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 (aminotransferases)

Salient features of Transamination

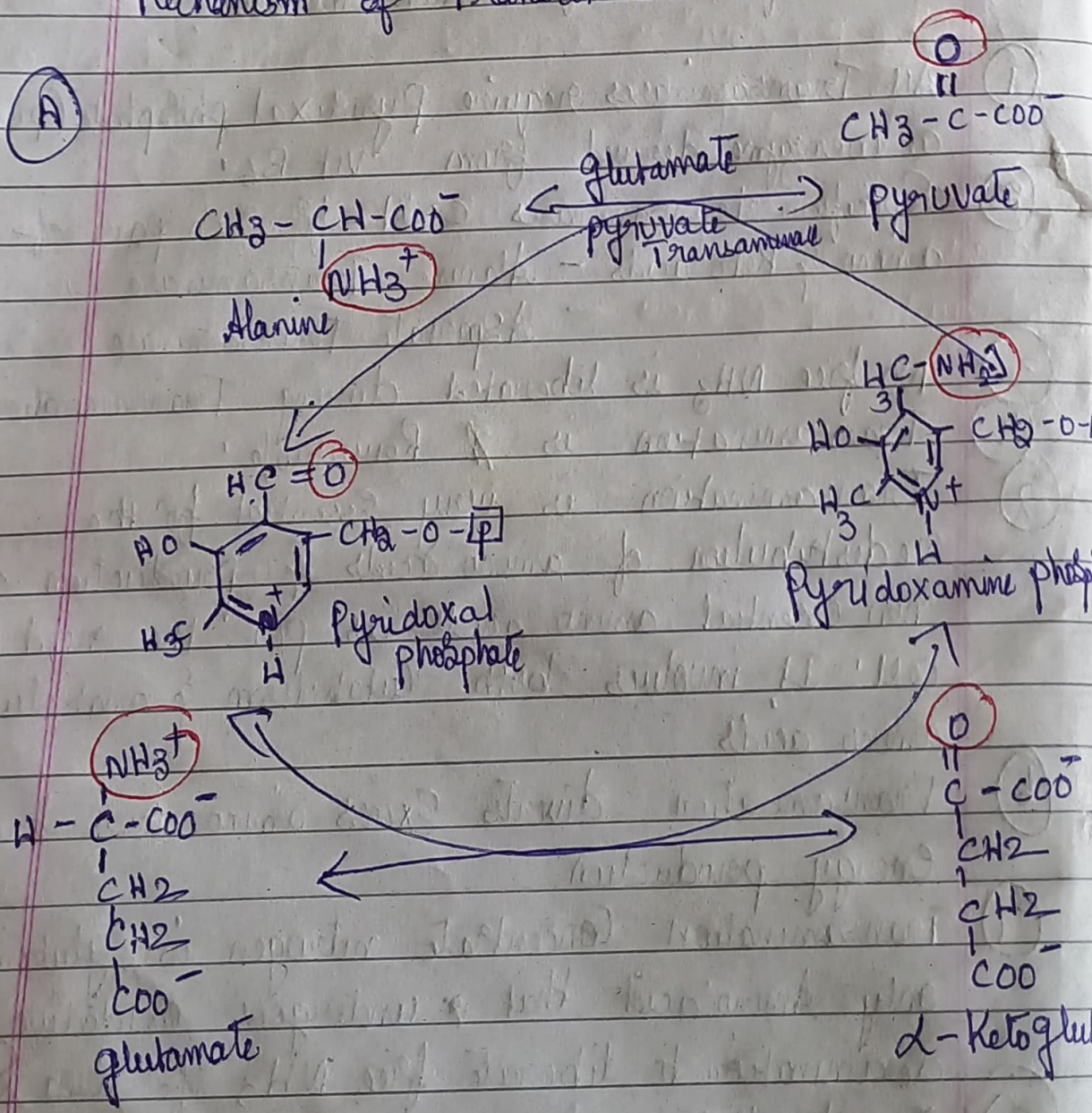
1. All Transaminases require Pyridoxal phosphate (PLP) a coenzyme derived from Vit B_6.
2. Specific transaminases exist for each pair of amino & Keto acids - Alanine Transaminase
   Aspartate Transaminase
3. No free NH_3 is liberated during Transamination
4. Transamination is Reversible.
5. Transamination is very Essential for the redistribution of amino acids & production of non-essential amino acids as per the need of cell. It involves both Catabolism & anabolism of amino acids
6. Transamination diverts Excess amino acids towards energy production
7. Transamination Concentrate nitrogen in glutamate.
8. only Amino acid that undergoes oxidative deamination to liberate free NH_3 for urea Synthesis
9. Except Lysine, Threonine, Proline & Hydroxy proline participate in Transamination.

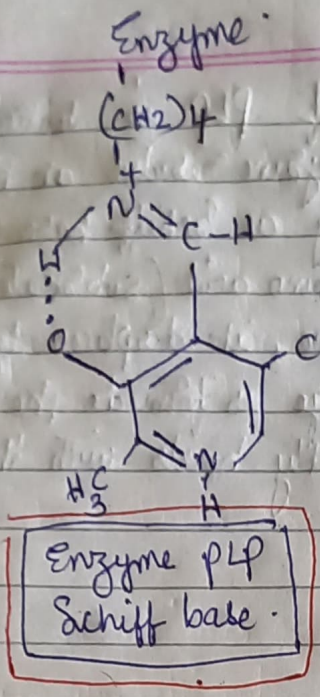
(9) Transamination ~~diverts the excess amino~~ is not restricted to α-amino groups. δ-amino group of Ornithine is transaminated

(10) Serum Transaminases are important for diagnostic and prognostic purposes.

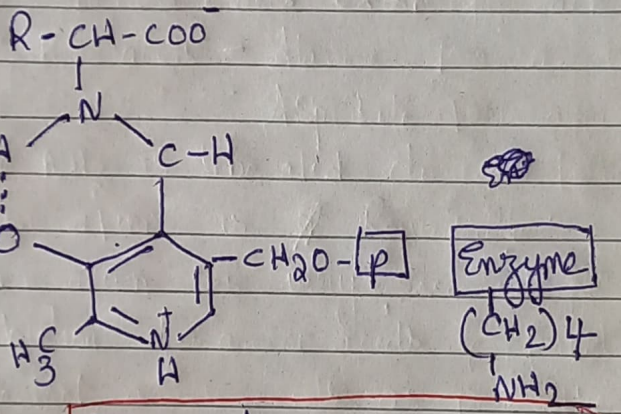
Mechanism of Transamination :-

(A)





Enzyme PLP Schiff base.



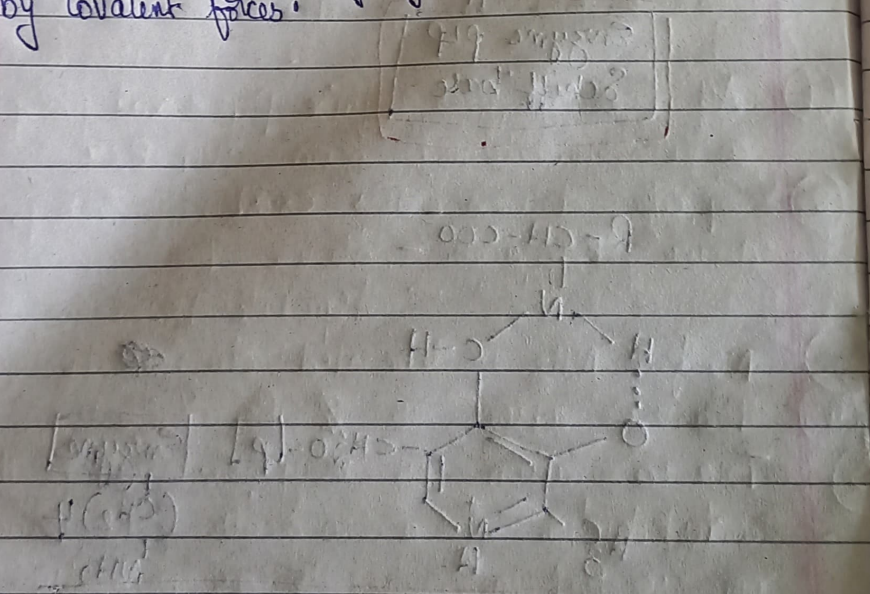
Amino acid PLP - Schiff base.

Transamination occurs in two stages:-

1) Transfer of the amino group to the coenzyme Pyridoxal phosphate (bound to the coenzyme) to form Pyridoxamine phosphate.

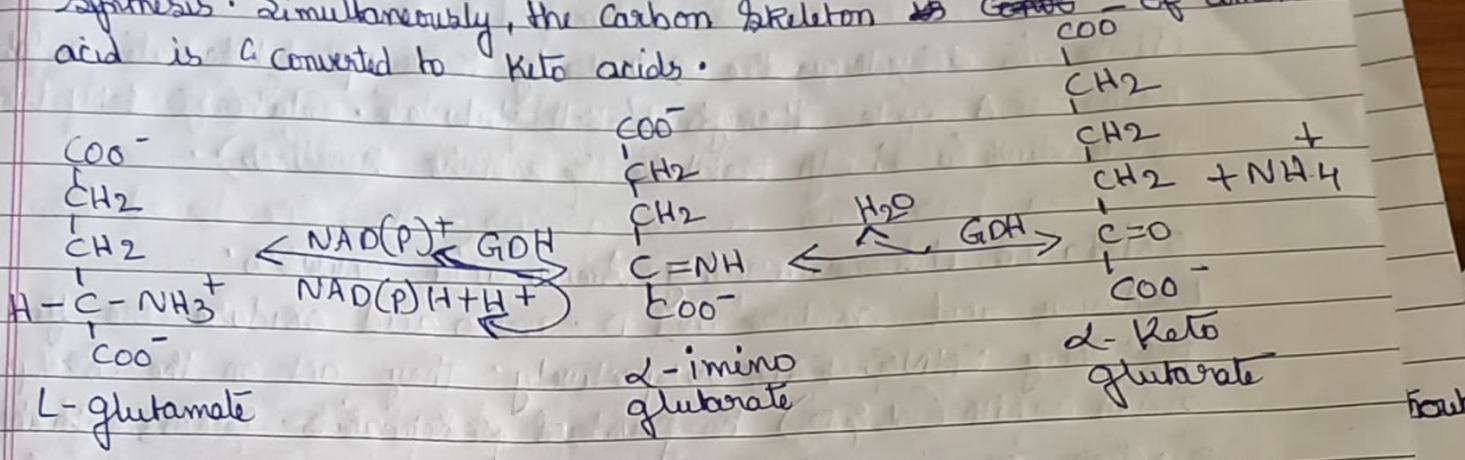
2) The amino group of Pyridoxamine phosphate is then transferred to a Keto acid to produce a new amino acid and the enzyme with PLP is regenerated

The aldehyde group of PLP is linked with ε-amino group of lysine residue at the active site of enzyme forming a Schiff base (imine linkage). when an amino acid (substrate) comes in contact with the enzyme, it displaces lysine & a new Schiff base linkage is formed. the amino acid PLP-Schiff base tightly binds with the enzyme by covalent forces.



Deamination

The removal of amino group from the amino acid as NH_3 is deamination. Deamination results in the liberation of NH_3 for urea Synthesis. Simultaneously, the carbon skeleton ~~is~~ Copies of amino acid is converted to keto acids.



It might be oxidative (or) non-oxidative.

Transamination & deamination occurs simultaneously mostly involving glutamate as central molecule. For this reason, this process is also called as Transdeamination.

I. Oxidative deamination :- It is the liberation of free ammonia from the amino group of amino acids coupled with oxidation. This occurs mostly in liver & Kidney. The purpose of oxidative deamination is to provide NH_3 for urea Synthesis & α-Keto acids for a variety of reactions, including energy generation.

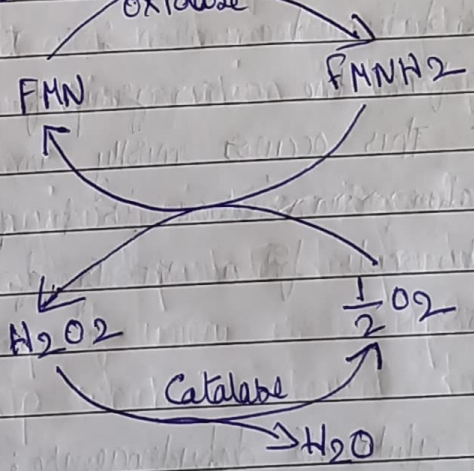
Role of glutamate dehydrogenase :- During transamination, the amino groups of most amino acids are transferred to α-Keto glutarate to produce glutamate. Thus, glutamate serves as a Collection Center for amino groups in the biological System. Glutamate rapidly undergoes oxidative deamination, Catalysed by glutamate dehydrogenase (GDH) to liberate NH_3. This enzyme can also utilize either NAD^+ (or) $NADP^+$ as Co-enzyme.

Conversion of glutamate to α-Ketoglutarate occurs through the formation of an intermediate, α-iminoglutarate.

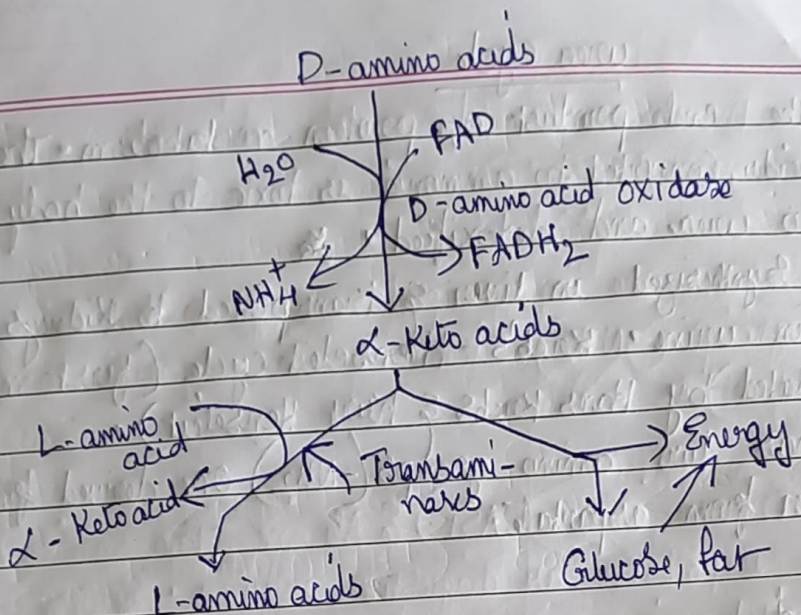
Glutamate dehydrogenase reversibly links up glutamate metabolism with TCA cycle through α-Keto glutarate. Glutamate dehydrogenase is involved in both Catabolic & anabolic reactions.

oxidative deamination by Amino acid oxidases.
L-Amino acid oxidase and D-amino acid oxidase are flavoproteins possessing FMN & FAD, respectively. They act on the corresponding amino acids (L & D) to produce α-Keto acids & NH_3. In this reaction O_2 is reduced to H_2O_2, which is later decomposed by Catalase.
The ~~activity~~ of L-amino acid oxidase do not act on glycine & dicarboxylic acids. D-amino acids are regularly taken in diet & are metabolized by the body.

L-Amino acid $\xrightarrow[\text{Oxidase}]{\text{L-amino acid}}$ α-Keto acid + NH_3



The α-Keto acids produced by the action of D-amino acid oxidase from D-amino acids undergo transamination to L-amino Keto acids may be oxidized to generate energy (ATP) act as prec for glucose & fat Synthesis. D-amino acid oxidase is important for its conversion of unnatural D-amino acids to L-am acids.



Non-oxidative deamination

Some of the amino acids are deaminated to liberate NH_3 without undergoing oxidation.

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

Serine, Threonine, Homoserine $\xrightarrow{\text{Dehydratase}}$ Respective α-Keto acids + NH_3

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

Cysteine $\xrightarrow{\text{Desulfhydrase}}$ Pyruvate + $NH_3 + H_2S$

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

Histidine $\xrightarrow{\text{Histidase}}$ urocanate + NH_3

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urea cycle

urea is the end product of protein metabolism. The nitrogen of amino acids, converted to ammonia is toxic to the body. It is converted to urea and detoxified.
urea is synthesized in liver & transported to kidneys for excretion in urine. Kreb's - Henseleit cycle (first metabolic cycle elucidated by Hans Krebs & Kurt Henseleit).
urea has two amino groups ($-NH_2$) one derived from NH_3 & the other from aspartate.

Synthesis of urea :-

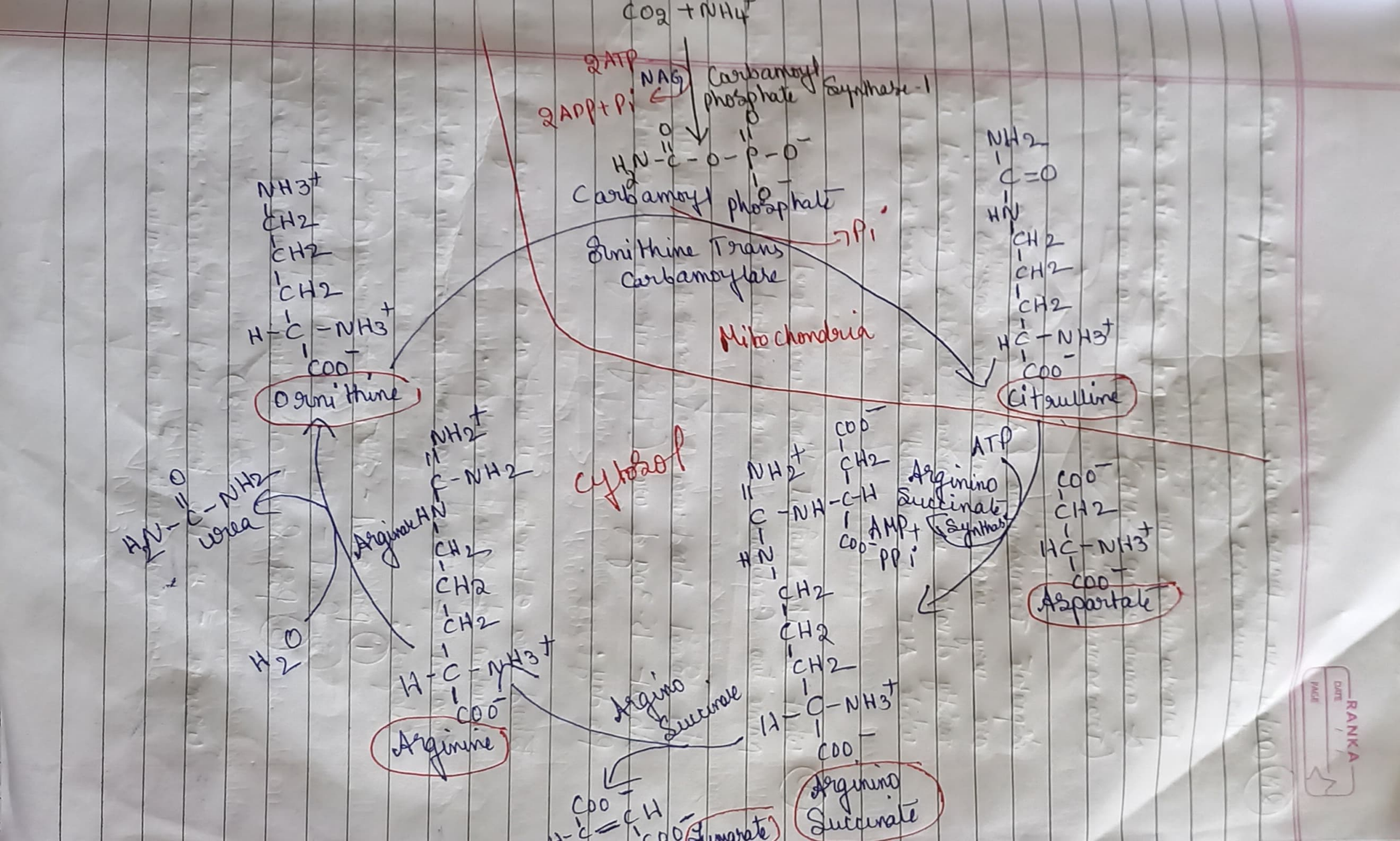
1) Synthesis of Carbamoyl phosphate :- Carbamoyl phosphate Synthase-I of mitochondria catalyses the condensation of NH_4^+ ions & CO_2 to form Carbamoyl phosphate. This step consumes 2 ATP & is irreversible & rate-limiting. Carbamoyl phosphate-I requires N-acetyl glutamate for its activity.

2) Formation of citrulline :- Citrulline is synthesized from Carbonyl phosphate & Ornithine by Ornithine transCarbamoylase Ornithine is regenerated and used in urea cycle. ~~Therefore, its role~~ Citrulline produced in this reaction is transported to cytosol by a transporter system.

3) Synthesis of ArginoSuccinate :- ArginoSuccinate 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 & pyrophosphate (PP_i).

4) Cleavage of ArginoSuccinate :- ArginoSuccinase cleaves arginoSuccinate to give arginine & fumarate. Arginine is the immediate precursor for urea. Fumarate Connects with TCA cycle & gluconeogenesis.

5) Formation of urea :- Arginase is the fifth & final enzyme that cleaves arginine to yield urea & Ornithine. Ornithine, Enters mitochondria & is reused in the urea cycle. Arginase activated by Co_2^{+} & Mn^{2+}.


$CO_2 + NH_4^+$
2ATP
2ADP + Pi
NAG
Carbamoyl phosphate Synthase-1
Carbamoyl phosphate
Pi
Ornithine Trans Carbamoylase
Mitochondria
Ornithine
Citrulline
Cytosol
Urea
H_2O
Arginase
Arginine
Argino Succinate
ATP
Argino Succinate Synthase
AMP + PPi
Aspartate
Fumarate
Argino Succinate

(2M) (1) What are Transaminases? Write the diagnostic importance of two transaminases?

Transaminases are a group of enzymes that catalyse transfer of amino group ($-NH_2$) from an amino acid to a Keto acid (Transamination).

Aspartate Transaminase (AST) & Alanine Transaminase (ALT) are very important as diagnostic enzymes. Serum AST is mostly elevated in cardiac disorders (Myocardial infarction) while ALT is increased in liver diseases (viral hepatitis)

(2) What is the normal blood urea level? ~~Name two conditions~~ name two conditions in which blood urea level is elevated.

The normal blood urea level concentration is 10-40 mg/dl. About 15-30g of urea (7-15g nitrogen) is excreted in urine per day.

Blood urea estimation is used as a screening test for the evaluation of kidney function.

Elevation of Blood urea may be broadly classified in 3 categories:

(1) pre renal - After major Surgery, Burns, ↑ed protein breakdown
(2) Renal - acute glomerulonephritis, polycystic kidney
(3) post Renal - obstruction in urinary tract (Tumors, stones)

"Uremia" - is the term used to indicate increased blood urea levels due to renal failure.

Name four Metabolic disorders of urea cycle with enzyme defect.

Defect	Enzyme Involved	Characteristics
Hyperammonemia Type-I	Carbamoyl phosphate Synthase-I	Highly elevated blood NH_3, Mental Retardation
Hyperammonemia Type-II	Ornithine Trans-Carbamoylase	Increased NH_3, & glutamine in blood & urine
Citrullinemia	Arginino Succinate Synthase	High blood Conc. of NH_3 & citrulline
Arginino Succinic aciduria	Arginino Succinase	elevated arginino Succinate in blood & urine.

Production of Bile pigments

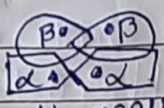
Erythrocytes have a life span of 120 days. Erythrocytes are taken up & degraded by the macrophages of Reticulo Endothelial System in Spleen & liver.

⇒ the Hemoglobin is cleaved to protein part globin & non-protein heme. the globin may be reutilized for the formation of Hb (or) degraded into individual amino acids

⇒ A Complex Microsomal enzyme Called heme oxygenase, utilizes NADPH & O_2 & cleaves the methenyl bridges between two pyrrole rings (A & B) to form Biliverdin (green pigment)

Aged Erythrocytes

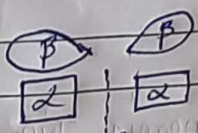
Macrophage



Haemoglobin

Other heme proteins
Apoproteins

Heme



Amino acids → Reutilized / degraded.

O_2 NADPH+H$^+$ → $NADP^+$

Heme oxygenase → Fe^{3+}, CO

Biliverdin

NADPH+H$^+$ → $NADP^+$ (Biliverdin Reductase)

Bilirubin

Blood

Bilirubin – albumin complex

LIVER

Bilirubin

2 UDP-glucuronate → (Bilirubin glucuronyl Transferase)

Bilirubin diglucuronide (to bile)

Intestine | Kidney

Microbial enzymes

UROBILINOGEN

Microbial enzymes

Kidney

Urobilin → to urine

Stercobilin → to faeces

Biliverdin reductase reduces methenyl bridges (Between pyrrole rings C & D) of Biliverdin & converts it into Bilirubin (yellow pigment).

Bilirubin is lipophilic & is insoluble in aqueous Solution Bilirubin is transported in the plasma in a bound form with albumin. This albumin - Bilirubin Complex enters liver Certain drugs & Antibiotics displace Bilirubin from albumin Due to this, bilirubin can enter CNS & cause damage to neurons

The albumin - Bilirubin Complex enters liver, bilirubin dissociates & taken up by hepatocytes through active transp Bilirubin Conjugates with 2 molecules of glucuronate & this reaction is Catalysed by bilirubin glucuronyl Transferase.

Now, the Bilirubin diglucuronide is in Conjugate form & is Completely water Soluble.

Conjugated Bilirubin enters into bile.

Bilirubin glucuronides are hydrolyzed in the intestine by bacterial enzymes namely β-glucuronidases & is Converted to urobilinogen (colourless). A small part enters circulation.

Urobilinogen is converted to urobilin (yellow coloured Compound) in the kidneys & is excreted.

→ Major part of urobilinogen is Converted by bacteria int Stercobilin.

→ The characteristic brown Colour of faeces is due to Stercobilin.