Protein Metabolism

INTRODUCTION

- Proteins are the most abundant organic compounds and constitute a major part of the dry body weight.
- These are nitrogen-containing macro-molecules consisting of L-alpha amino acids as the repeating units.

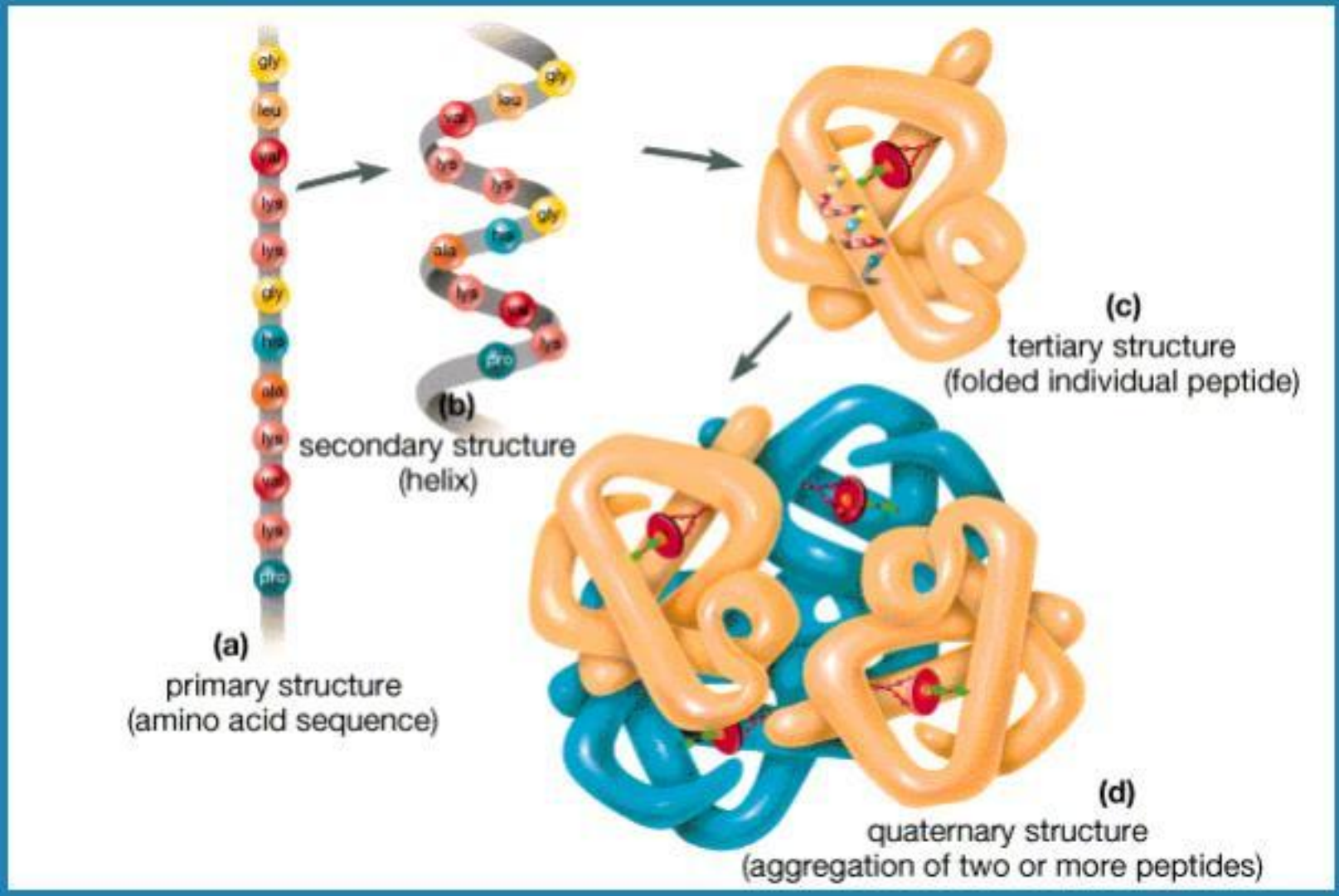
- Proteins are made from 20 different amino acids,9 of which are essential.
- Each amino acid has an amino group, an acid group, a hydrogen atom, and a side group.
- It is the side group that make each amino acid unique.
- The sequence of amino acids in each protein determines it's unique shape and function.
- **Amino acids** are a group of organic compounds containing two functional groups-amino and carboxyl. The amino group (-NH2) is basic while the carboxyl group (-COOH) is acidic in nature.

• Elemental composition of proteins

- Proteins are predominantly constituted by five major elements in the following proportion:

Carbon: 50-55%
Hydrogen: 6-7.3%
Oxygen: 19-24%
Nitrogn:13-19%
Sulfur:0-4%

Four Levels of Protein Structure



Functions of Proteins

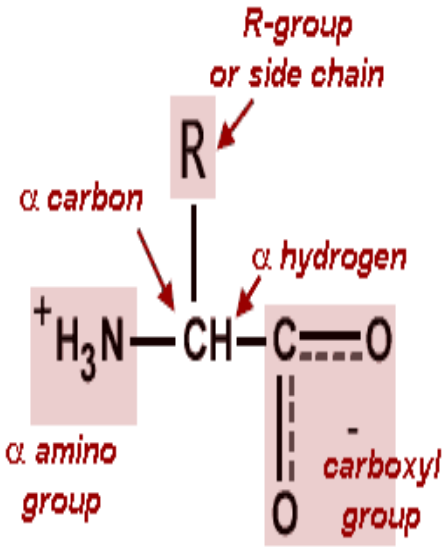
Proteins perform many different functions.

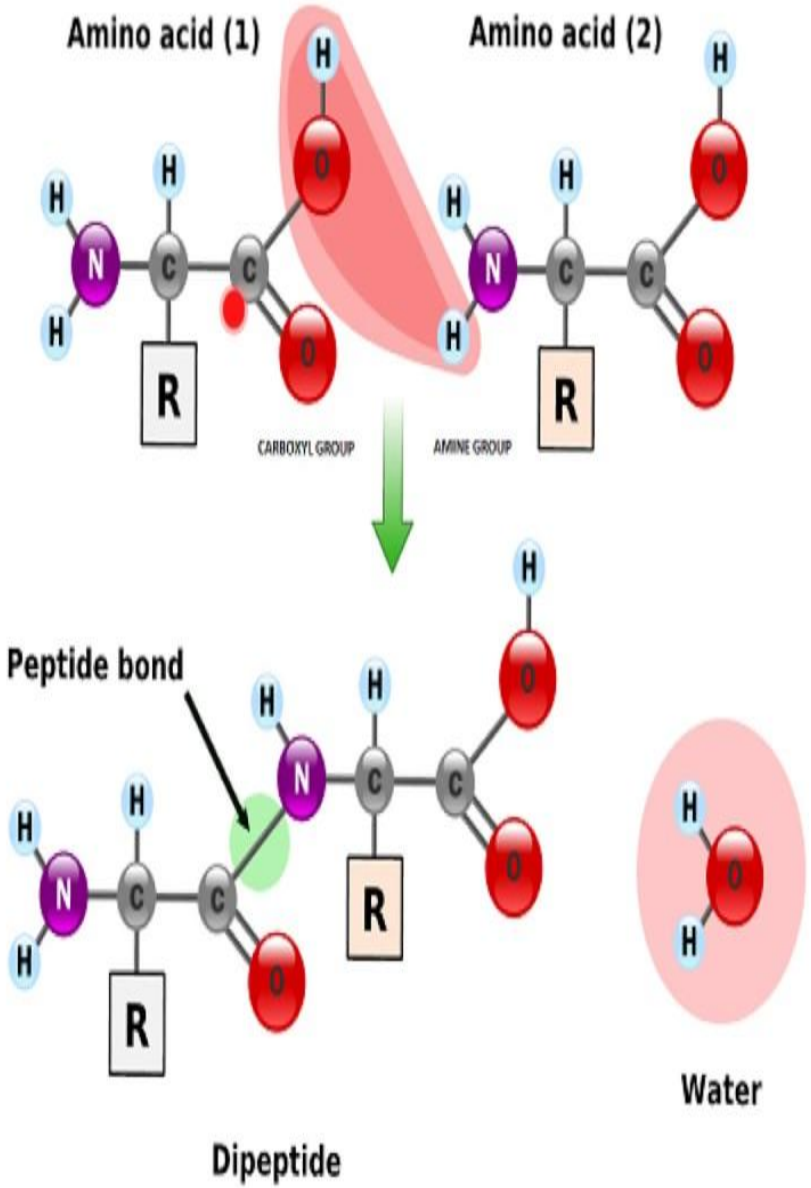
Table 20.1 Classification of Some Proteins and their Functions

Class of Protein	Function in the Body	Examples
Structural	Provide structural components	*Collagen* is in tendons and cartilage. *Keratin* is in hair, skin, wool, and nails.
Contractile	Movement of muscles	*Myosin* and *actin* contract muscle fibers.
Transport	Carry essential substances throughout the body	*Hemoglobin* transports oxygen. *Lipoproteins* transport lipids.
Storage	Store nutrients	*Casein* stores protein in milk. *Ferritin* stores iron in the spleen and liver.
Hormone	Regulate body metabolism and nervous system	*Insulin* regulates blood glucose level. *Growth hormone* regulates body growth.
Enzyme	Catalyze biochemical reactions in the cells	*Sucrase* catalyzes the hydrolysis of sucrose. *Trypsin* catalyzes the hydrolysis of proteins.
Protection	Recognize and destroy foreign substances	*Immunoglobulins* stimulate immune responses.

WORKING HORSES OF THE CELL

Amino Acid Structure and Peptide Bond Formation





The general structure of an amino acid has an amino group, a hydrogen, a carboxyl group, and a side chain

The side chain is the most important in determining reactivity of the amino acid

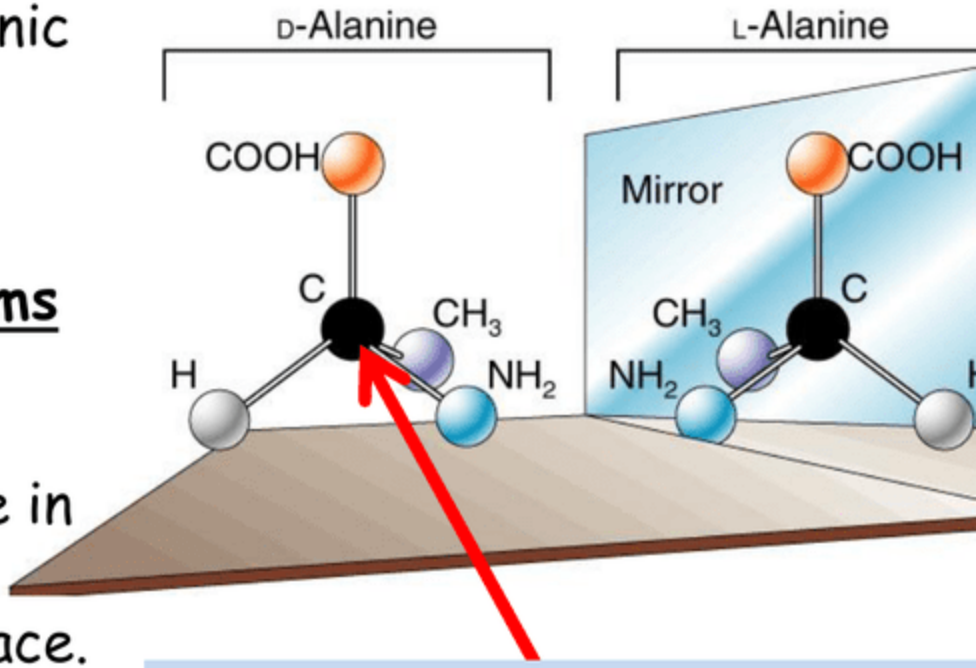
The amino acid is read from the n terminus to the c terminus

Each amino acid has abbreviations

Peptide bonds are formed when two amino acids come together

Optical Isomerism

- Optical isomerism arises in organic molecules that contain a c**arbon atom attached to 4 different atoms or groups.**
- ***A carbon with 4 different atoms or groups attached is called*** a **chiral centre.**
- If a molecule has a chiral centre in its structure, two mirror image arrangements are possible in space. They are non-superimposable mirror images of each other: they are **optical isomers.**

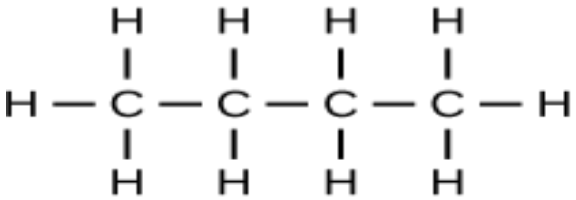


Chiral Carbon attached to different atoms or groups

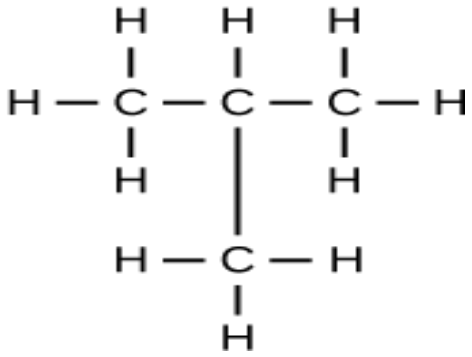
- **Optical isomers rotate plane polarised light in opposite directions:** one rotates light clockwise and the other anticlockwise.
- A mixture containing equal amounts of each isomer is known as a **racemic mixture**. A racemic mixture has no effect on plane polarised light because the rotations cancel each other out.

Structural Isomers

Butane



Isobutane



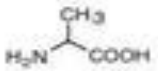
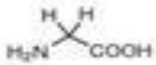
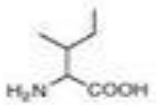
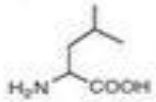
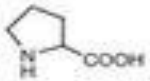
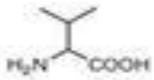
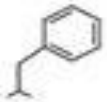
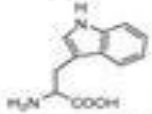
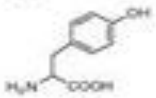
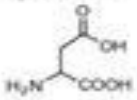
ALL AA are L-ALPHA AMINO ACIDS

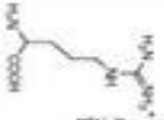
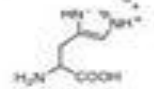
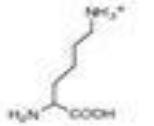
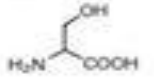
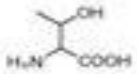
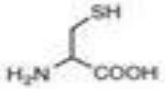
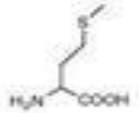
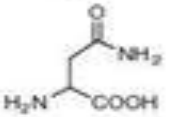
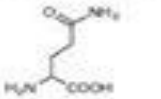
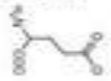
L-NH2 group is on left side
Alpha carbon has amino group

C4-C3-C2-COOH

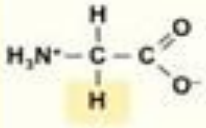
NH2-C-COOH alpha carbon has amino group

CLASSIFICATION OF AMINO ACIDS

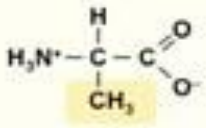
Class	Name	Atomic Structure
Aliphatic	Alanine	
	Glycine	
	Isoleucine	
	Leucine	
	Proline	
	Valine	
Aromatic	Phenylalanine	
	Tryptophan	
	Tyrosine	
Acidic	Aspartic Acid	

Class	Name	Atomic Structure
Basic	Arginine	
	Histidine	
	Lysine	
Hydroxylic	Serine	
	Threonine	
Sulfur-Containing	Cysteine	
	Methionine	
Amidic (containing amide group)	Asparagine	
	Glutamine	
Acidic	Glutamic Acid	

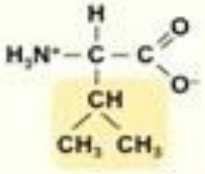
NON-POLAR (Hydrophobic)



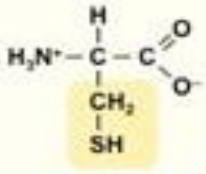
Glycine
(Gly / G)



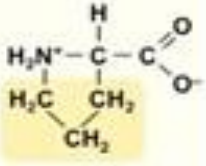
Alanine
(Ala / A)



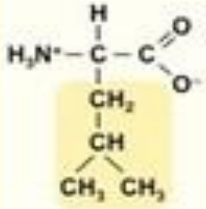
Valine
(Val / V)



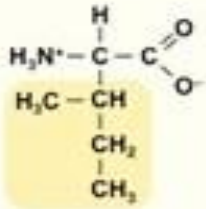
Cysteine
(Cys / C)



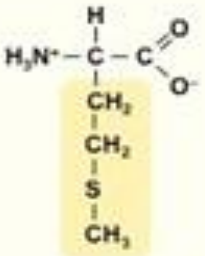
Proline
(Pro / P)



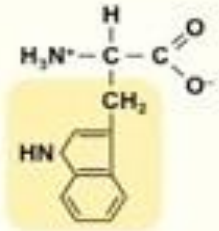
Leucine
(Leu / L)



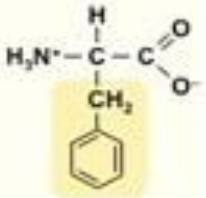
Isoleucine
(Ile / I)



Methionine
(Met / M)

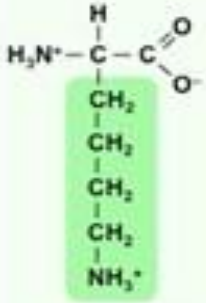


Tryptophan
(Trp / W)

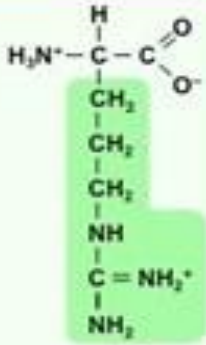


Phenylalanine
(Phe / F)

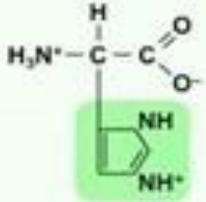
+ CHARGE



Lysine
(Lys / K)

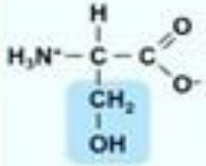


Arginine
(Arg / R)

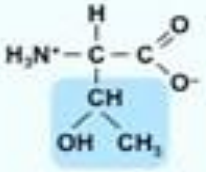


Histidine
(His / H)

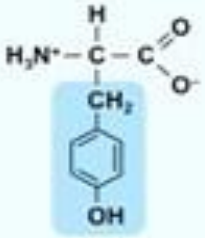
POLAR (Hydrophilic)



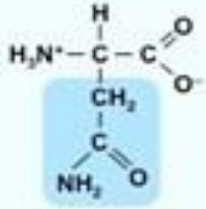
Serine
(Ser / S)



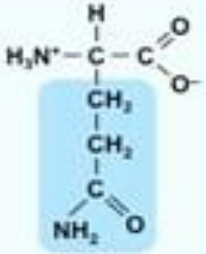
Threonine
(Thr / T)



Tyrosine
(Tyr / Y)

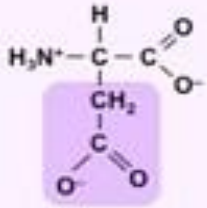


Asparagine
(Asn / N)

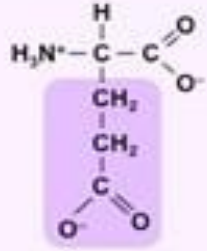


Glutamine
(Gln / Q)

– CHARGE

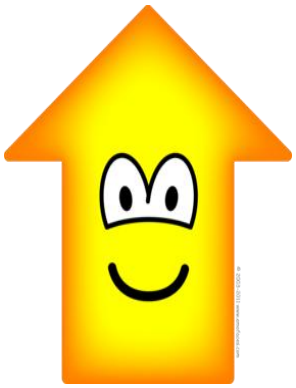


Aspartic Acid
(Asp / D)



Glutamic Acid
(Glu / E)

9
HILL
MP
TTV



Essential	Non-Essential
Histidine	Alanine
Isoleucine	Arginine
Leucine	Asparagine
Lysine	Aspartic acid
Methionine	Cysteine
Phenylalanine	Glutamic acid
Threonine	Glutamine
Tryptophan	Glycine
Valine	Proline
	Selenocysteine
	Serine
	Tyrosine

Table 2.1 - Essential and non-essential amino acids

In recent years, a 21st amino acid namely selenocysteine has been added.

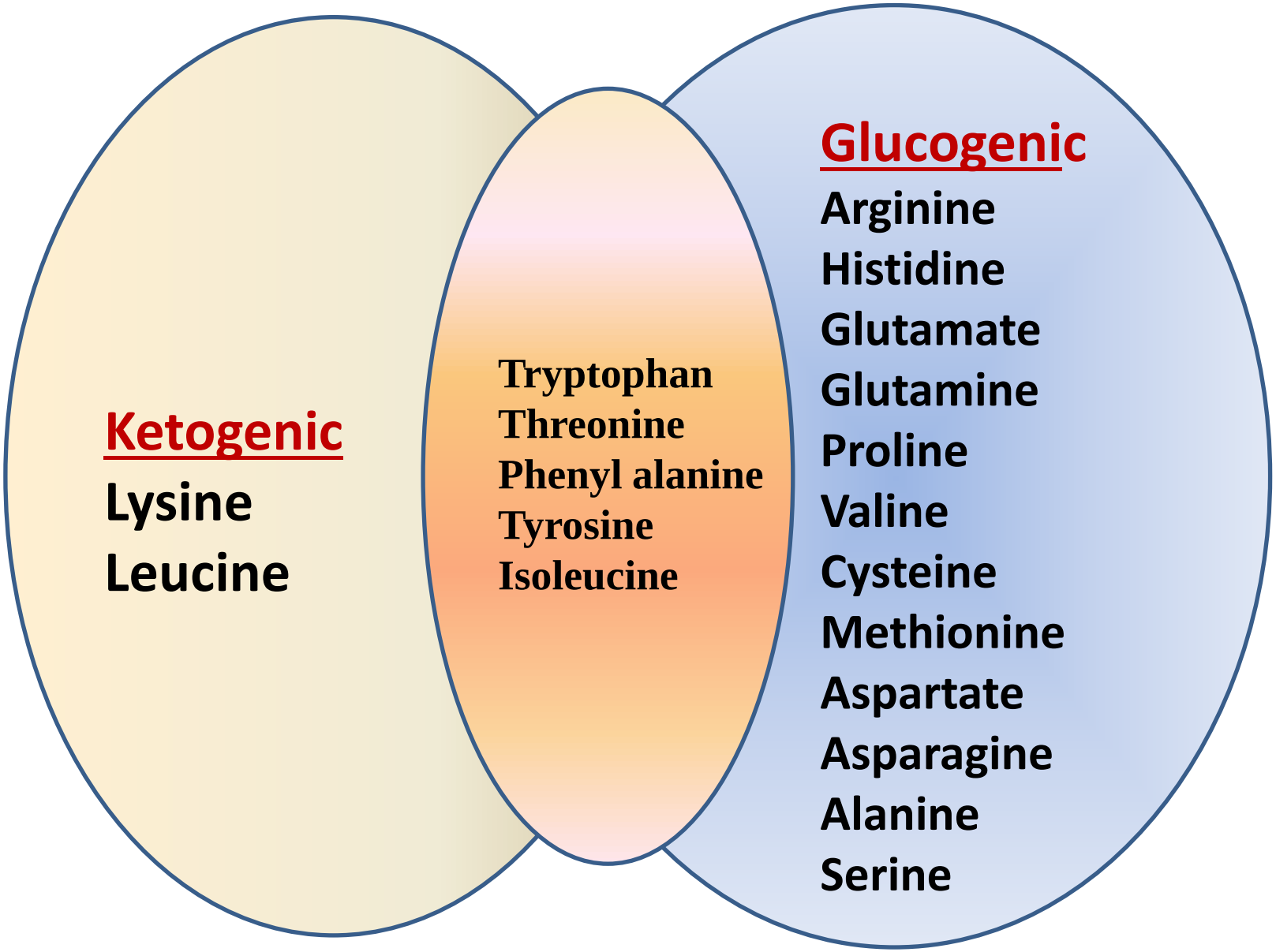
*** Histidine and Arginine are semi-essential amino acid**

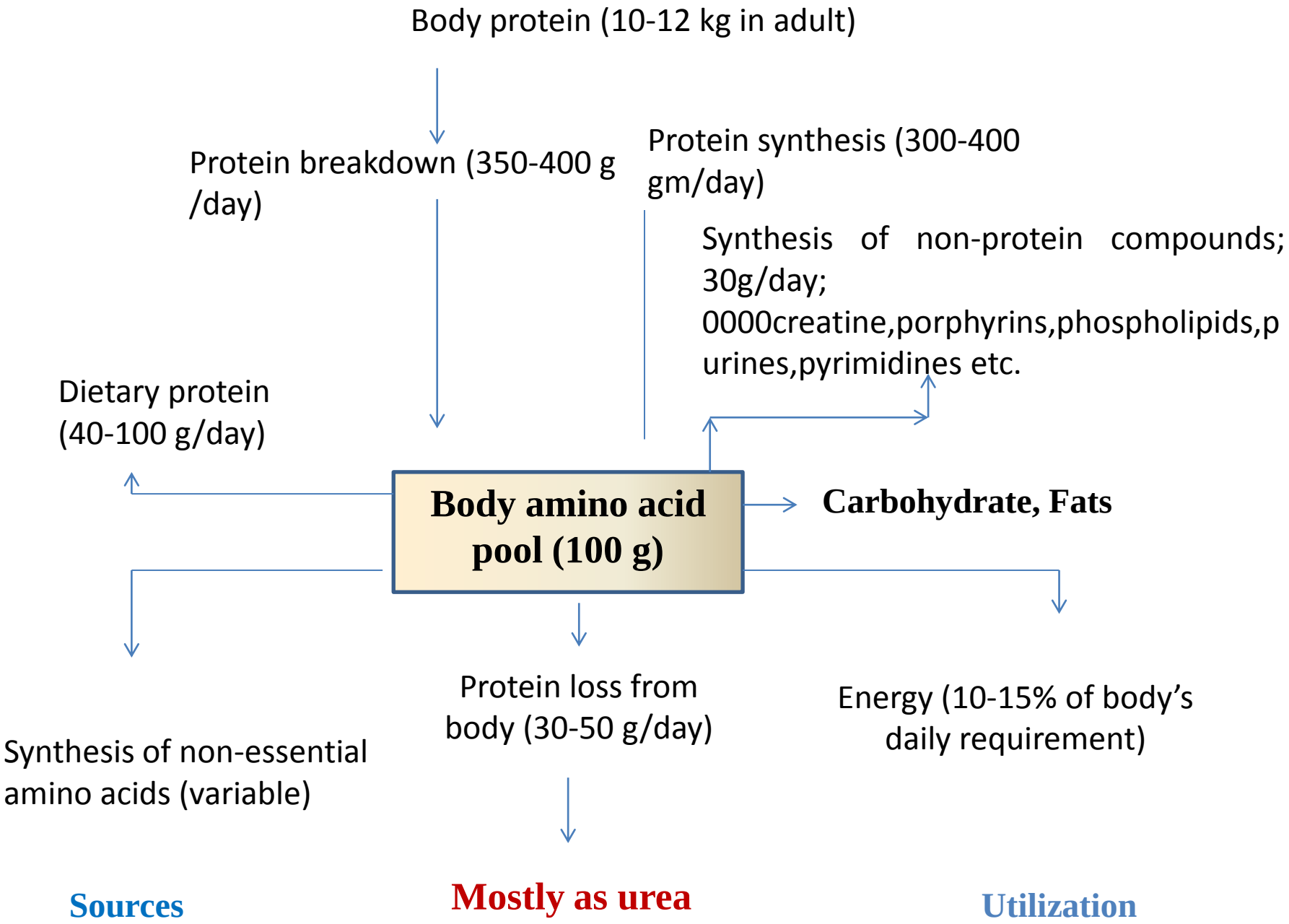
Animal proteins of high biological values (e.g., milk, liver and egg proteins)

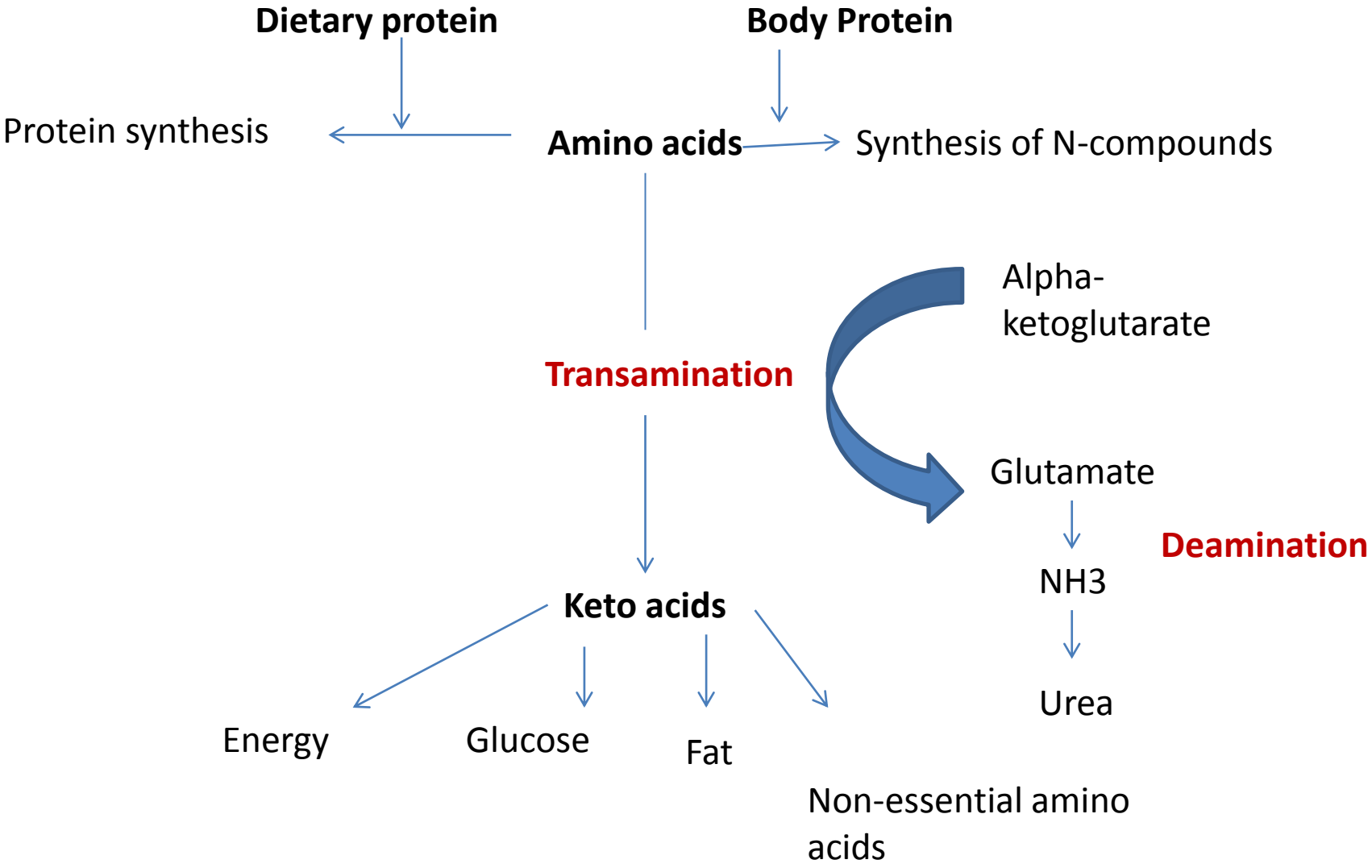
- **Amino acid pool**

- An adult has about 100 g of free amino acids which represent the amino acid pool of the body.
- Glutamate and glutamine together constitute about 50%,and essential amino acids about 10% of the body pool (100g).

METABOLIC FATE




Body protein (10-12 kg in adult)
Protein breakdown (350-400 g /day)
Protein synthesis (300-400 gm/day)
Synthesis of non-protein compounds; 30g/day; 0000creatine,porphyrins,phospholipids,purines,pyrimidines etc.
Dietary protein (40-100 g/day)
Body amino acid pool (100 g)
Carbohydrate, Fats
Protein loss from body (30-50 g/day)
Energy (10-15% of body's daily requirement)
Synthesis of non-essential amino acids (variable)
Sources
Mostly as urea
Utilization



AN OVERVIEW OF AMINO ACID METABOLISM

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

Transamination

The transfer of an amino (-NH2) group from an amino acid to alpha 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).

Salient features of transamination

All transaminases require pyridoxal phosphate (PLP), a coenzyme derived from vitamin B6.

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.

There is no free NH3 liberated, only the transfer of amino group occurs.

Transamination is reversible.

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 and anabolism of amino acids.

Salient features of transamination

- All transaminases require PLP.
- No free NH_3 liberated, only the transfer of amino group.
- Transamination is reversible.
- There are multiple transaminase enzymes which vary in substrate specificity.
- AST & ALT make a significant contribution for transamination.

- **Transamination is important for redistribution of amino groups & production of non-essential amino acids.**
- **It diverts excess amino acids towards the energy generation.**
- **Amino acids undergo transamination to finally concentrate nitrogen in glutamate.**

- Glutamate undergoes oxidative deamination to liberate free NH_3 for urea synthesis.
- All amino acids except, lysine, threonine, proline & hydroxyproline participate in transamination.
- It involves both anabolism & catabolism, since – reversible.

AA_1 + α- KG ⟷ $ketoacid_1$ + Glutamate

Alanine + α- KG ⟷ Pyruvate + Glutamate

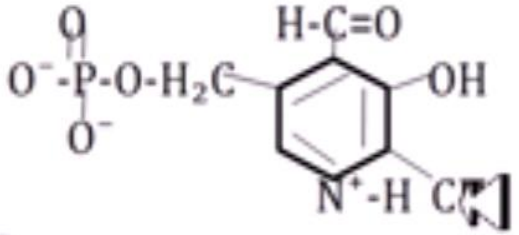
Aspartate + α- KG ⟷ Oxaloacetetae + Glutamate

d) **PROPERTIES OF THE ENZYME TRANSAMINASE / AMINOTRANSFERASE :**

- They are highly specific for α-keto glutarate which accepts the α-NH_2 group.
- They are highly stereospecific towards L-amino acids, which acts the α-NH_2 group donor.
- This reaction is freely reversible, having an equilibrium constant (K_{eq}) of about 1.
- This enzyme requires PLP as the coenzyme.

e) **ABOUT PYRIDOXAL PHOSPHATE / PLP / PALPO :**

- It is a derivative of pyridoxine or vitamin B_6.
- STRUCTURE –



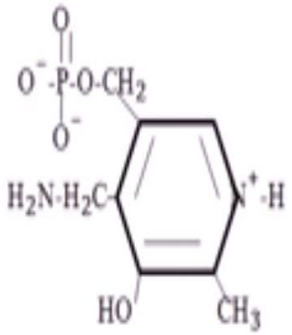
PLP

- METABOLIC ROLE –

i. It is the coenzyme of the transaminase or aminotransferase enzyme.

ii. The α-amino group which is donated by the donor L-amino acid (aā 1) is accepted by PLP, when the latter gets converted to pyridoxamine phosphate or PMP or PAMPO. This pyridoxamine phosphate will in turn donate this accepted α-NH_2 group to acceptor α-keto acid (α-keto acid 2)

that itself gets converted to the product L-amino acid (aâ 2). The aâ 1 by losing the α-NH_2 group, will get converted to the product α-keto acid 1.

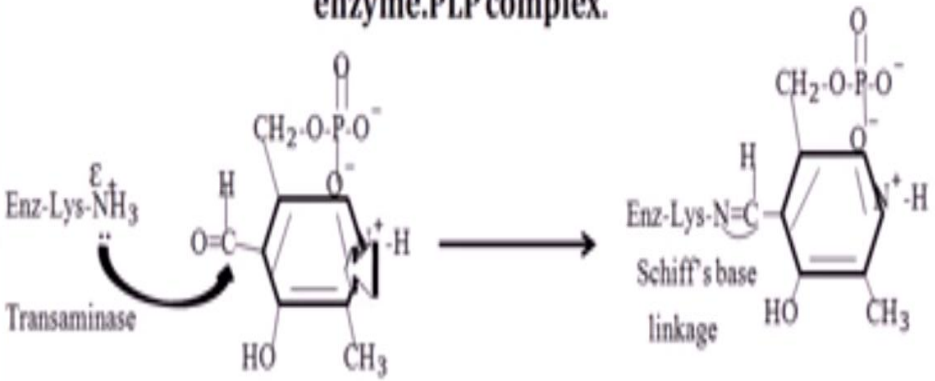


PMP

iii. PLP remains bound to the enzyme transaminase by 2 ways –

- By non-covalent interactions
- By covalent interactions

PLP is bound to the enzyme via covalent interactions Through imine or Schiff's base linkage to the ε-NH_2 group of a Lys residue present at the active site of the enzyme, to form a compound called **internal aldamine** or **enzyme.PLP complex.**



PLP

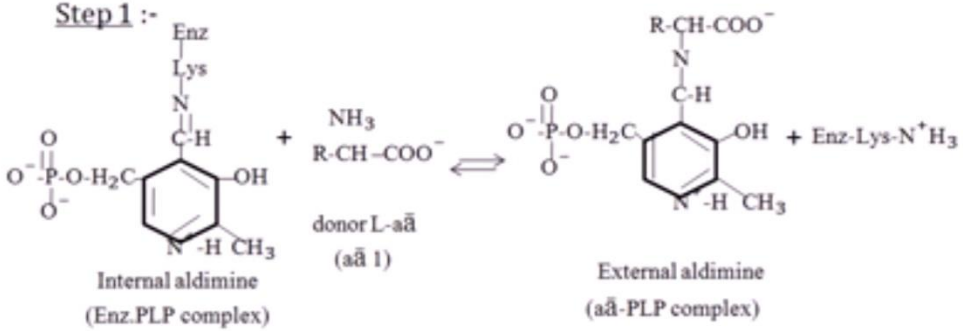
Internal aldimine

f) **MECHANISM OF THE STEPS OF TRANSAMINATION:**

- Internal aldimine (Enz.PLP complex)
- External aldimine (Aā.PLP complex)

i. First half-reaction

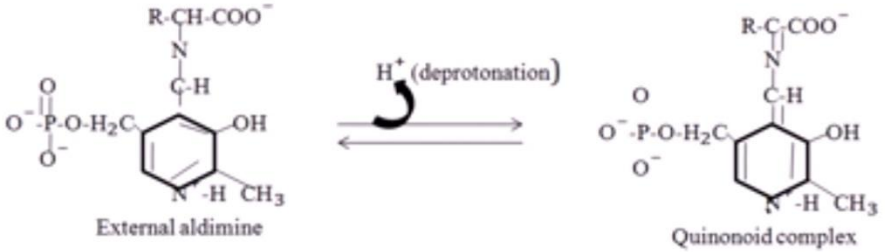
Step 1 :-



Internal aldimine (Enz.PLP complex)

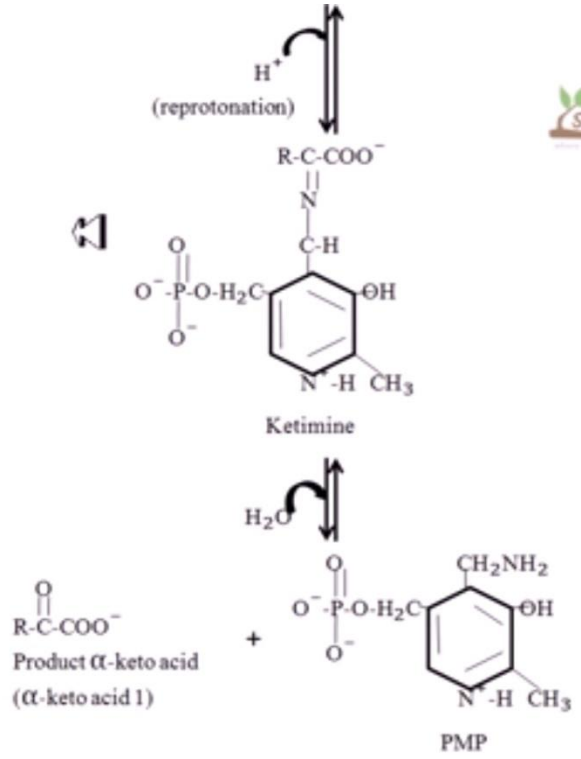
External aldimine (aā-PLP complex)

Step 2 :- [Mechanism proposed by Snell and Brunstein]



External aldimine

Quinonoid complex


H+
(reprotonation)
R-C-COO⁻
N
C-H
O⁻-P-O-H2C
OH
O⁻
N+-H CH3
Ketimine
H2O
R-C-COO⁻
Product α-keto acid
(α-keto acid 1)
+
CH2NH2
OH
PMP

Net Reaction of the First Half-Reaction :-

Internal aldimine + Donor L-aā 1 + External aldimine + Quinonoid complex + Ketimine

⇕

External aldimine + Quinonoid complex + Ketimine + Product α-keto acid 1 + PMP

Internal aldimine + Donor L-aā 1 ⇌ Product α-keto acid 1 + PMP

ii. ## Net Reaction of the Second Half-Reaction

Acceptor α-keto acid + PMP ⇌ L-aā + Internal aldimine

(α-keto acid 2) (aā 2)

iii. ## Net Reaction of Transamination

Internal aldimine + Donor L-aā 1 + Acceptor α-keto acid 2 + PMP

⇕

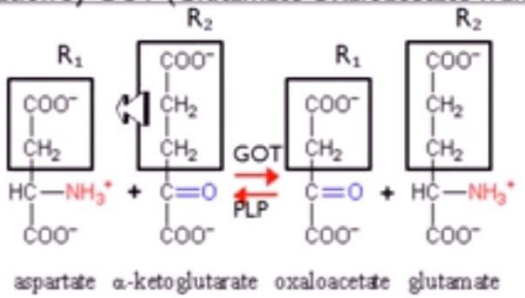
Product α-keto acid 1 + PMP + Product L-aā 2 + Internal aldimine

---

Donor L-aā 1 + Acceptor α-keto acid 2 ⇌ Product α-keto acid 1 + Product L-aā 2

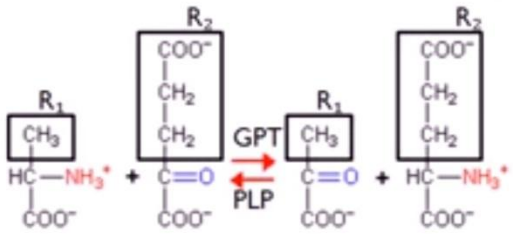
g) EXAMPLES OF TRANSAMINATION :

- Transamination by GOT (Glutamate Oxaloacetate Transaminase) –



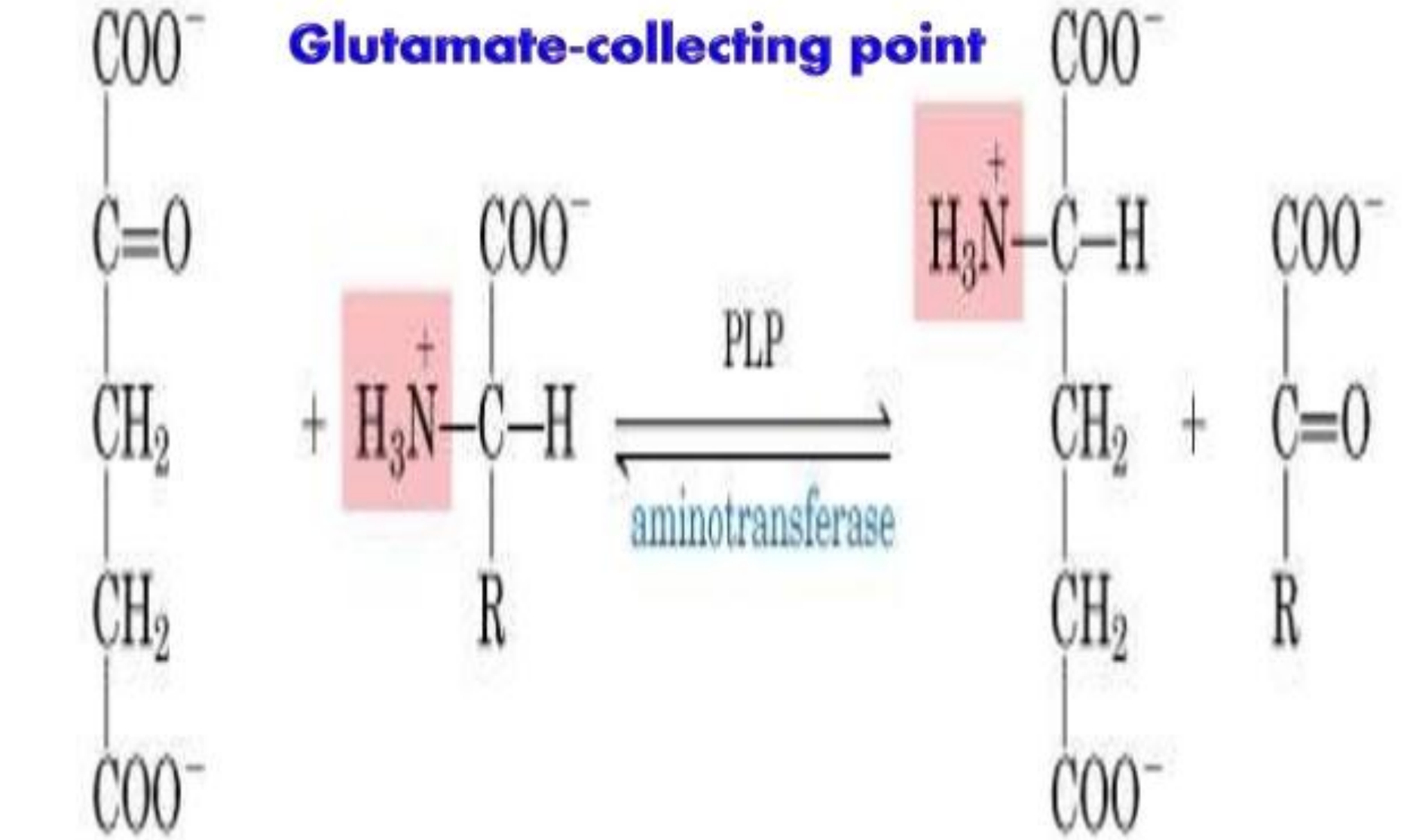
aspartate α-ketoglutarate oxaloacetate glutamate

- Transamination by GPT (Glutamate Pyruvate Transaminase) –

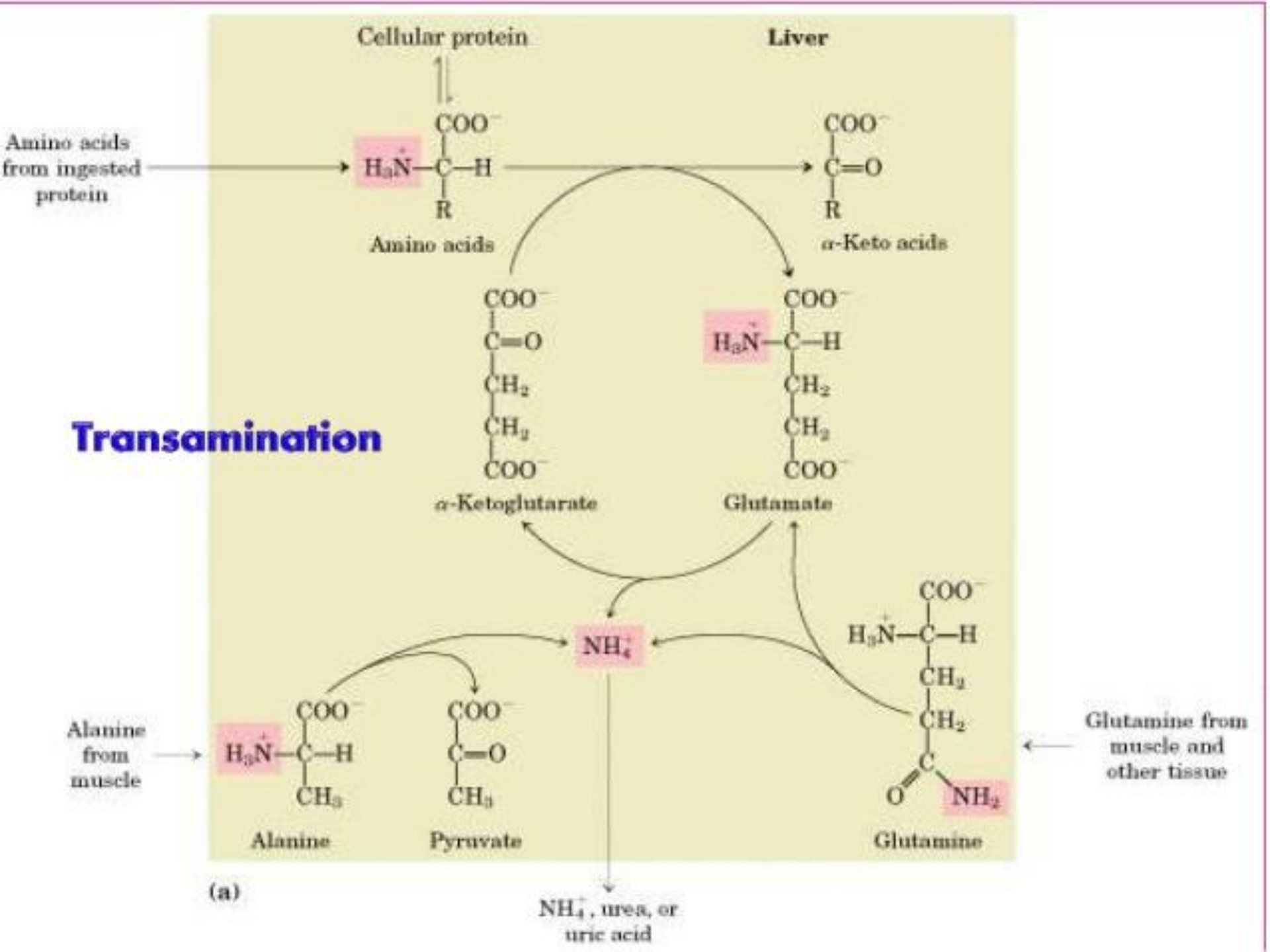


alanine α-ketoglutarate pyruvate glutamate

Glutamate-collecting point



α-Ketoglutarate L-Amino acid L-Glutamate α-Keto acid


Transamination
Cellular protein
Liver
Amino acids from ingested protein
Amino acids
α-Keto acids
α-Ketoglutarate
Glutamate
NH4+
Alanine from muscle
Alanine
Pyruvate
Glutamine from muscle and other tissue
Glutamine
(a)
NH4+, urea, or uric acid

Mechanism of transamination

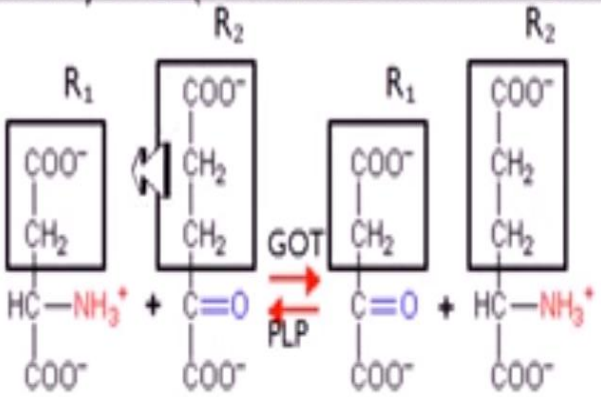
- **Step: 1**
- **Transfer of amino group from AA_1 to the coenzyme PLP to form pyridoxamine phosphate.**
- **Amino acid1 is converted to Keto acid2.**
- **Step: 2**
- **Amino group of pyridoxamine phosphate is then transferred to a keto $acid_1$ to produce a new AA_2 & enzyme with PLP is regenerated.**

Clinical Significance

- Enzymes, present within cell, released in cellular damage into blood.
- ↑ AST - Myocardial Infarction (MI).
- ↑ AST, ALT – Hepatitis, alcoholic cirrhosis.
- Muscular Dystrophy.

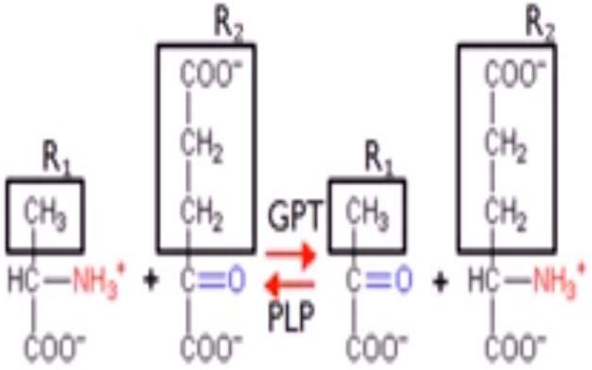
g) EXAMPLES OF TRANSAMINATION :

- Transamination by GOT (Glutamate Oxaloacetate Transaminase) –



aspartate α-ketoglutarate oxaloacetate glutamate

- Transamination by GPT (Glutamate Pyruvate Transaminase) –



alanine α-ketoglutarate pyruvate glutamate

Trans-deamination

- The amino group of most of the amino acids is released by a coupled reaction, trans-deamination.
- Transamination followed by oxidative deamination.
- Transamination takes place in the cytoplasm.

- The amino group is transported to liver as glutamic acid, which is finally oxidatively deaminated in the mitochondria of hepatocytes.

Deamination

- The removal of amino group from the amino acids as NH_3 is deamination.
- Deamination results in the liberation of ammonia for urea synthesis.
- The carbon skeleton of amino acids is converted to keto acids.
- Deamination may be either oxidative or non-oxidative

- Only liver mitochondria contain glutamate dehydrogenase (GDH) which deaminates glutamate to α-ketoglutarate & ammonia.
- It needs NAD^+ as co-enzyme.
- It is an allosteric enzyme.
- It is activated by ADP & inhibited by GTP.

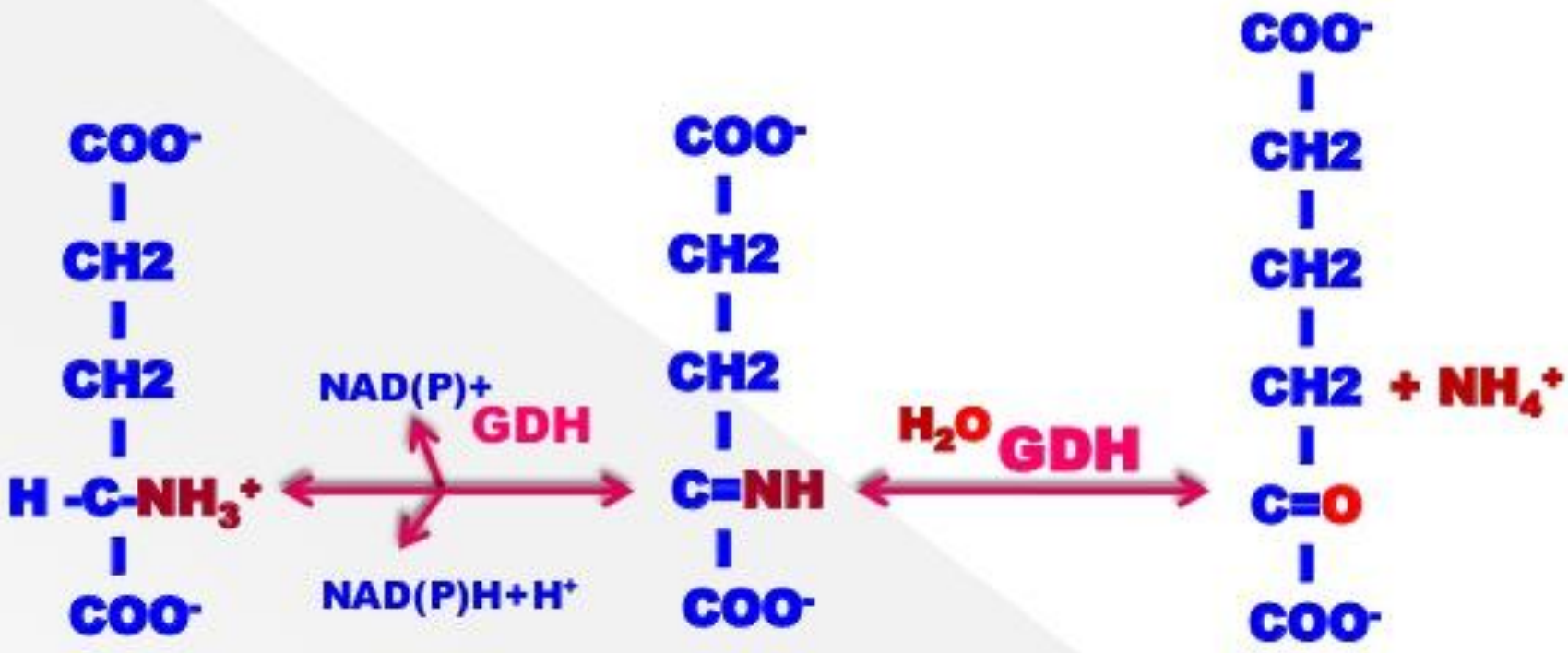
Oxidative Deamination

- **Oxidative deamination** is the liberation of free ammonia from the amino group of amino acids coupled with oxidation.
- **Site**: Mostly in liver & kidney.
- 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

- Glutamate is a 'collection centre' for amino groups.
- Glutamate rapidly undergoes oxidative deamination.
- Catalysed by GDH to liberate ammonia.
- It can utilize either NAD^+ or $NADP^+$.
- This conversion occurs through the formation of an α-iminoglutarate

Oxidation of glutamate by GDH



L-Glutamate

α- Iminoglutarate

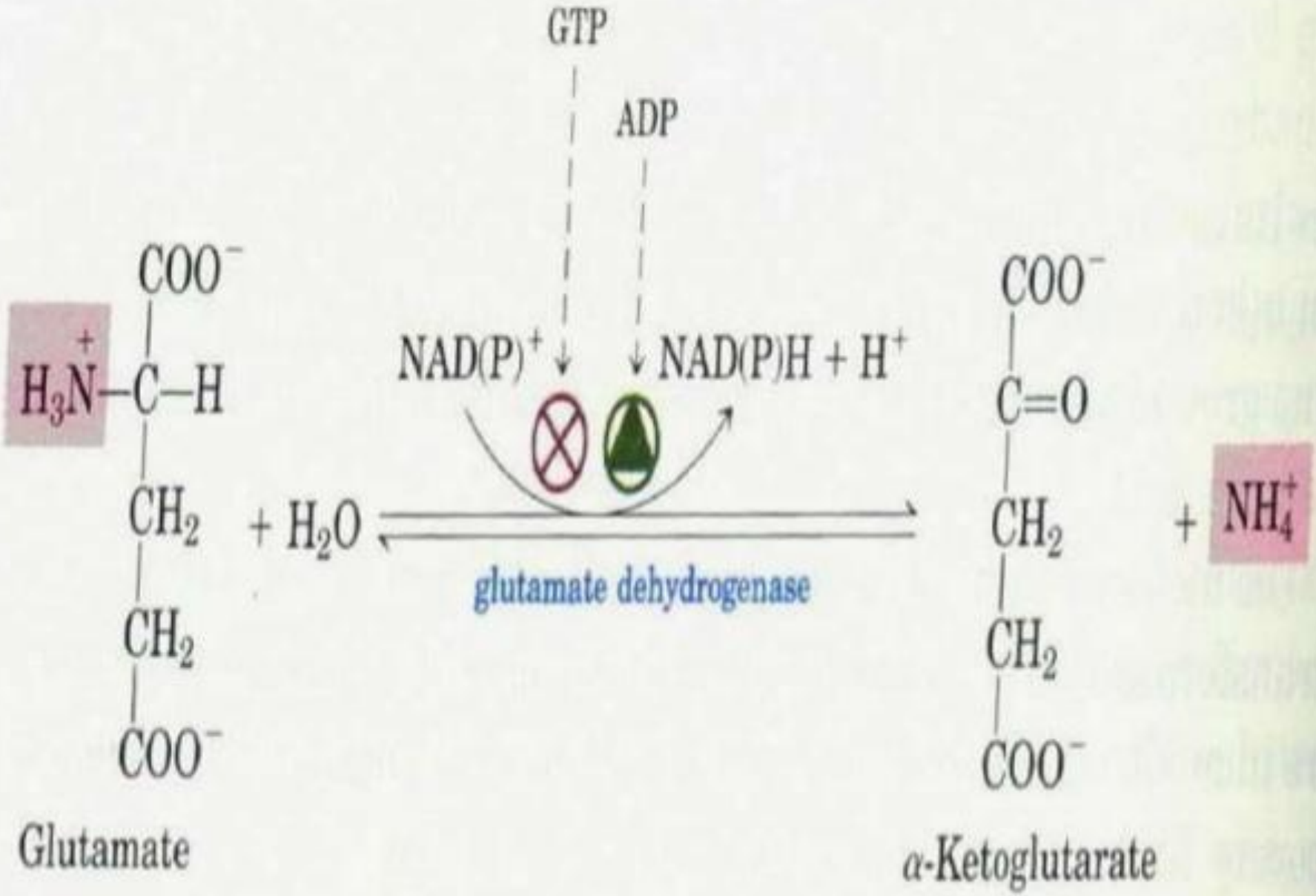
α- ketoglutarate

Metabolic Significance

- Reversible Reaction
- Both Anabolic & Catabolic.
- Regulation of GDH activity:
- Zinc containing mitochondrial, allosteric enzyme.
- Consists of 6 identical subunits.
- Molecular weight is 56,000.

Allosteric regulation

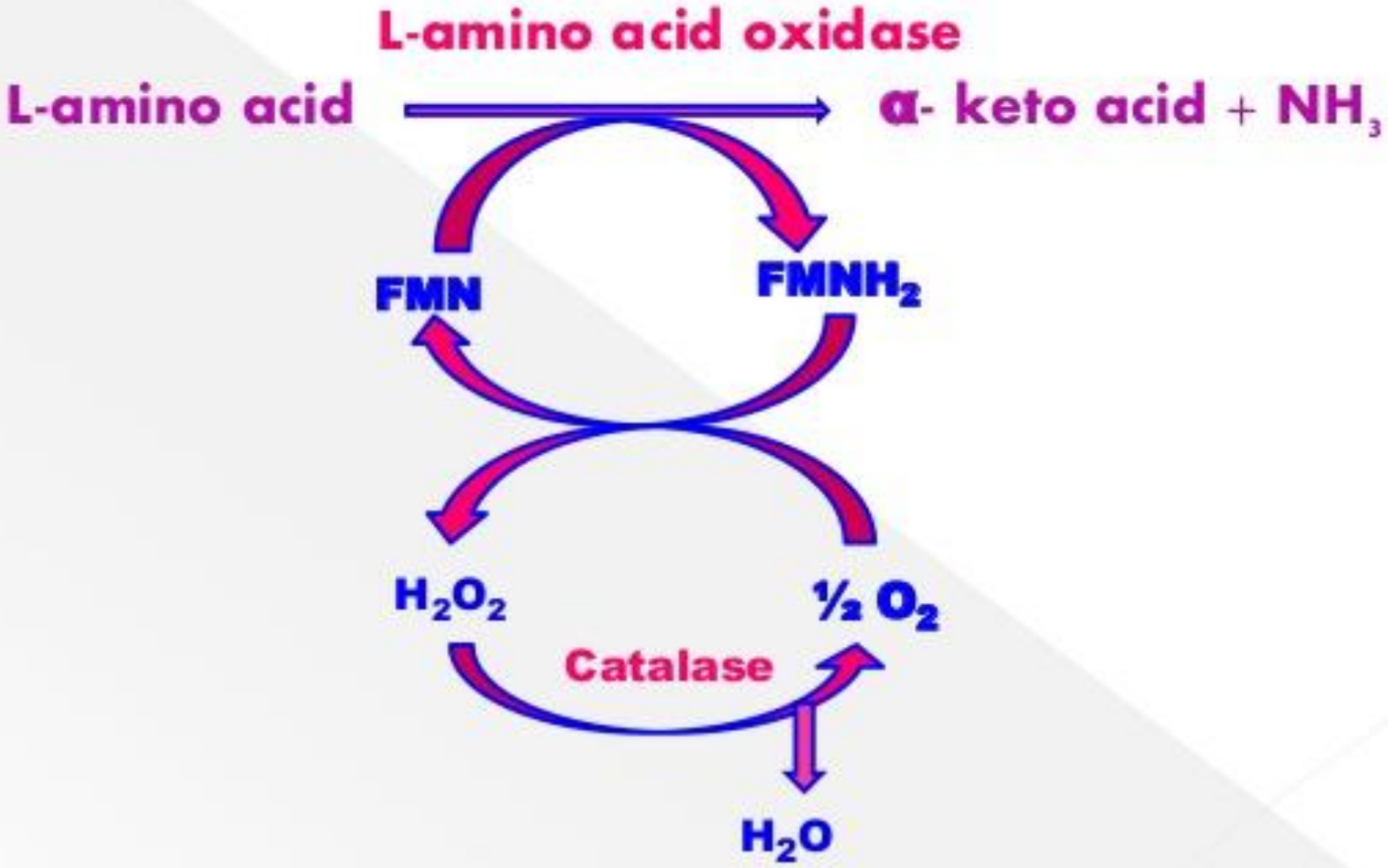
- GTP & ATP – allosteric inhibitors.
- GDP & ADP - allosteric activators.
- ↓ Energy - ↑ oxidation of A.A.
- Steroid & thyroid hormones inhibit GDH.


GTP
ADP
NAD(P)+
NAD(P)H + H+
+ H2O
glutamate dehydrogenase
+ NH4+
Glutamate
α-Ketoglutarate

Amino acid Oxidases

- L-amino acid oxidase & D-Amino acid oxidase.
- Flavoproteins & Cofactors are FMN & FAD.
- Act on corresponding amino acids to produce α-keto acids & NH_3
- Site: Liver, kidney, Peroxisomes.
- Activity of L-Amino acid oxidase is low.
- Plays a minor role in Amino acid catabolism.

Oxidative deamination of amino acids



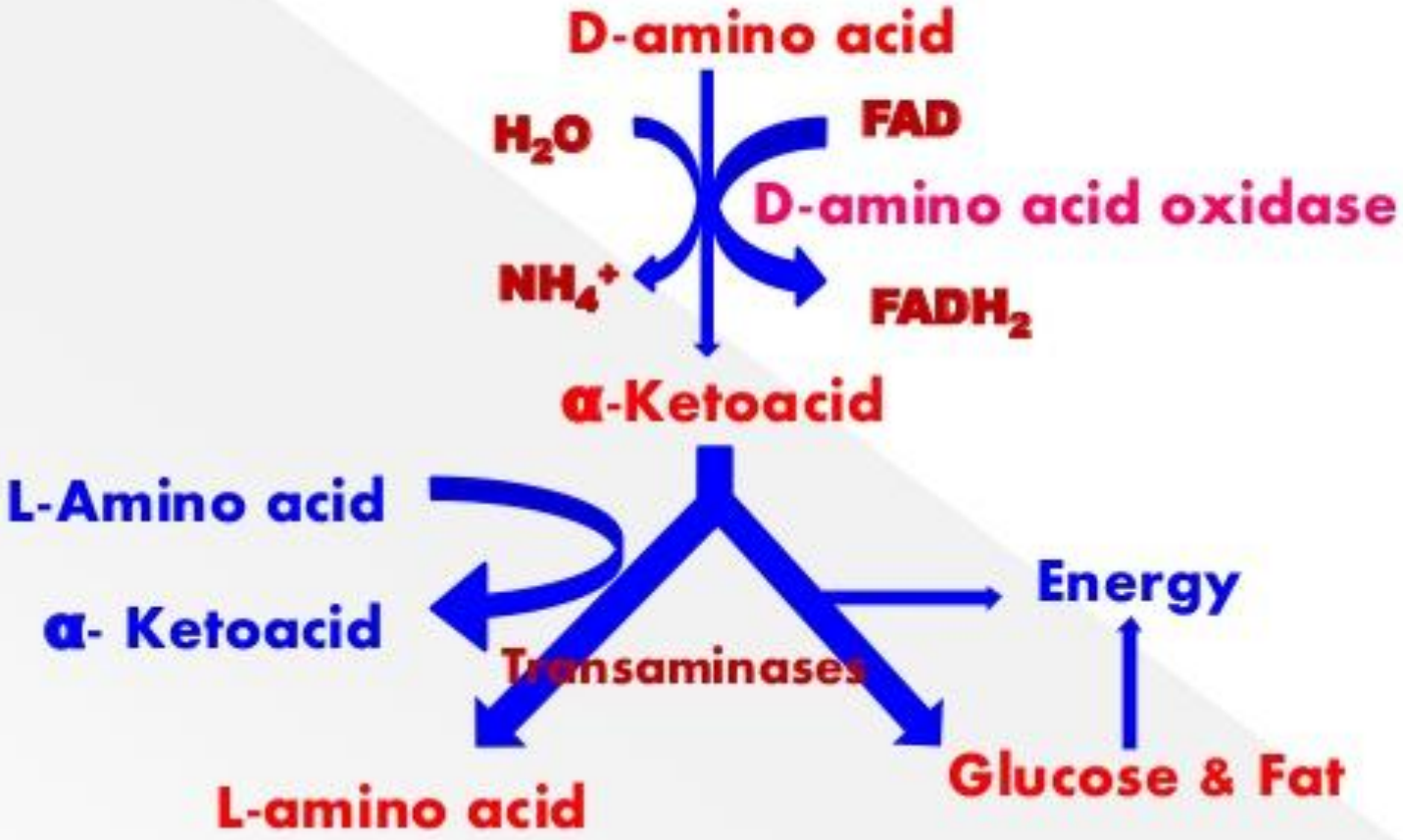
- L-Amino acid Oxidase acts on all Amino acids, except glycine & dicarboxylic acids.
- Activity of D-Amino oxidase is high than that of L-Amino acid oxidase
- D-Amino oxidase degrades D-Amino acids in bacterial cell wall.

Fate of D-amino acids

- D-amino acids are found in plants & microorganisms.
- They are not present in mammalian proteins.
- D-amino acids are taken in the diet/bacterial cell wall, absorbed from gut - D-Amino acid oxidase converts them to respective α-keto acids.

- The α-ketoacids undergo transamination to be converted to L-amino acids which participate in various metabolic pathways.
- Keto acids may be oxidized to generate energy or serve as precursors for glucose & fat synthesis.

Metabolic fate of D-amino acids



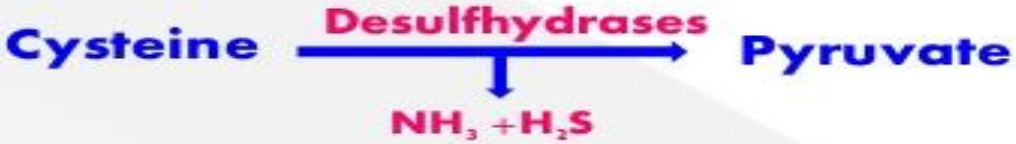
Non-Oxidative deamination

- Direct deamination, without oxidation.
- Amino acid Dehydratases:
- Serine, threonine & homoserine are the hydroxy amino acids.
- They undergo non-oxidative deamination catalyzed by PLP-dependent dehydratases

Non-Oxidative deamination

Amino acid Desulfhydrases

- **Cysteine & homocysteine undergo deamination coupled with desulfhydration to give keto acids.**



- **Deamination of histidine:**

- Transamination diverts the excess amino acids towards energy generation.
- 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 NH3 for urea synthesis.
- All amino acids except lysine, threonine, proline and hydroxy- proline participate in transamination.
- Transamination is not restricted to alpha amino groups only. For instance, delta-amino group of ornithine is transaminated.
- Serum transaminases are important for diagnostic and prognostic purposes.

h) PRACTICAL / CLINICAL APPLICATIONS OF TRANSAMINATION:

Transaminase enzymes are used in diagnostic examination of heart and liver damage (through SGOT, SGPT, and SCK)as assaying these particular enzymes gives a measure of heart and liver damage.

- In diagnosing heart damage –

Occlusion of coronary artery by myocardial infarction lipid deposition can lead to local oxygen starvation and ultimately the degeneration of a localized portion of heart tissue. This process is called myocardial infraction. Such a damage will cause the aminotransferase enzymes to leak out from the injured heart cells into the blood stream. Therefore, measurement of concentration in blood of these aminotransferases (GOT and GPT) by SGOT and SGPT tests respectively and of another enzyme of the heart called creatinine kinase (CK, also called creatinine phosphokinase) by SCK can provide information regarding the severity and stage of damage to the heart.

- In diagnosing liver damage –

SGOT and SGPT tests are also important in industrial medicines to determine whether people exposed to carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) and also other solvents used in chemicals, dry cleaning and other industries are suffering from liver damage. These solvents cause liver degeneration with the resulting leakage of the aminotransferase enzymes from the hepatocytes into the blood stream. Aminotransferases because they are very active in the liver and because their activity can be detected in very small amounts, are most useful in monitoring people exposed to such industrial chemicals.

Metabolism of Ammonia

- Formation of ammonia
- Transport and storage of glutamine
- The transport of ammonia between various tissues and the liver mostly occurs in the form of glutamine and alanine and not as free ammonia.
- Alanine is important for NH3 transport from muscle to liver by glucose-alanine cycle.
- Role of glutamine: Glutamine is a storehouse of NH3.
- Functions of ammonia
- Disposal of ammonia
  - Ammoniotelic
  - Uricotelic
  - Ureotelic
- Toxicity of ammonia
  - Hyperammonemia

- **Thank You**