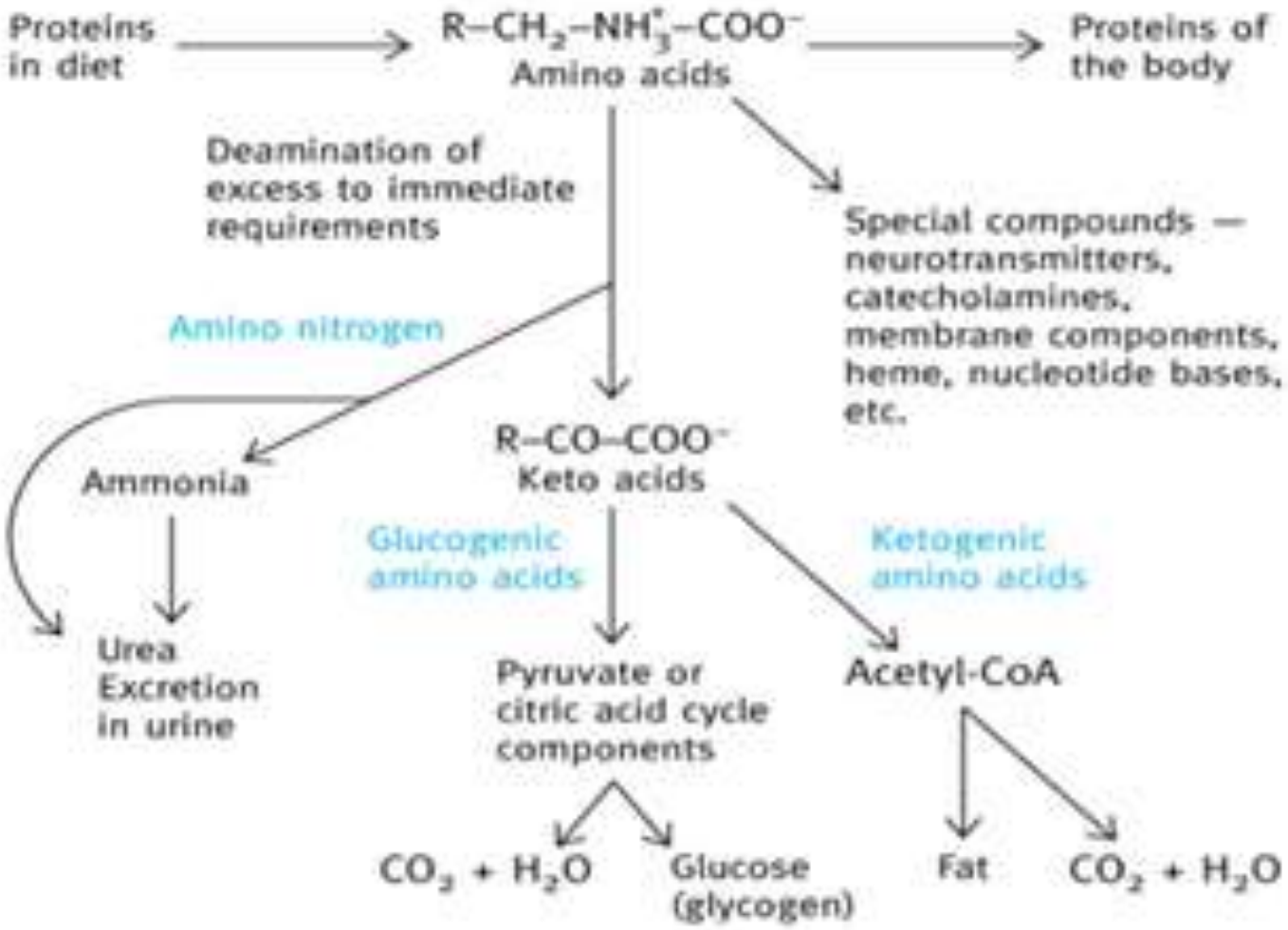
METABOLISM OF AMINO ACIDS —GENERAL ASPECTS

The amino acids undergo certain common reactions like ***transamination*** followed by ***deamination*** for the liberation of ***ammonia***. The amino group of the amino acids is utilized for the formation of ***urea*** which is an excretory ***end product*** of protein metabolism. The carbon skeleton of the amino acids is first converted to keto acids (by transamination) which meet one or more of the following fates.

1. Utilized to generate energy.
2. Used for the synthesis of glucose.
3. Diverted for the formation of fat or ketone bodies.
4. Involved in the production of non-essential amino acids.

AMINO ACID METABOLISM



TRANSAMINATION

The ***transfer of an amino ($-NH_2$) group from an amino acid to a keto acid*** is known as transamination. This process involves the interconversion of a pair of amino acids and a pair of keto acids, catalysed by a group of enzymes called ***transaminases*** (recently, ***aminotransferases***).

1. Transfer of the amino group to the coenzyme pyridoxal phosphate (bound to the coenzyme) to form pyridoxamine phosphate.

2. The amino group of pyridoxamine phosphate is then transferred to a keto acid to produce a new amino acid and the enzyme with PLP is regenerated.

All the transaminases require ***pyridoxal phosphate*** (PLP), a derivative of vitamin B_6. The aldehyde group of PLP is linked with ε-amino group of lysine residue, at the active site of the enzyme forming a ***Schiff base*** (imine linkage). When an amino acid (substrate) comes in contact with the enzyme, it displaces lysine and a new Schiff base linkage is formed. The amino

Salient features of transamination

1. All transaminases require ***pyridoxal phosphate*** (PLP), a coenzyme derived from vitamin B_6.

2. Specific transaminases exist for each pair of amino and keto acids. However, only two—namely, aspartate transaminase and alanine transaminase—make a significant contribution for transamination.

3. There is no free NH_3 liberated, only the transfer of amino group occurs.

4. Transamination is ***reversible*** (***Fig. 15.3***).

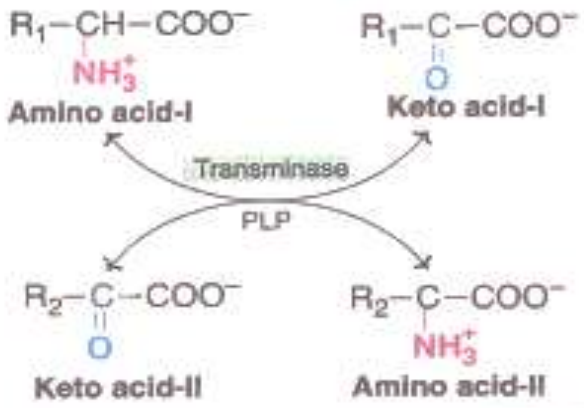


Fig. 15.3 : Transamination reaction.

5. Transamination is very important for the redistribution of amino groups and ***production of non-essential amino acids***, as per the requirement of the cell. It involves both catabolism (degradation) and anabolism (synthesis) of amino acids.

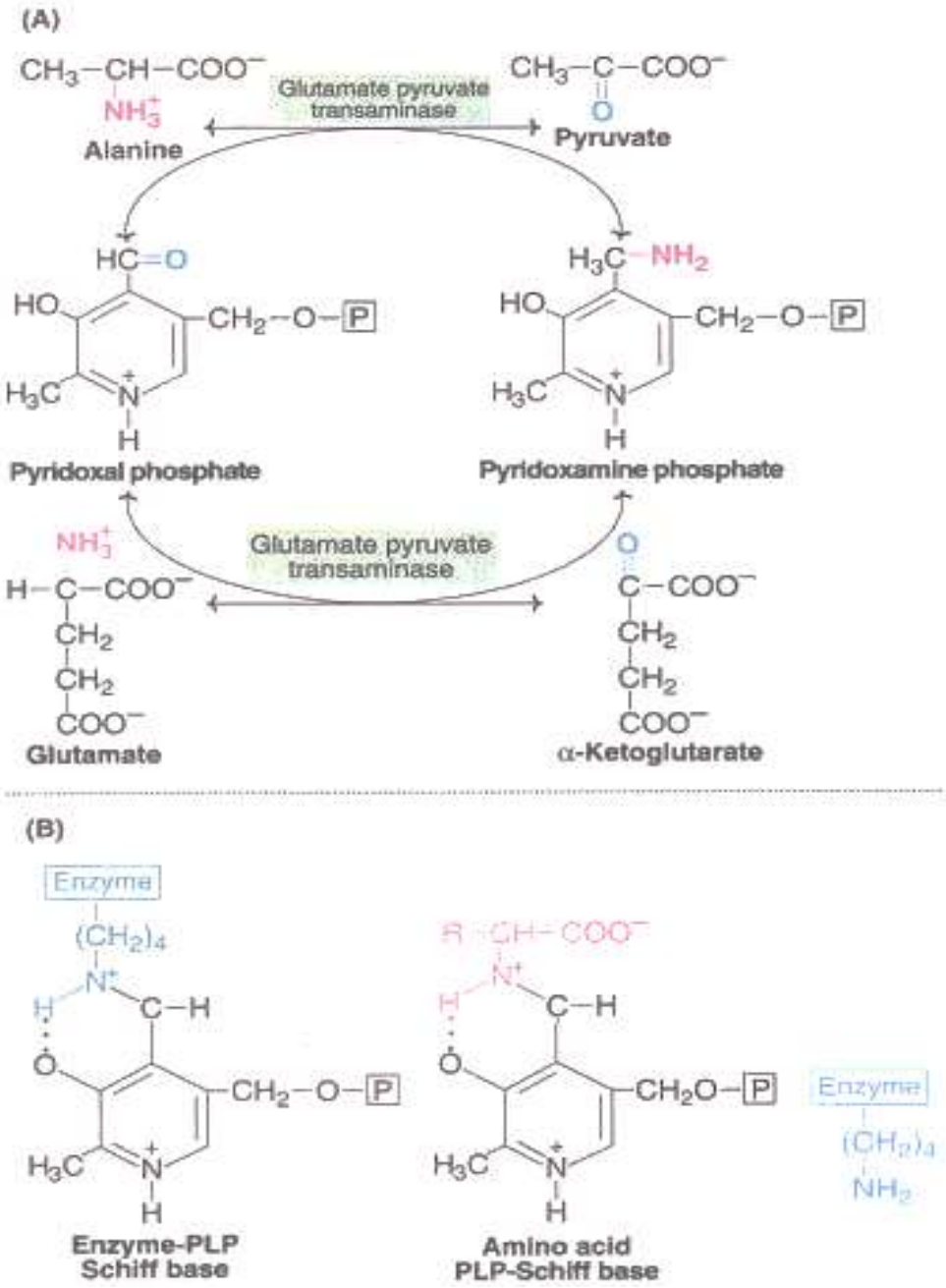
6. Transamination diverts the excess amino acids towards ***energy generation***.

7. The amino acids undergo transamination to finally concentrate nitrogen in glutamate. ***Glutamate*** is the only amino acid that undergoes oxidative deamination to a significant extent to liberate free NH_3 for urea synthesis.

8. All amino acids except lysine, threonine, proline and hydroxyproline participate in transamination.

9. Transamination is not restricted to α-amino groups only. For instance, δ-amino group of ornithine is transaminated.

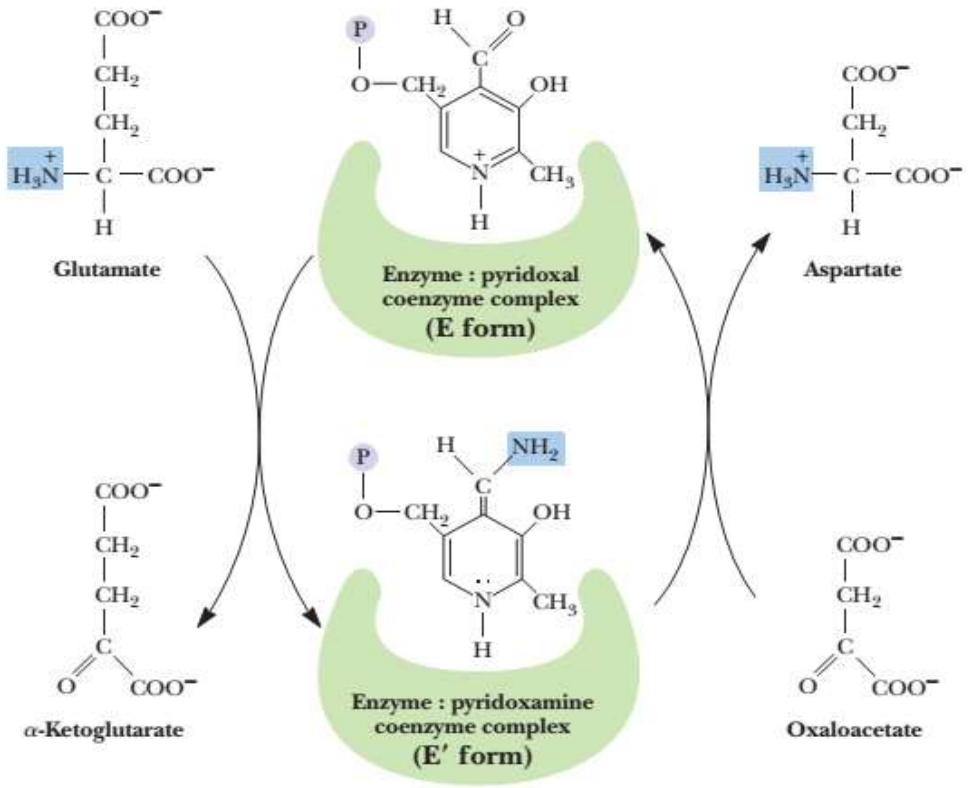
10. Serum transaminases are important for diagnostic and prognostic purposes.



Aminotransferases Show Double-Displacement Catalytic Mechanisms One class of enzymes that follow a ping-pong–type mechanism are *aminotransferases* (previously known as transaminases). These enzymes catalyze the transfer of an amino group from an amino acid to an α-keto acid. The products are a new amino acid and the keto acid corresponding to the carbon skeleton of the amino donor:

$$\text{amino acid}_1 + \text{keto acid}_2 \longrightarrow \text{keto acid}_1 + \text{amino acid}_2$$

A specific example would be *glutamate: aspartate aminotransferase.* Figure 13.23 de-



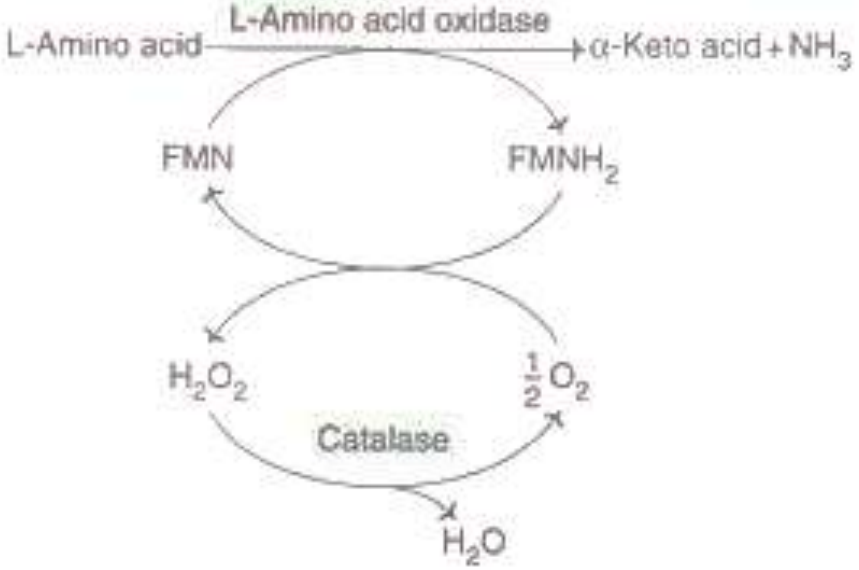
DEAMINATION

The ***removal of amino group*** from the amino acids as NH_3 is deamination. Transamination (discussed above) involves only the shuffling of amino groups among the amino acids. On the other hand, deamination results in the liberation of ammonia for urea synthesis. Simultaneously, the carbon skeleton of amino acids is converted to keto acids. Deamination may be either oxidative or non-oxidative.

Although transamination and deamination are separately discussed, they occur simultaneously, often involving glutamate as the central molecule. For this reason, some authors use the term ***transdeamination*** while describing the reactions of transamination and deamination, particularly involving glutamate.

I. Oxidative deamination

Oxidative deamination is the ***liberation of free ammonia*** from the amino group of amino acids ***coupled with oxidation***. This takes place mostly in liver and kidney. The purpose of oxidative deamination is to provide NH_3 for urea synthesis and α-keto acids for a variety of reactions, including energy generation.



Role of glutamate dehydrogenase : In the process of transamination, the amino groups of most amino acids are transferred to α-keto-glutarate to produce glutamate. Thus, ***glutamate*** serves as a '***collection centre' for amino groups*** in the biological system. Glutamate rapidly undergoes oxidative deamination, catalysed by glutamate dehydrogenase (GDH) to liberate ammonia. This enzyme is unique in that it can utilize either NAD^+ or $NADP^+$ as a coenzyme. Conversion of glutamate to α-ketoglutarate occurs through the formation of an intermediate, α-iminoglutarate (*Fig.15.5*).

Glutamate dehydrogenase catalysed reaction is important as it reversibly links up glutamate metabolism with TCA cycle through α-keto-glutarate. GDH is involved in both catabolic and anabolic reactions.

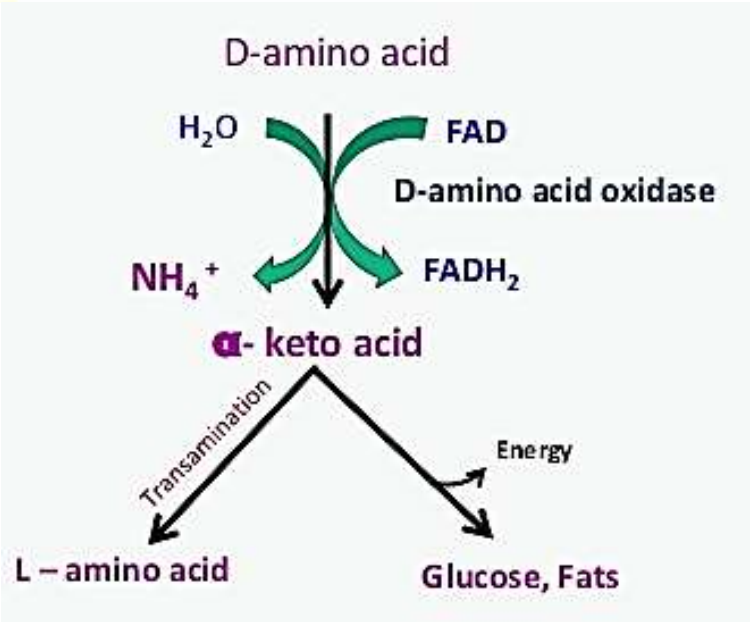
Regulation of GDH activity : Glutamate dehydrogenase is a zinc containing mito-chondrial enzyme. It is a complex enzyme consisting of six identical units with a molecular weight of 56,000 each. GDH is controlled by allosteric regulation. ***GTP*** and ***ATP inhibit*** whereas ***GDP*** and ***ADP activate*** glutamate dehydrogenase. Steroid and thyroid hormones inhibit GDH.

After ingestion of a protein-rich meal, liver glutamate level is elevated. It is converted to α-ketoglutarate with liberation of NH_3. Further, when the cellular energy levels are low, the degradation of glutamate is increased to provide α-ketoglutarate which enters TCA cycle to liberate energy.

Oxidative deamination by amino acid oxidases : L-Amino acid oxidase and D-amino acid oxidase are flavoproteins, possessing FMN and FAD, respectively. They act on the corresponding amino acids (L or D) to produce α-keto acids and NH_3. In this reaction, oxygen is reduced to H_2O_2, which is later decomposed by catalase (***Fig.15.6***).

The activity of ***L-amino acid oxidase*** is much low while that of ***D-amino acid oxidase*** is high in tissues (mostly liver and kidney). L-Amino acid oxidase does not act on glycine and dicarboxylic acids. This enzyme, due to its very low activity, does not appear to play any significant role in the amino acid metabolism.

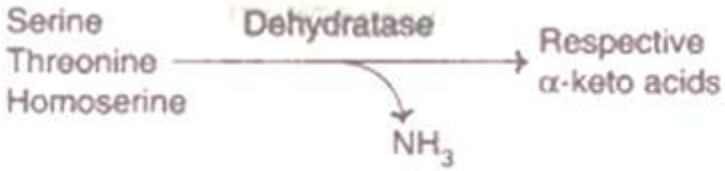
Fate of D-amino acids : D-Amino acids are found in plants and microorganisms. They are, however, not present in the mammalian proteins. But D-amino acids are regularly taken in the diet and metabolized by the body. D-Amino acid oxidase converts them to the respective α-keto acids by oxidative deamination. The α-keto acids so produced undergo transamination to be converted to L-amino acids which participate in various metabolisms. Keto acids may be oxidized to generate energy or serve as precursors for glucose and fat synthesis. Thus, D-amino acid oxidase is important as it initiates the first step for the conversion of unnatural D-amino acids to L-amino acids in the body (***Fig.15.7***).



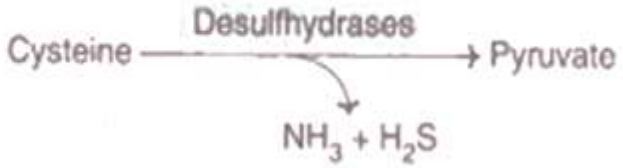
NON- OXIDATIVE DEAMINATION

Some of the amino acids can be deaminated to liberate NH_3 without undergoing oxidation

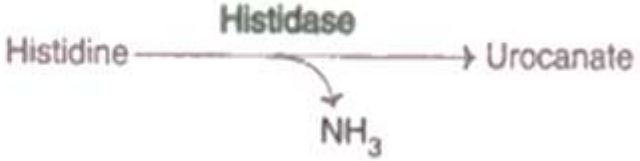
(a) **Amino acid dehydrases :** Serine, threonine and homoserine are the hydroxy amino acids. They undergo non-oxidative deamination catalysed by PLP-dependent dehydrases (dehydratases).



(b) **Amino acid desulfhydrases :** The sulfur amino acids, namely cysteine and homocysteine, undergo deamination coupled with desulfhydration to give keto acids.

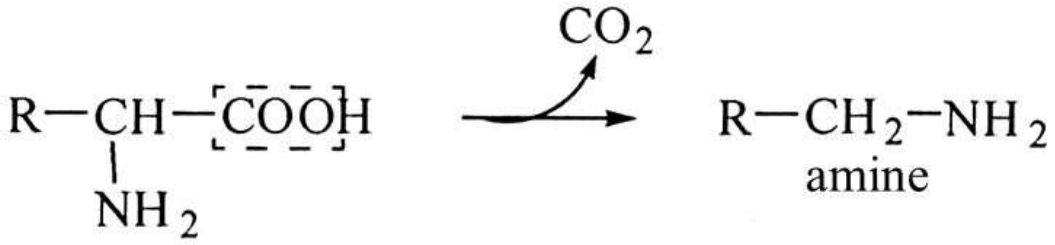


(c) **Deamination of histidine :** The enzyme histidase acts on histidine to liberate NH_3 by a non-oxidative deamination process.



Decarboxylation of amino acids

Decarboxylation – removal of *carbon dioxide* from amino acid with formation of *amines*.

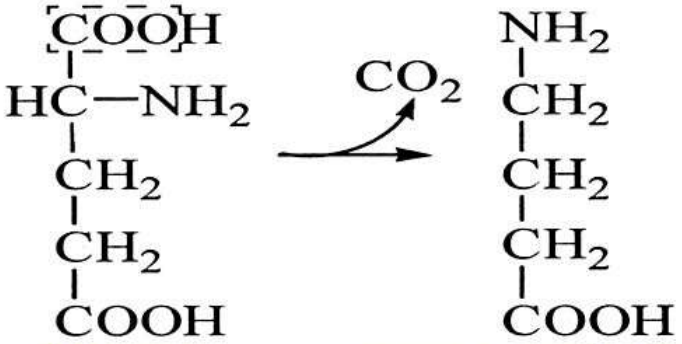


Usually amines have high physiological activity (hormones, neurotransmitters etc).

Enzyme: ***decarboxylases***
Coenzyme – **pyrydoxalphosphate**

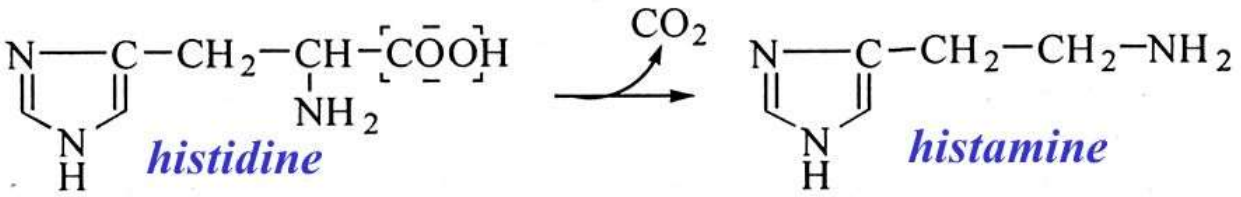
Significance of amino acid decarboxylation

1. Formation of physiologically active compounds



glutamate *gamma-aminobutyric acid (GABA)*

GABA – mediator of nervous system



histidine *histamine*

Histamine - mediator of inflammation, allergic reaction.

Reactions due to carboxyl group

1) **Decarboxylation–** Amino acids undergo alpha decarboxylation to form corresponding amines. Examples–

Glutamic acid ⟶ GABA
Histidine ⟶ Histamine
Tyrosine ⟶ Tyramine

2) **Formation of amide linkage**

- Non α carboxyl group of an acidic amino acid reacts with ammonia by condensation reaction to form corresponding amides

Aspartic acid ⟶ Asparagine
Glutamic acid ⟶ Glutamine

UREA CYCLE

Urea is the ***end product of protein metabolism*** (amino acid metabolism). The nitrogen of amino acids, converted to ammonia (as described above), 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***. The individual reactions, however, were described in more detail later on by Ratner and Cohen.

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 (***Figs.15.9 and 15.10***).

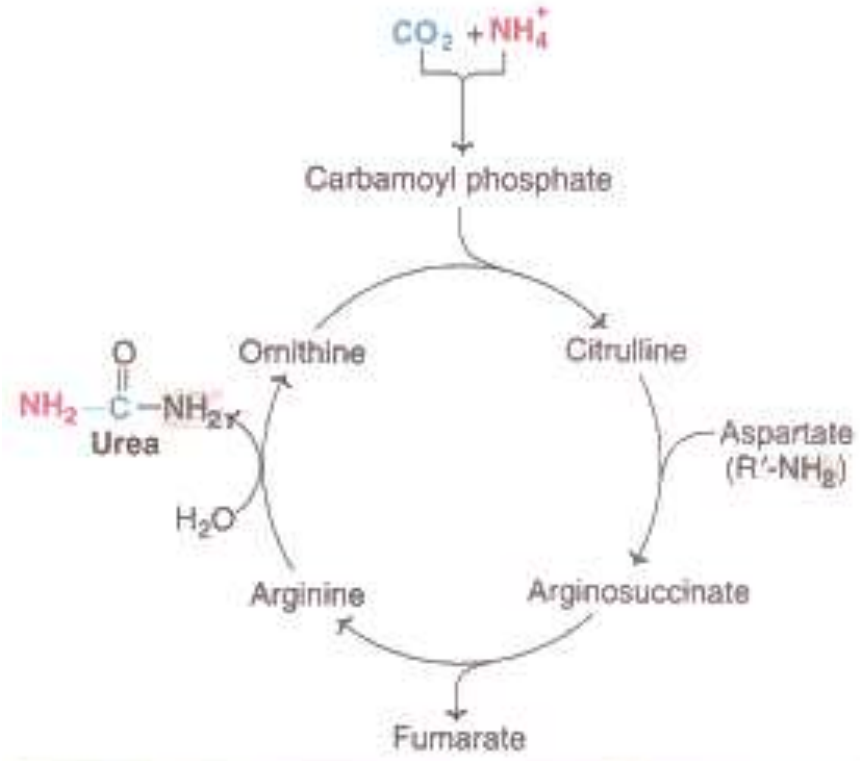


Fig. 15.9 : *Outline of urea cycle. (**Note :** In the synthesis of urea one amino group comes from ammonium ion while the other is from aspartate; carbon is derived from CO_2. This is represented in colours.)*

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 :** Argino-succinate 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).

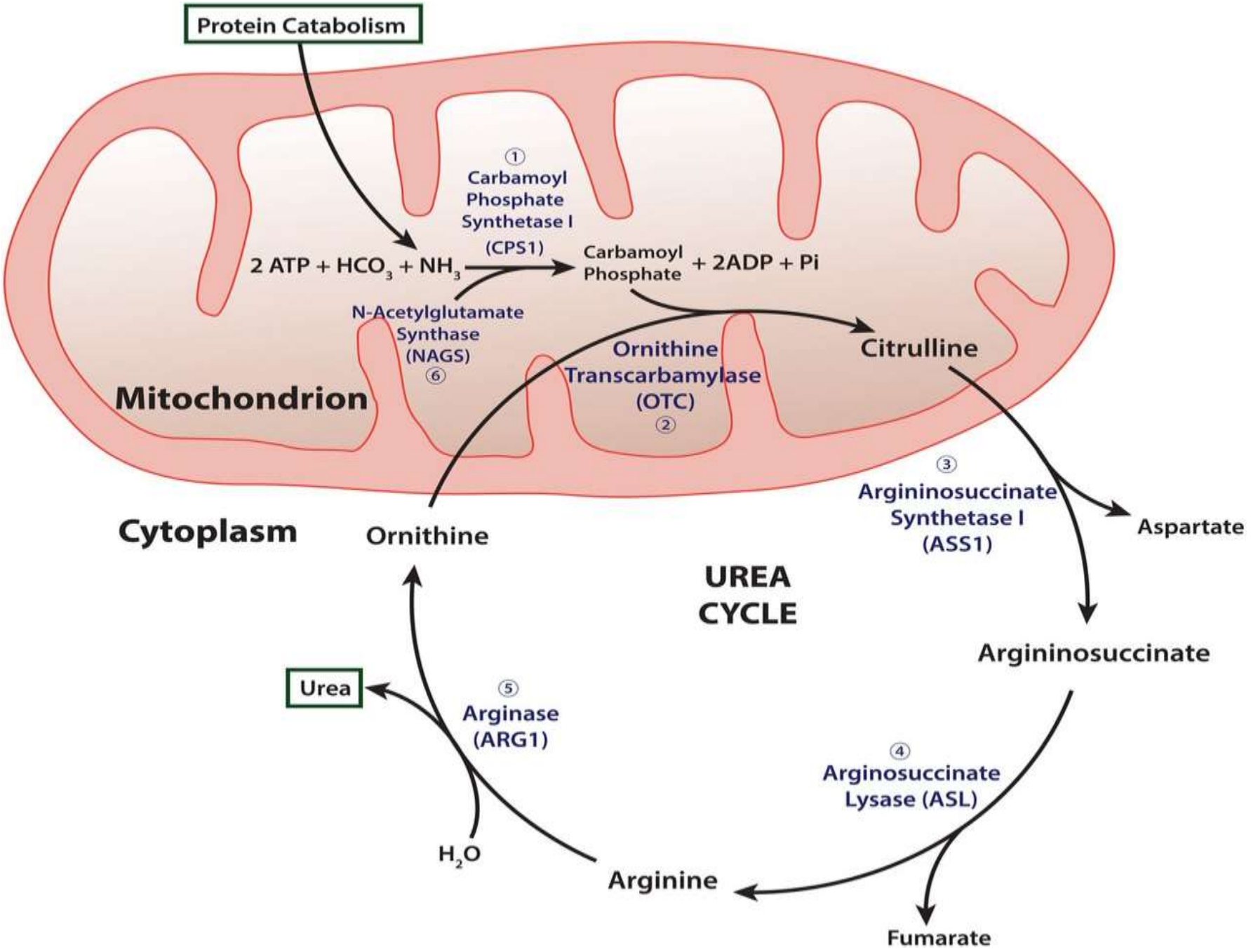
4. **Cleavage of arginosuccinate :** Argino-succinase cleaves arginosuccinate to give arginine and fumarate. Arginine is the immediate precursor for urea. Fumarate liberated here provides a connecting link with TCA cycle, gluconeogenesis etc.

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+}. Ornithine and lysine compete with arginine (competitive inhibition). ***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***.

$NH_4^+ + CO_2 + \text{Aspartate} + 3ATP \longrightarrow \text{Urea} + \text{Fumarate} + 2\ ADP + 2\ Pi + AMP + PPi$

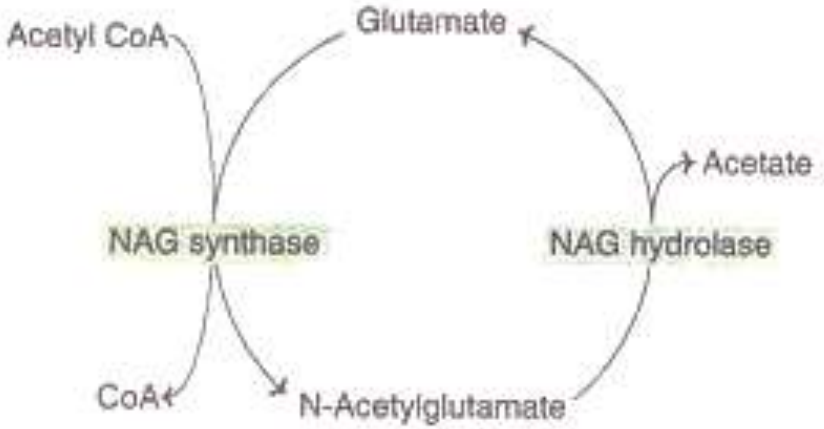

Protein Catabolism
Mitochondrion
Cytoplasm
① Carbamoyl Phosphate Synthetase I (CPS1)
2 ATP + HCO3 + NH3
Carbamoyl Phosphate + 2ADP + Pi
N-Acetylglutamate Synthase (NAGS) ⑥
Ornithine Transcarbamylase (OTC) ②
Citrulline
③ Argininosuccinate Synthetase I (ASS1)
Aspartate
Ornithine
UREA CYCLE
Argininosuccinate
Urea
⑤ Arginase (ARG1)
H2O
④ Arginosuccinate Lysase (ASL)
Arginine
Fumarate

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). It is synthesized from glutamate and acetyl CoA by synthase and degraded by a hydrolase (***Fig.15.11***).

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.



Disposal of urea

Urea produced in the liver freely diffuses and is transported in blood to ***kidneys***, and excreted. A small amount of urea enters the intestine where it is broken down to CO_2 and NH_3 by the bacterial enzyme urease. This ammonia is either lost in the feces or absorbed into the blood. In renal failure, the blood urea level is elevated (uremia), resulting in diffusion of more urea into intestine and its breakdown to NH_3. Hyperammonemia (increased blood NH_3) is commonly seen in patients of kidney failure. For these patients, oral administration of antibiotics (neomycin) to kill intestinal bacteria is advised.

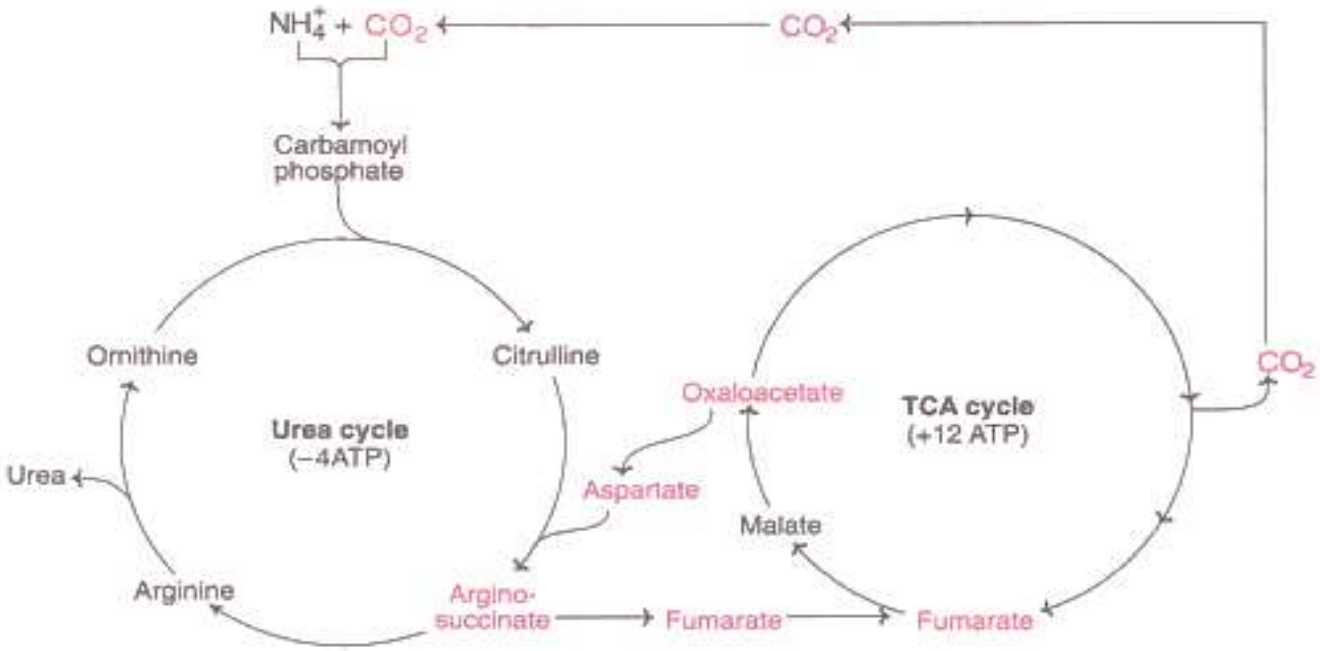
Integration between urea cycle and TCA cycle

Urea cycle is linked with TCA cycle in three different ways (***Fig.15.12***). 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.



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