STUDY MATERIAL

VIVEKANANDA COLLEGE THAKURPUKUR

NAAC ACCREDITED GRADE—'A'

Subject: Zoology
Topic: Metabolism of Amino Acids
(as per C.U. Zoology Hons. Syllabus 2016, Paper IV-Unit I-Group B)

Name of the Teacher:

Dr. Samita Kundu

Theme of the topic

This topic is aimed to understand the

- Steps of amino acid metabolism
- Pathways of nitrogen metabolism
  - Transamination
  - Deamination
    - Oxidative deamination
    - Non-oxidative deamination
- Fate of ammonia

Steps of amino acid metabolism

- ✓ **First phase:** Removal of α-amino groups (by transamination and oxidative deamination), forming ammonia and the corresponding α-keto acids (the carbon-skeletons of amino acids)
- ✓ **Second phase:** The carbon skeletons of the α-keto acids are converted to common intermediates of energy producing metabolic pathways. These compounds are ultimately metabolized to CO_2 and water, glucose, fatty acids or ketone bodies.

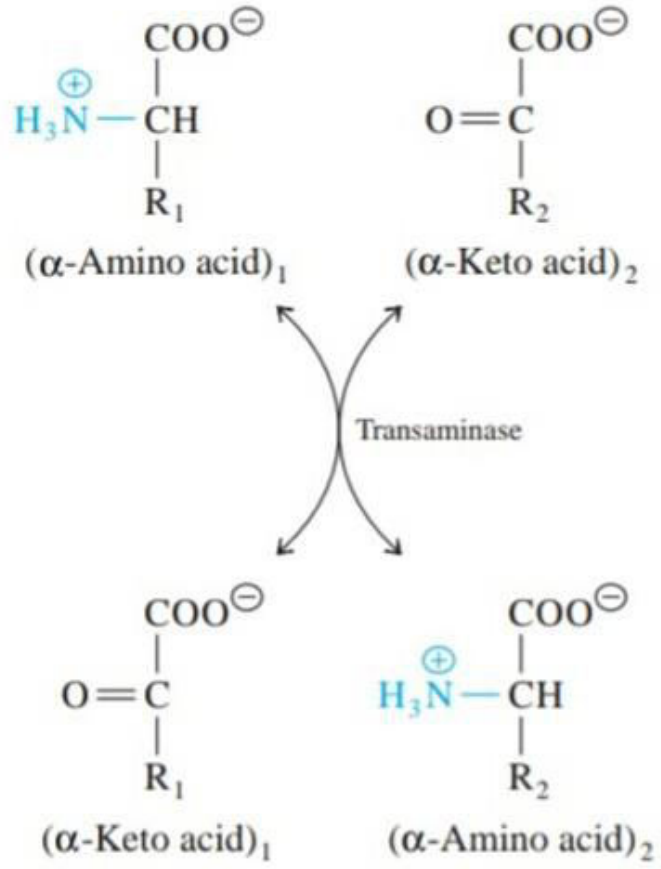
Need for removal of α-amino group

- The first part of amino acid metabolism is basically the metabolism (catabolism) of ***Nitrogen-*** containing molecules (here, removal of the α-amino group, $-NH_2$)
- The presence of the α-amino group prevents amino acids from oxidative breakdown. So removal of the α-NH_2 group is an obligatory step and is essential for producing energy.
- N-catabolism consists of removal of α -NH_2 group as NH_3 and conversion of this NH_3 to excretory end products like urea and uric acid.
- Removal of this α -NH_2 group is accomplished by basically 2 processes –
  - *Trans*amination (*transfer* of -NH_2 group)
  - *De*amination (*removal* of -NH_2 group)

Pathways of Nitrogen catabolism

- **Transamination**

Refers to the *transfer* of the *amino* group of an Amino acid (α-Amino $acid_1$) to a keto acid (α-Keto $acid_2$), changing that keto acid (α-Keto $acid_2$) into a new amino acid (α-Amino $acid_2$) and the original Amino acid (α-Amino $acid_1$) into a new keto acid (α-Keto $acid_1$).



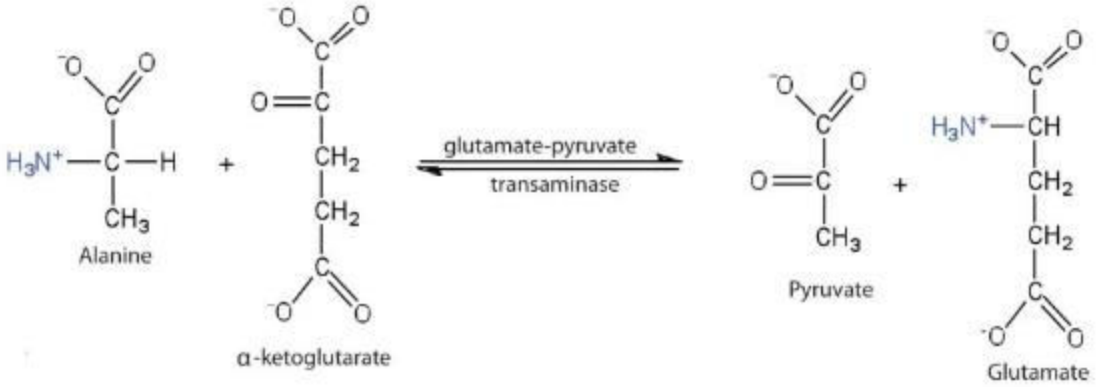
- The enzymes catalyzing transamination reactions are known as *transaminases* (or *aminotransferases)*

- Reactions occur in the mitochondria and cytoplasm of liver, kidney, heart, testes and brain.

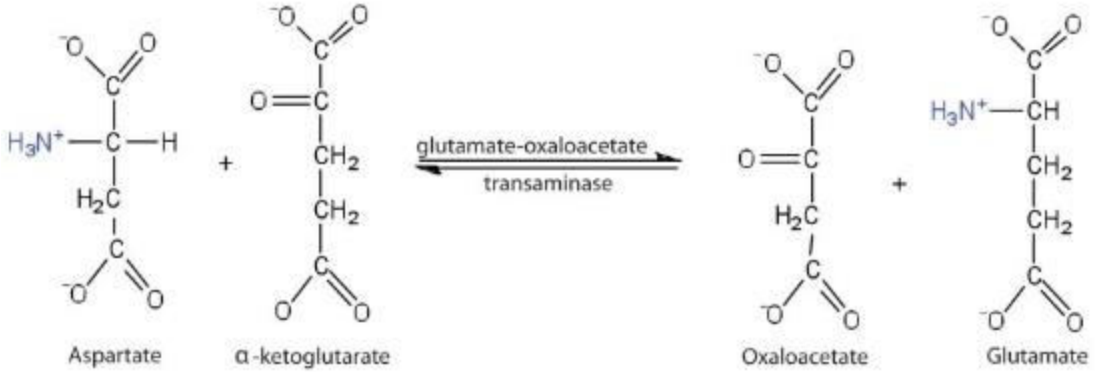
- Transaminations are reversible reactions that can serve in both formation of amino acid from keto acid and catabolism of former to the latter.

❑ ***Examples:***

- Alanine transaminase (ALT/GPT) catalyzes the transamination of alanine to pyruvate.



- Aspartate transaminase (AST/GOT) catalyzes the transamination of aspartate to oxaloacetate.

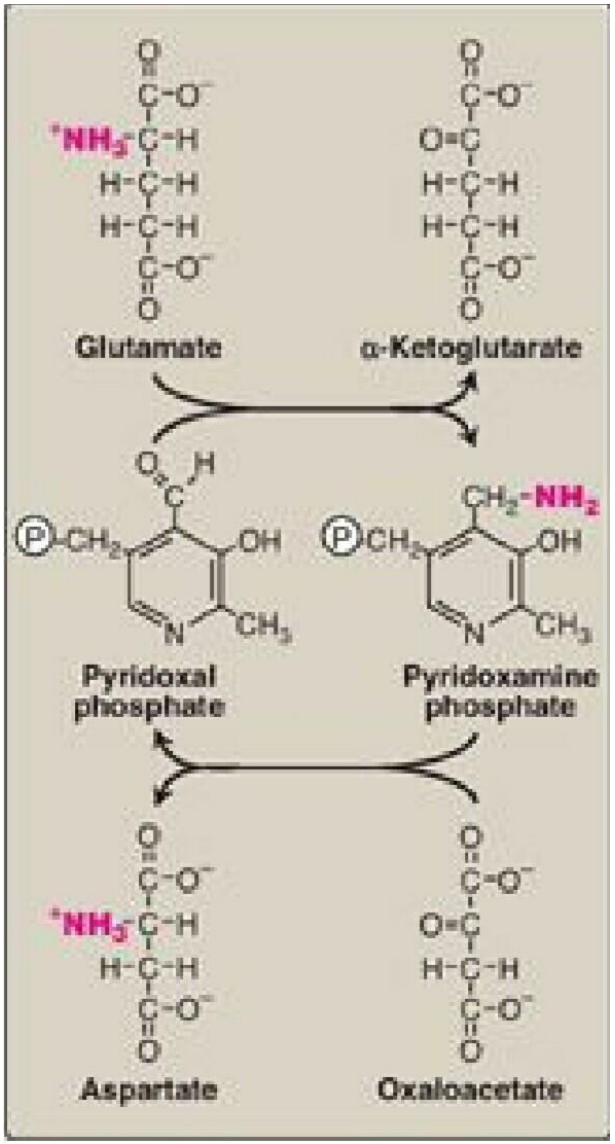


❑ *Mechanism:*

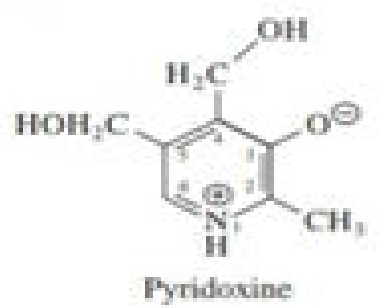
- ✓ Transaminases require pyridoxal phosphate (PLP) as the prosthetic group.
- ✓ Double displacement (ping-pong) type of bisubstrate reaction where 2 substrates, amino acid and α-keto acid bind separately and successively with the prosthetic group of the enzyme.
- ✓ Transaminases transfer $-NH_2$ group to pyridoxal part of PLP to generate pyridoxamine phosphate (PMP), which then reacts with an α-keto acid to form amino acid and regenerating the original aldehyde (PLP) form of the coenzyme.

(i) α-Amino $acid_1$ + enzyme-PLP ↔ α-Keto $acid_1$ + enzyme-PMP

(ii) α-Keto $acid_2$ + enzyme-PMP ↔ α-Amino $acid_2$ + enzyme-PLP

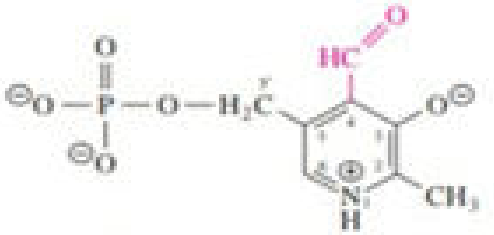


❖ *Pyridoxal phosphate (PLP)*



Pyridoxine

- PLP is a derivative of Vitamin B_6 (Pyridoxine).
- PLP contains a hydroxymethyl (-CH_2OH) group at position 4 of the pyridine ring.

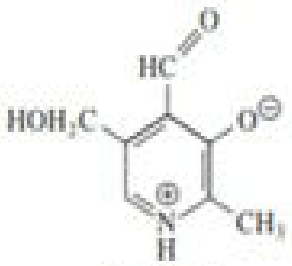


Pyridoxal 5'-phosphate (PLP)

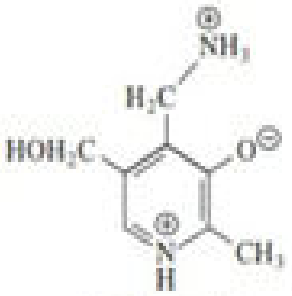
- In PLP this group has been oxidized to an aldehyde.
- The -CH_2OH group at position 5 is phosphorylated.

- PLP functions as an intermediate carrier of –NH_2 groups at the active site of transaminases.

- It undergoes reversible transformation between its aldehyde form PLP, which can accept an –NH_2 group from an amino acid, and its aminated form PMP, which can donate its –NH_2 group to a keto acid.



Pyridoxal

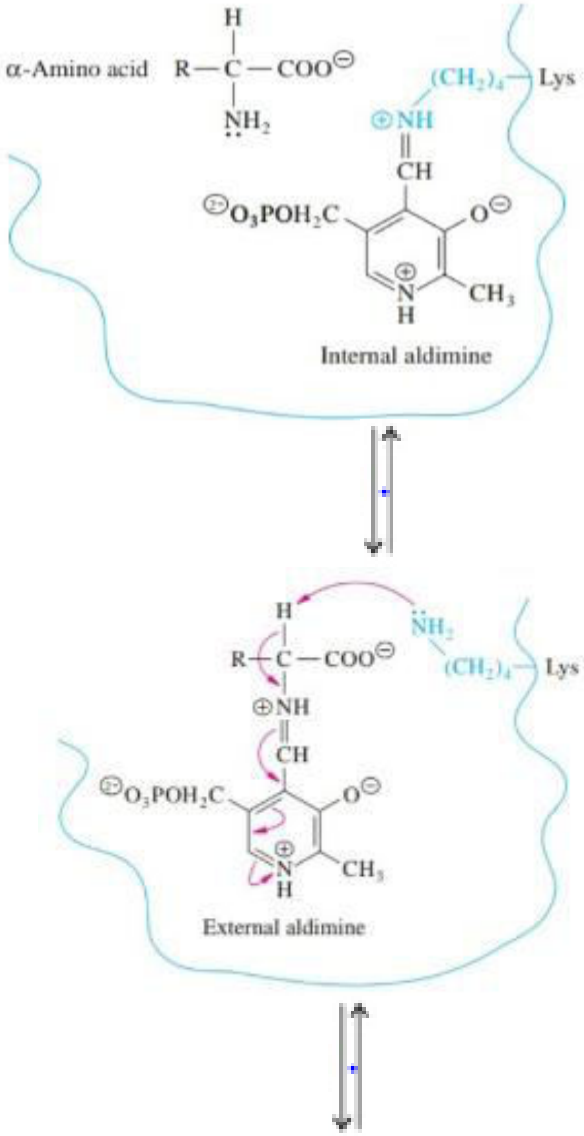


Pyridoxamine

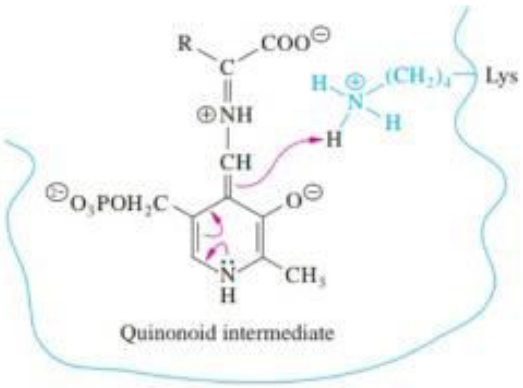
❖ *Steps of transamination*

- ✓ PLP is generally covalently bound to the active site of transaminase through a Schiff base (aldimine, *with a double bond between -N and carbonyl-C of PLP*) linkage to the €-amino group of a Lysine residue of the enzyme.

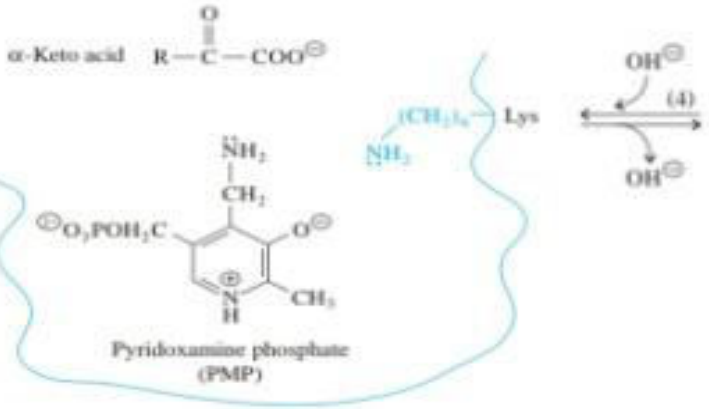
  [A Schiff base is a condensation product between an amino group (-NH_2) and a carbonyl group (C=O)]
- ✓ A new Schiff base linkage is formed on addition of an amino acid substrate. The α-NH_2 group of the amino acid substrate displaces the €-NH_2 group of the active site Lys.
- ✓ Thus, *an internal aldimine becomes an external aldimine*, with release of the Lys amino group of the enzyme.

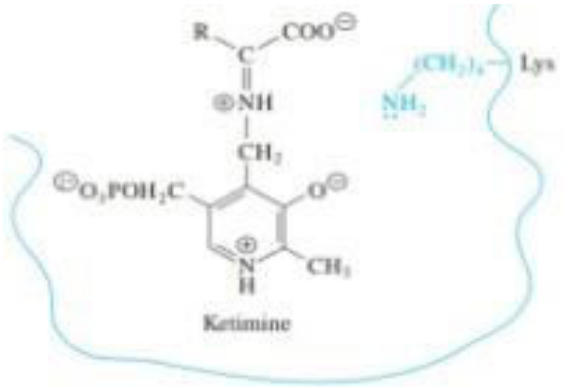


- ✓ A bond to the α-Carbon is broken, removing a proton and leaving behind a free electron pair on the α-C. This carbanion intermediate is very unstable.
- ✓ The electrophilic N of the pyridine ring of PLP acts as an electron sink, drawing electrons away from the amino acid and providing a resonance-stabilized carbanion with a quinonoid structure.



- ✓ Reprotonation yields a ketimine, (*with a double bond between -N and -C_α of the substrate*).





- ✓ The α-keto acid-PMP Schiff base (ketimine) is hydrolyzed to PMP and an α-keto acid.
- ✓ The second half occurs by a reversal of the above steps to regain enzyme-PLP.

- ***Significance:***

i. Transamination is both an anabolic as well as a catabolic process.

ii. Effect of transamination is to collect amino ($-NH_2$) groups from different amino acids in the form of L-glutamate.

iii. Glutamate in turn functions as $-NH_2$ group donor for biosynthetic pathways or excretion pathways that eliminate nitrogenous waste products.

iv. The most abundant keto acid formed is α-ketoglutaric acid, an intermediate in the TCA cycle.

v. The keto acids may also be used for the complete metabolism into carbon dioxide and water by the TCA cycle.

vi. Redistributes amino-N among amino acids and available keto acids, forming new non-essential amino acids.

vii. Produces gluconeogenic products like α-ketoglutarate, pyruvate and oxaloacetate.

viii. Serum transaminases (SGPT/SGOT) are widely used as clinical markers of tissue damage, particularly liver and cardiac muscle.

- **Deamination**

✓ Refers to the *removal* of the *amino* (-NH_2) group of an amino acid as free ammonia (NH_3)

✓ It is of 2 types:

A. Oxidative deamination

- Liberates the amino (-NH_2) group as free NH_3 along with simultaneous oxidation of the carbon skeleton.
- Occurs primarily in the liver and kidney mitochondria.
- Catalyzed mainly by two non-specific flavoprotein enzymes called amino acid oxidases and also by glycine oxidase (for glycine) and glutamate dehydrogenase *(explained later)*.

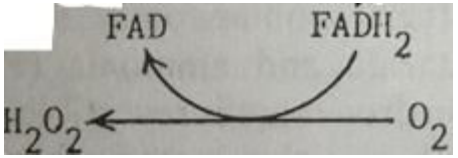
o D-amino acid oxidase oxidatively deaminates D-amino acids and carries FAD (*F*lavin *A*denine *D*inucleotide)

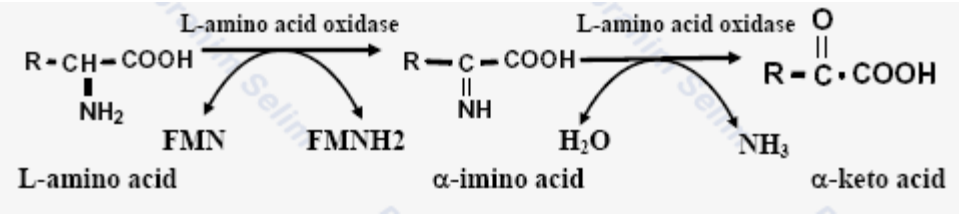
o L-amino acid oxidase oxidatively deaminates L-amino acids and requires FMN (*F*lavin *M*ono *N*ucleotide).

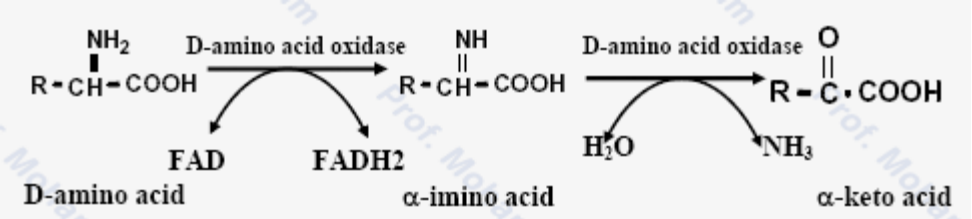
D(/L)-Amino acid + FAD(/FMN) + $H_2O \rightarrow$ α-Keto acid + NH_3 + $FADH_2$(/$FMNH_2$)

Mechanism of action:

- Amino acid oxidases oxidize an amino acid to the corresponding imino acid by transferring reducing equivalents (H^+ and *e*) from the amino acid to the flavin nucleotide.
- The imino acid reacts spontaneously with water to give an α-keto acid and NH_3.
- The reduced flavin nucleotide (e.g., $FADH_2$/$FMNH_2$) is reoxidized directly by molecular O_2, producing H_2O_2.







- ***Significance:***

i. Deamination breaks down amino acids into ammonia and α-keto acids.

ii. The α-keto acids can be used for a variety of reactions like biosynthetic precursor and energy generation.

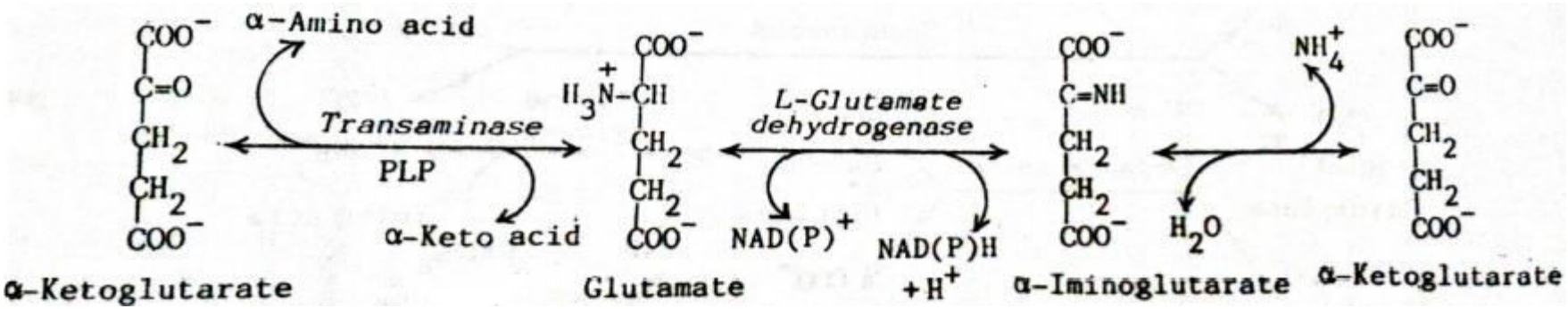
iii. The ammonia gets out of the system through urea synthesis.

➢ Transdeamination:

- (Transamination followed by oxidative deamination)
- Occurs in the liver cytoplasm and mitochondria.
- ***Mechanism***:

1. *Transamination*: A transaminase first transfers the amino group of an amino acid to α-ketoglutarate, producing a new α-keto acid and glutamate.
2. *Oxidative deamination*: (i) L-Glutamate dehydrogenase first uses NAD^+ in mitochondria (or $NADP^+$ in cytosol) as the electron acceptor to oxidize glutamate to α-iminoglutarate.

   (ii) α-iminoglutarate is then spontaneously hydrolyzed into α-ketoglutarate and ammonia.



- ***Significance***:

i. This reaction is reversible and may also synthesize glutamate by the reductive amination of α-ketoglutarate with free ammonia

ii. Also serves to deaminate other amino acids through glutamate and to provide α-ketoglutarate for the TCA cycle.

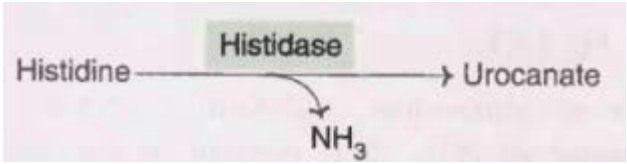
iii. Oxidative deamination occurs primarily on glutamic acid as glutamate is the end product of many transamination reactions

iv. The interconversion of α-ketoglutarate and glutamate lies at the very heart of nitrogen metabolism. These molecules serve as the collection and receiving agent for nitrogen.

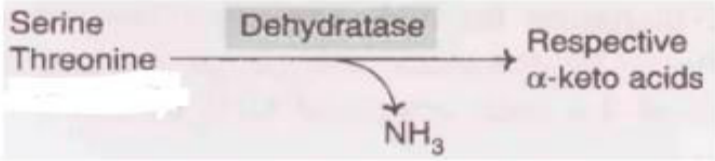
v. This reaction links glutamate metabolism with TCA cycle via α-ketoglutarate.

B. Non-oxidative deamination

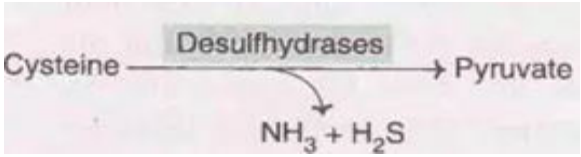
- Deamination of amino acids i.e removal of the amino group of amino acids as ammonia, but involving no oxidation of the carbon skeleton.
- **Amino acid lyases**: these are C-N lyases that deaminate significant amounts of histidine (histidase) and aspartic acid (aspartate ammonia lyase)



- **Amino acid dehydratases**: PLP containing enzymes that catalyze the dehydration followed by deamination of hydroxy amino acids like serine (by serine dehydratase) and threonine (by threonine dehydratase).



- **Amino acid desulfhydrases**: PLP containing enzymes that catalyzes desulfhydration and then deamination of sulfur containing amino acid like cysteine (by cysteine desulfhydrase)



- **Amino acid amide hydrolases:** Catalyze hydrolytic deamination of amino acid amides like glutamine and asparagine, releasing their amide groups as ammonia and changing them respectively to glutamate and aspartate.

Fate of ammonia

✓ Ammonia is highly toxic, so needs to be rapidly eliminated from the body.

- **Metabolic fate**

1. Mainly converted to urea in the liver by urea cycle.
2. Formation of glutamine (a non-toxic form of ammonia that can be transported to liver, kidneys and intestine via blood)
3. Amination of α- keto acid to form α-amino acid.

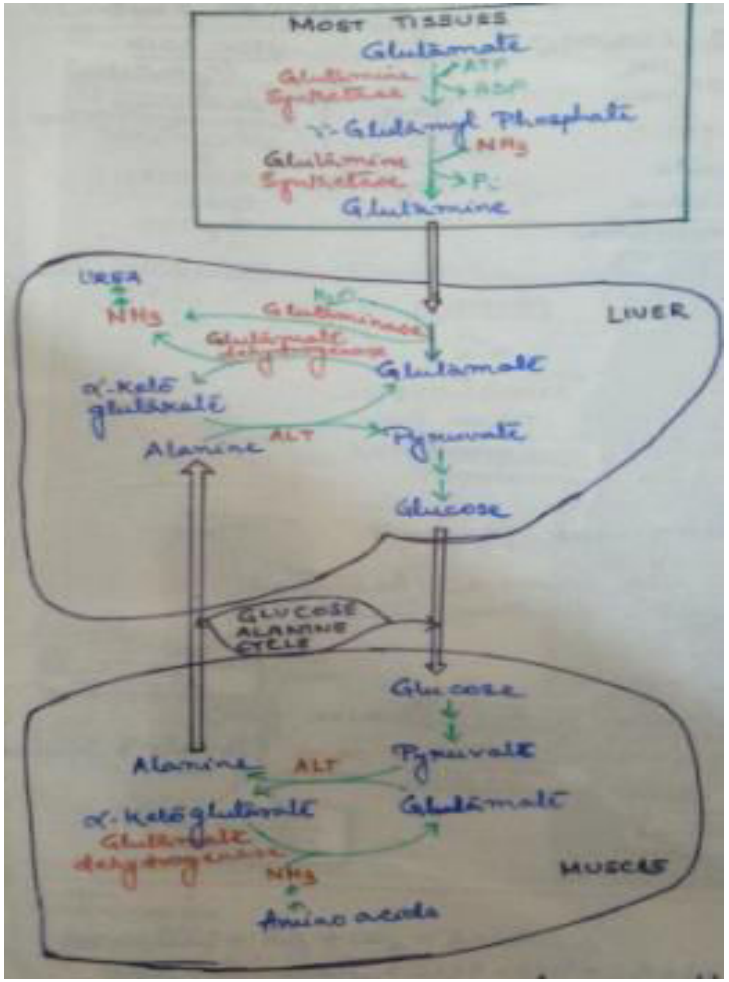


Fig: Transport of ammonia in peripheral tissues

References

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