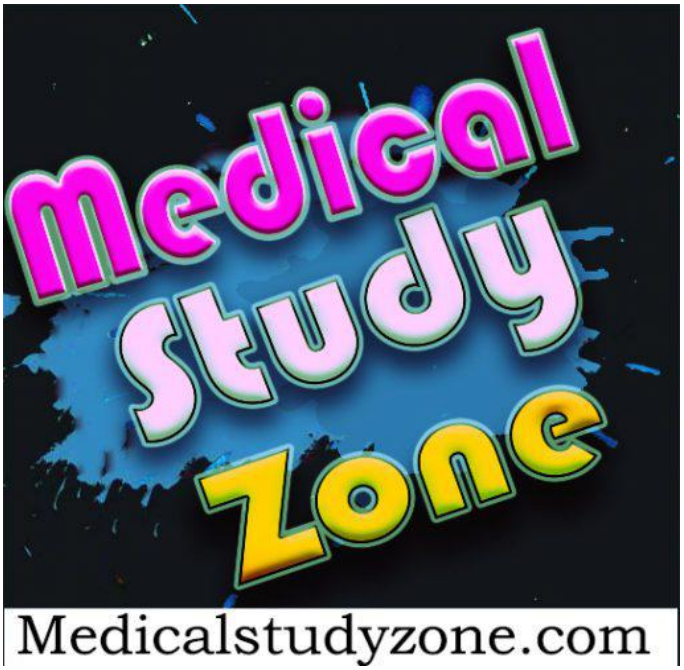
INDEX



CONCEPTS

FORMULAS 1 2 3 4

"Four Formulas"

Smile Formula 1:

Which pathway is Anabolic and which pathway is Catabolic?

ANABOLIC PATHWAYS	CATABOLIC PATHWAY
1. HMP (Pentose Phosphate Pathway) 2. Glycogenesis 3. Fat Synthesis (Fatty Acid Synthesis, Triglyceride Synthesis, Cholesterol Synthesis) 4. Lipoprotein Lipase enzyme	1Glycolysis 2.Link Reaction (3C → 2C) 3 Glycogenolysis 4 β- Oxidation of Fatty Acids 5 Gluconeogenesis 6 Ketone Body Synthesis / utilization 7 Hormone Sensitive lipase enzyme

Smile Formula 2:

Insulin and Glucagon activates which pathways or enzymes?

→ Insulin activates all anabolic pathways enzymes.

→ Insulin also activates two catabolic pathway enzymes i.e. Glycolysis and Link Reaction.

→ Glucagon activates all catabolic pathway enzymes but, glucagon does not activate two catabolic pathway enzymes i.e. Glycolysis and Link Reaction, as these are activated by Insulin.

Smile Formula 3:

Which hormone causes Phosphorylation and which causes Dephosphorylation?

→ Enzymes which are activated by Insulin (Anabolic Enzyme) are always active in Dephosphorylated state.

→ Enzymes which are activated by Glucagon (Catabolic enzymes) are always active in Phosphorylated state.

ONE EXCEPTION: ATP Citrate Lyase is anabolic enzyme but it is active in phosphorylated state.

Smile Formula 4:

Which pathway occurs in which compartment of the cell?

→ All Anabolic Pathways occur in Cytoplasm
→ All Catabolic Pathways occur in Mitochondria

→ But two Catabolic Pathways also occur in cytoplasm i.e. Glycolysis and Glycogenolysis.

Pathways which occur in both Mitochondria and Cytoplasm

1. Gluconeogenesis
2. Urea cycle
3. Haem synthesis

TCA, ETC (Vital Pathways occur in MITOCHONDRIA)

HOW TO USE FORMULAS

(Q) Which of the following does not occur in mitochondria?

a. Beta oxidation
b. Fatty acid synthesis →Cytoplasm
c. DNA synthesis
d. Protein synthesis

Ans - b

(Q) Which of the following is active in dephosphorylated state? [PGI]

a. Glycogen Synthase	→	**Synthesis**	→	**Anabolic**
b. Pyruvate Carboxylase	→	Gluconeogenesis	→	Catabolic
c. Glycogen phosphorylase	→	Break down	→	Catabolic
d. Acetyl CoA Carboxylase	→	**FA Synthesis**	→	**Anabolic**
e. Pyruvate dehydrogenase	→	**Link Synthesis**	→	**Catabolic**

Ans – a, d, e

(Q) Insulin promotes lipogenesis by all except

a. Decreasing cAMP

b. Increasing Glucose uptake

c. Inhibiting Pyruvate Dehydrogenase

d. Increasing Acetyl CoA

Ans – c

(Q) Mitochondria are involved in all except

a. ATP Production

b. Apoptosis

c. Tri carboxylic Acid Cycle

d. Cholesterol Synthesis

Ans – d

(Q) Hormone Sensitive lipase is not activated by

a. Insulin → **Lipoprotein Lipase** → **Anabolic**

b. Glucagon
c. Catecholamines
d. Thyroid
} → Hormone Sensitive lipase → catabolic

Ans – a

(Q) Which of the following is not seen in low insulin – Glucagon Ratio?

a. Gluconeogenesis → catabolic

b. Glycogen Breakdown → catabolic

c. Ketogenesis → catabolic

d. Glycogen Storage → **Anabolic**

→ Low Insulin → catabolic

Ans – d

(Q) Which of the following is active in dephosphorylated State?

a. Glycogen Synthase → **Anabolic**

b. Pyruvate carboxylase → catabolic

c. Glycogen Phosphorylase → catabolic

d. PEPCK → catabolic

Ans – a

(Q) All occur in mitochondria except? [PGMEE 2015]

a. Glycolysis

b. TCA Cycle

c. ETC

d. Ketogenesis

Ans – a

(Q) The biosynthesis of the enzymes Pyruvate Carboxylase is repressed by

a. Insulin

b. Cortisol

c. Glucagon

d. Ketogenesis

Ans - a

(Q) Which of the following is active in phosphorylated state?

a. Glycogen Synthase

b. Glycogen Phosphorylase

c. Acetyl Co A Carboxylase

d. G6PD Enzyme

Ans – b

SOURCES OF BLOOD GLUCOSE

➔ SOURCES OF BLOOD GLUCOSE

1. Food
2. Liver Glycogen [12-18Hrs]
3. Gluconeogenesis [Requires High Energy]

→ Main/preferred fuel for the body?

- CARBOHYDRATES [Fed]
  ↓
  FATS [Fasting]
  ↓
  PROTEINS [Starving]

FUEL IN FED, FASTING AND STARVATION

SUBSTRATES UTILIZED FOR ENERGY PRODUCTION

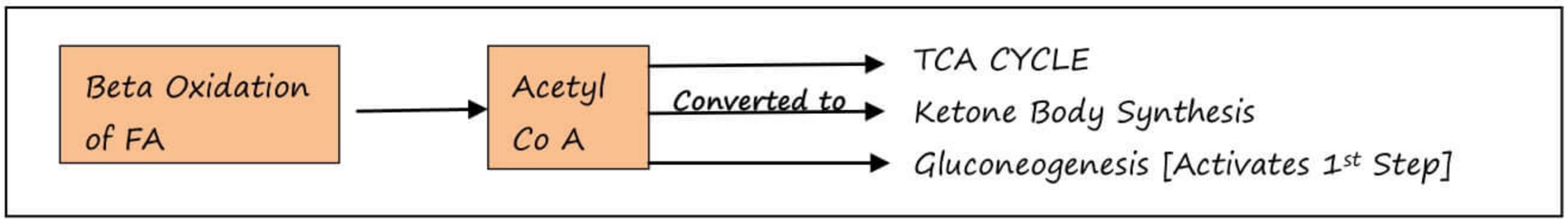
	FED	FASTING	STARVATION
BRAIN	Glucose	Glucose	Ketone Bodies
HEART	Fatty Acids	Fatty Acids	Ketone Bodies
LIVER	Glucose	Fatty Acids	Amino Acids
MUSCLE	Glucose	Fatty Acids	Fatty Acids and KB
ADIPOSE TISSUE	Glucose	Fatty Acids	FA
RBC	Glucos	Glucose	Glucose

Fetal Heart
Heart Failure
} Glucose

FASTING STATE

During Fasting / Starvation

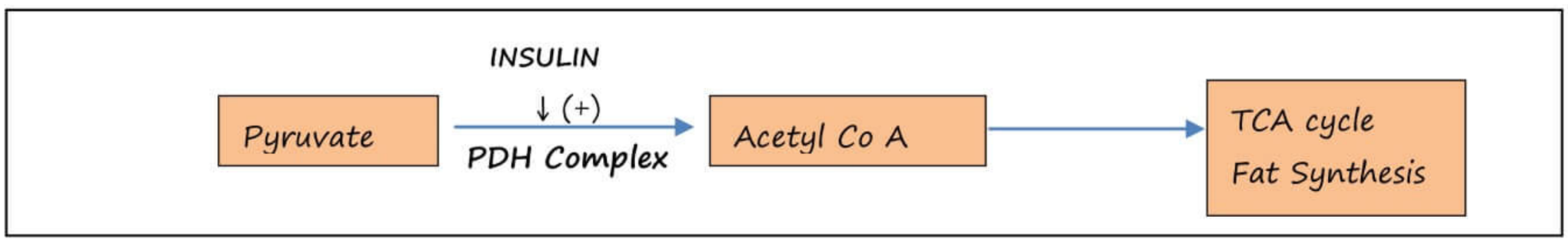
Adipose Tissue
↓ Hormone sensitive Lipase
Fatty Acids
↓
Blood
↓
Liver



Sequence of Fate of Acetyl CoA → 1. TCA CYCLE
→ 2. KB SYNTHESIS
→ 3. GLUCONEOGENESIS

ACETYL Co A

FED STATE



FASTING / STARVATION

Adipose Tissue

↓ **Hormone Sensitive Lipase**

Fatty Acids

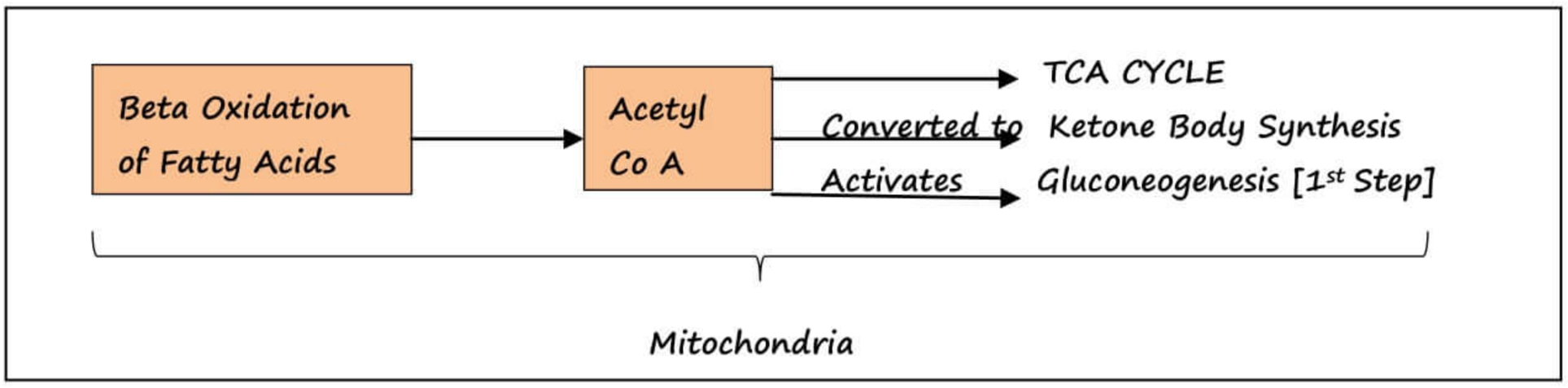
↓

Blood

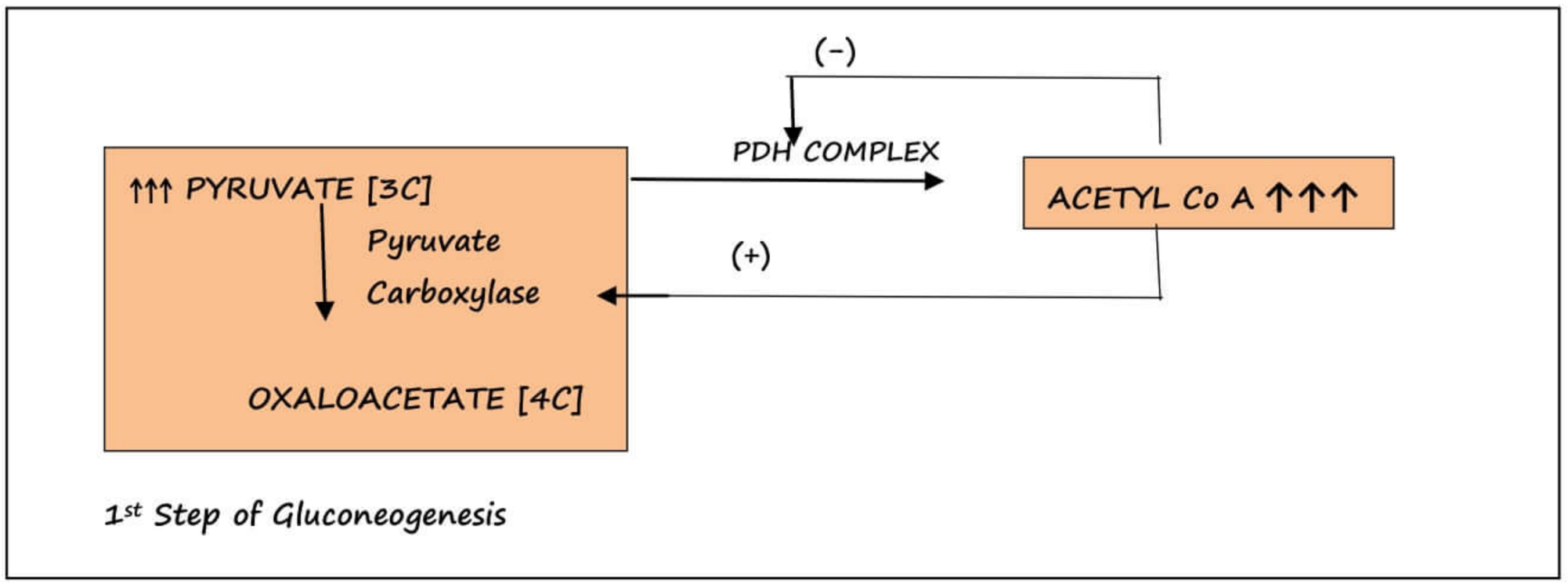
↓

Liver

↓



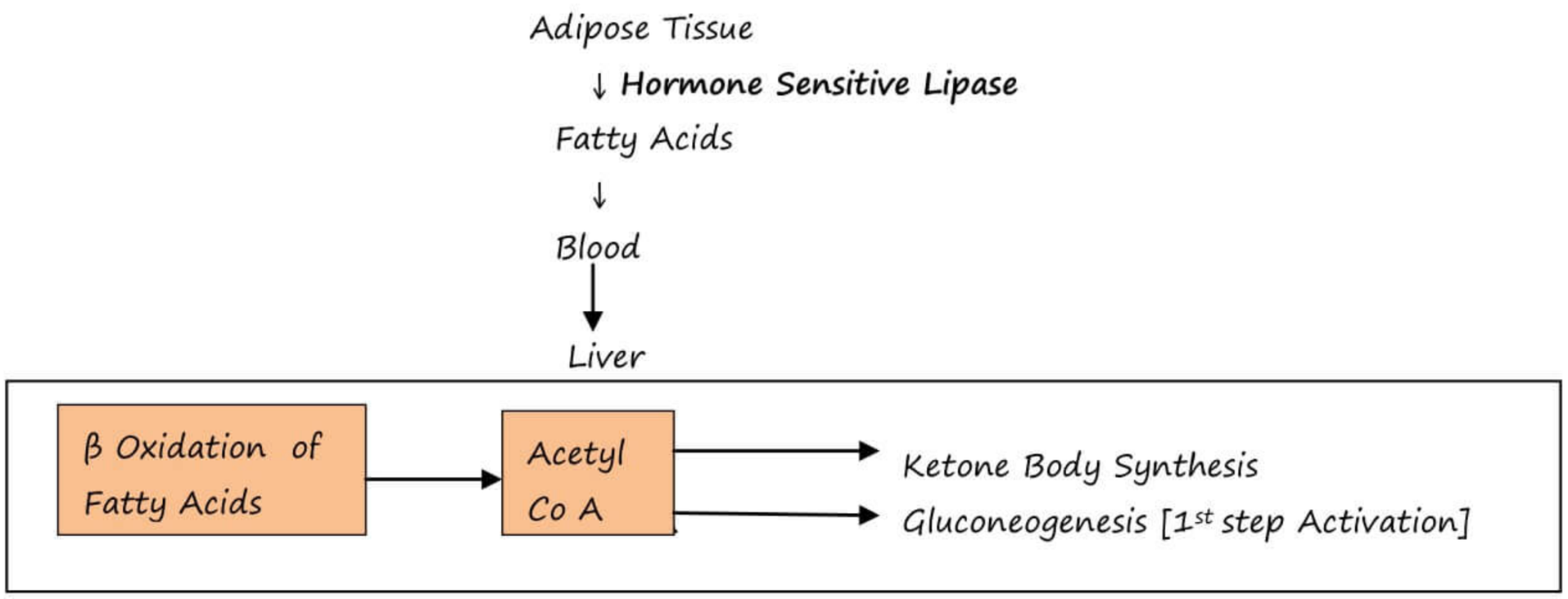
➔ Lots of Acetyl CoA [by β- Oxidation]



- Acetyl CoA is never Glucogenic.
- Acetyl CoA is not the first substrate of TCA cycle.
- Not the intermediate of TCA cycle and not the carrier of TCA cycle.

DIABETES

- Diabetes is fasting or starvation like state



- Only one Anabolic Process is happening in Diabetes [Fat formation in Liver].
- Dur to excess Acetyl CoA fat formation occurs in liver and this fat is converted into endogenous triglyceride and this travels in the blood in the form of VLDL.
- This excess Acetyl CoA will from cholesterol in the body.

CELL ORGANELLES

PROKARYOTES	EUKARYOTES
→ Simple	→ More Complex
→ Circular & double stranded DNA	→ Linear & Double stranded DNA
→ No organelle	→ Membrane bound organelle
→ Plasma membrane do not have receptor	→ Plasma membrane is having receptor
→ Cell wall (+), Chemically complex	→ Cell wall (+) present only in Eukaryotic Fungi & Plant
→ Nucleoid (No membrane)	→ Membrane Bound Nucleus
→ DNA is not attached to Histone	→ DNA attached to Histone
→ Transcription & Translation both occur simultaneously in cytoplasm	→ Transcription occurs in nucleus & Translation occurs in cytoplasm
→ Ribosome are Smaller	→ Large Ribosome
→ Lacks cytoskeleton	→ Eukaryotic cell have large cytoskeleton

Cell Organelle

→ Eukaryotic

→ Membrane Bound

Ribosome

→ Not membrane Bound

Not considered organelle

Nucleus

→ Largest Organelle

→ Nucleus rich in rRNA & disappear during cell division

→ Nucleus have big pore in membrane. as its help in transport of molecule

Mitochondria

→ Energy Production

→ Mitochondrial DNA

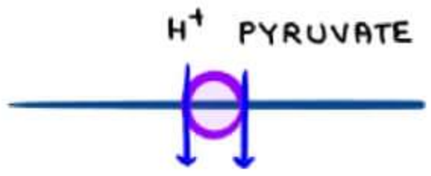
Self replicating

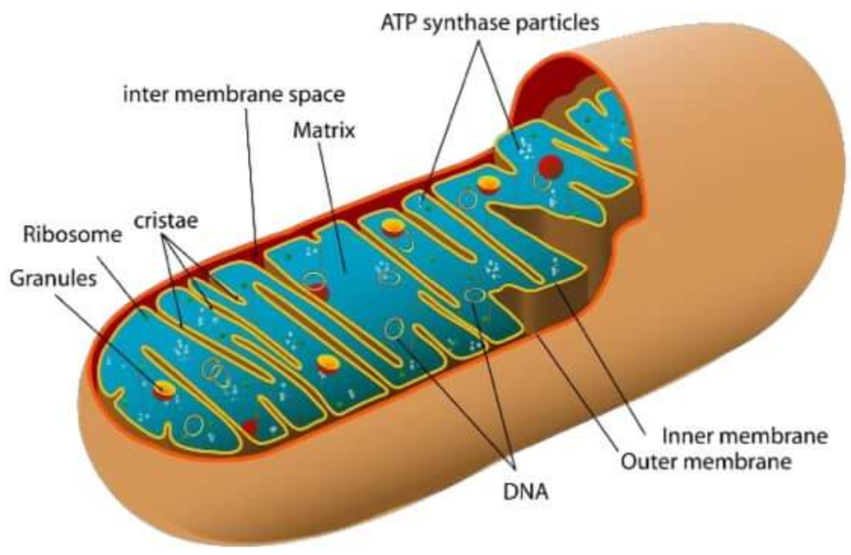
→ Outer membrane → Many pore

→ Inner membrane → Semi Permeable

→ Cristae → ↑ Surface area

→ IMM have many transport mechanisms like (Symport)





→ IMM is semi permeable because of Phospholipid - Cardiolipin (4 Fatty Acid)

OMM	→	Lipid metabolism
IMM Space	→	Nucleotide metabolism, CytC
IMM	→	PDH, ETC
Matrix	→	TCA Enzyme
Lysosome	→	Digestive

LYSOSOMES

→ Digestive Organelles

→ Macromolecular break down by hydrolase

→ Optimal PH <5

Lysosomal Storage Diseases

1. MPS (Mucopolysaccharidosis)

2. I- cell Disease
3. Pompe's Disease / Type II GSD
4. Cystinosis – S – containing Amino acids
5. Sphingolipidosis (SLP)
6. Wolman's Disease

Peroxisomes → Microbodies/ Glyoxisomes

→ Single membrane

→ No ATP Produce

→ H_2O_2 regularly formed in Peroxisomes. Peroxisomes have enzymes like Catalase & Peroxidase to breakdown to H_2O_2

Function

1. Synthesis of Plasmalogen which is most abundant Phospholipid in myelin
2. Oxidation of very long chain fatty acid
3. α - Oxidation

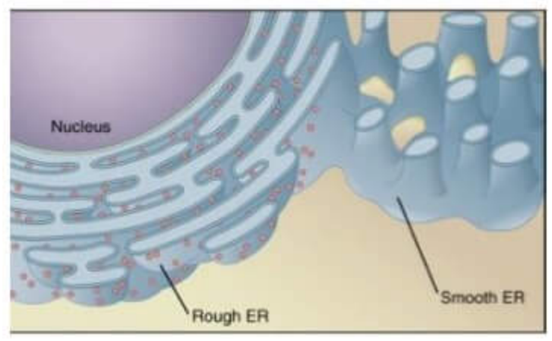
Peroxisomes Biogenesis Disorder

→ All effect CNS

→ Defect in Formation of Peroxisomes

Most severe PBD → Zellweger syndrome

Endoplasmic Reticulum → Continuation of nuclear membrane



Two Types

RER

→ Attach to ribosome

→ Protein synthesis which are exported outside the cell
E.g. Insulin

→ Which are required for outer Membrane of Nucleus, Lysosomes

SER

→ Not attach to ribosome

→ Detoxification of drugs

→Lipid Synthesis

→ Ca^{+2} Sequestration & Release

Sarcoplasmic Reticulum →SER in the myocytes of smooth & striated muscles

Microsomes → They are residues formed on disruption of cell (ER) in vitro conditions.

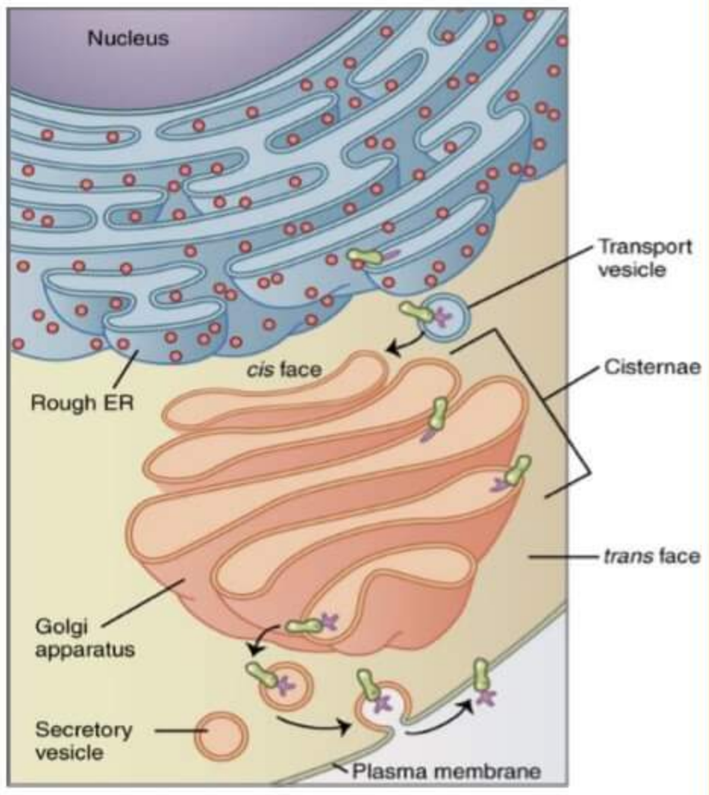
Golgi Apparatus

→ Gift Packing, Protein Stored, Glycosylation

→ Phosphate is attached

RER → Golgi Apparatus → Targeted site → O glycosylation → ER

→ Defect in Golgi apparatus & tagging in protein is known as protein targeting disorder



E.g.

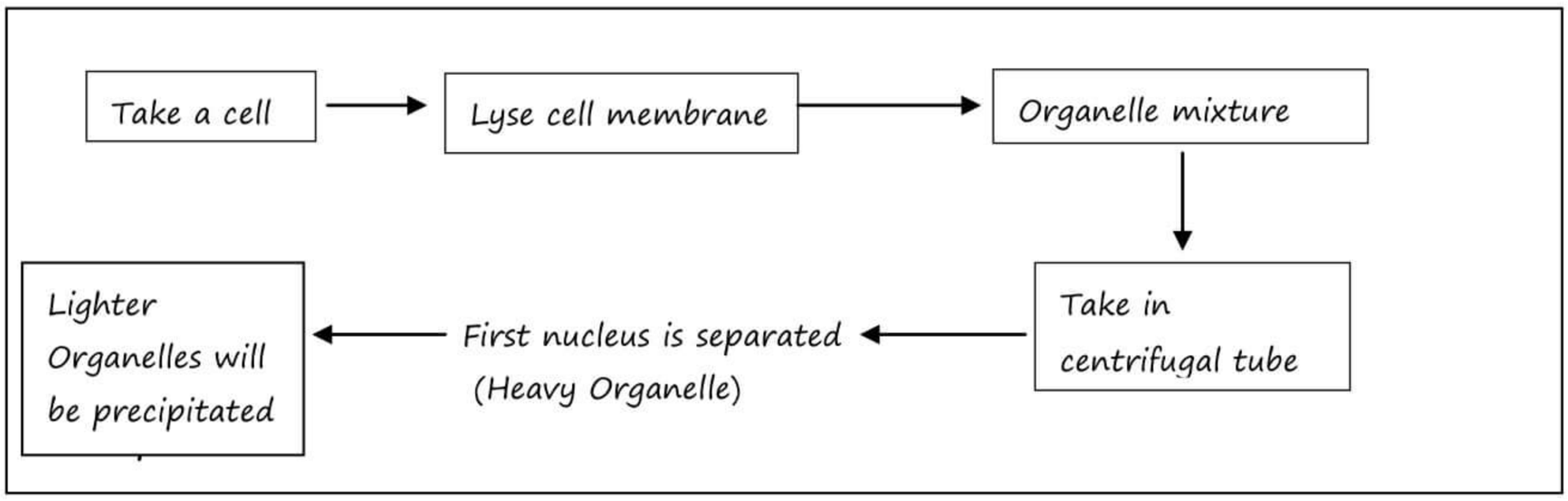
1. I Cell disease: Defect in formation of Mannose -6- PO_4 on hydrolyse
2. Primary Hyperoxaluria
3. Familial Hypercholesterinaemia
4. Zellweger Syndrome
5. Cystic Fibrosis

Ribosome

→ Free / Cytosolic ribosomes

→ Protein Synthesis

Sucrose Density Gradient Centrifuge



Separation Dependent on size and weight

Markers for Various Organelles

Plasma Membrane	→	Na^+ K^+ ATPase, 5'Nucleotidase, Adenyl Cyclase
Golgi Apparatus	→	Galactosyl Transferase
Cytosol	→	Lactate DH

Ribosome	→	r RNA
ER	→	Glucose -6-Phosphatase
Peroxisomes	→	Catalase
Nucleus	→	DNA, RNA Polymerase
Mitochondria	→	Succinate DH, Glutamate DH
Lysosome	→	Acid Phosphatase

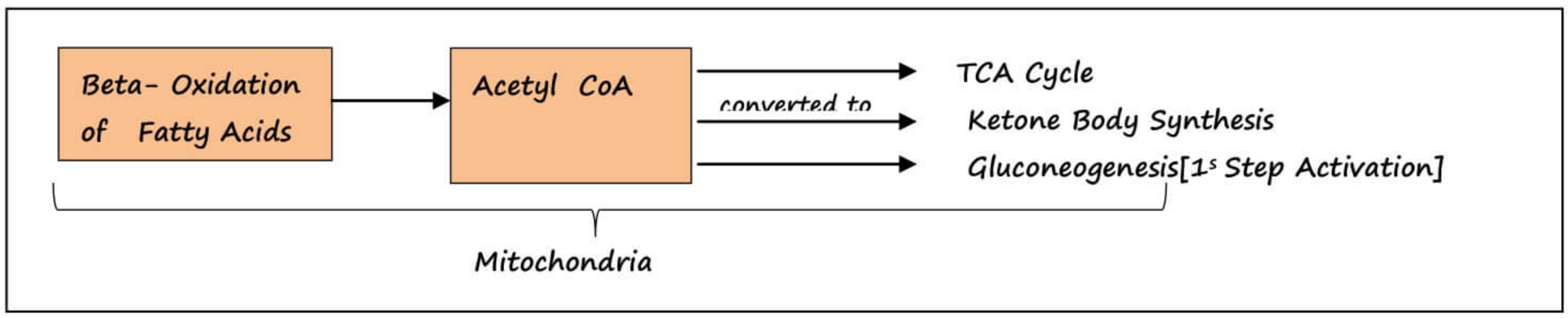
FATS AND CARBOHYDRATE INTERCONVERSION

SOURCES OF BLOOD GLUCOSE

1. Food
2. Glycogen
3. Gluconeogenesis [lots of energy required]

FASTING /STARVATION

Adipose tissue
↓ Hormone Sensitive Lipase
Fatty Acids
↓
Blood
↓



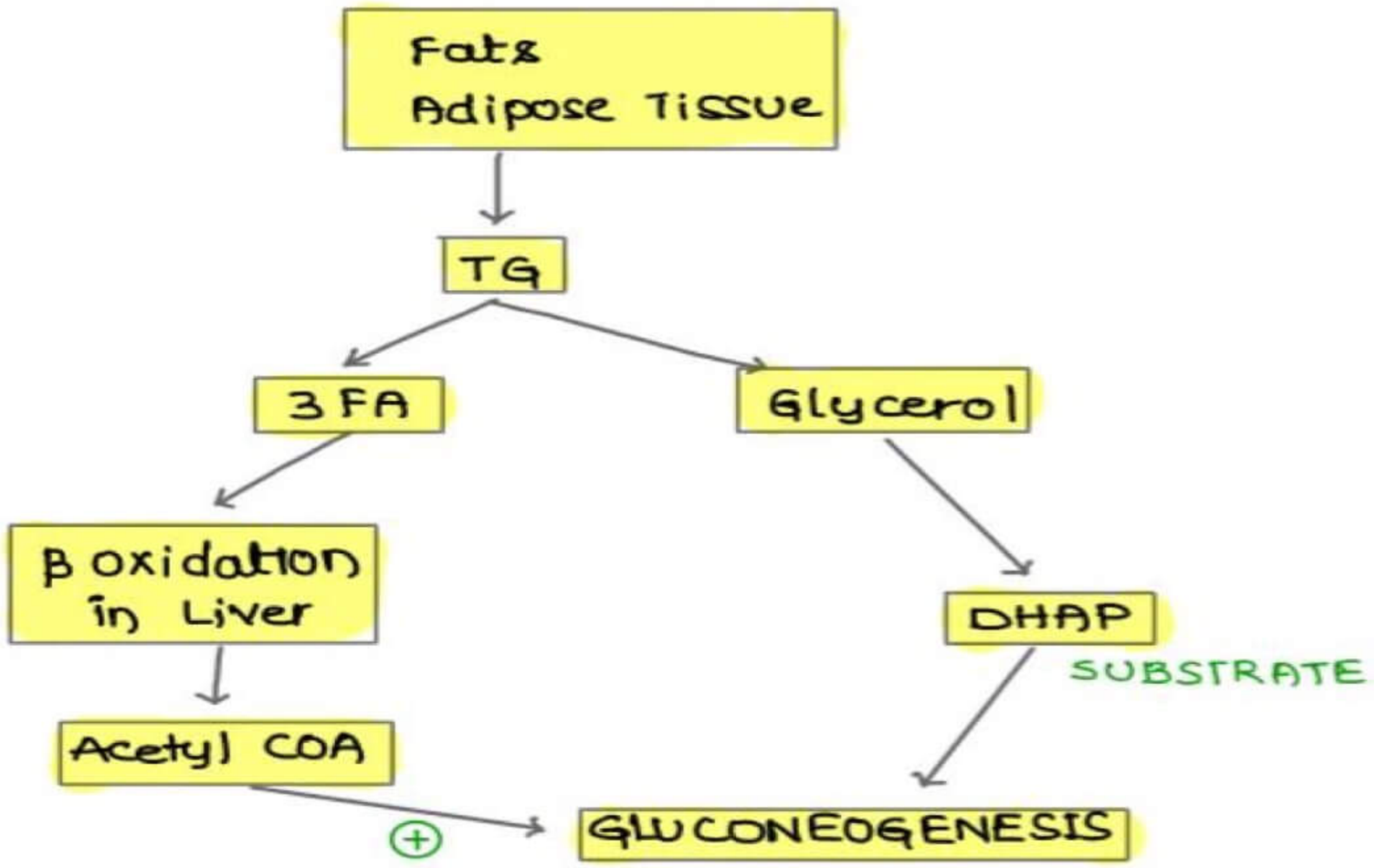
➔ **LINK REACTION** [PDH Complex] is IRREVERSIBLE → Fats can never be converted to carbohydrates

EXCEPTIONS

1. Glycerol
2. Propionic Acid

} Breakdown products of FATS can be converted to carbohydrate

➔ Acetyl Co A is never glucogenic, But indirectly can be glucogenic.



CARBOHYDRATES (BASICS)

Fed → Diet → 60% - 70% Carbohydrates
15% - 20% Fats
Rest proteins

→ USAGE OF DIFFERENT NUTRIENTS BY BODY FROM DIET (In Sedentary Life style)

- CARBOHYDRATES
  - 50% → Utilised for energy production
  - 50% → Stored
    - 10% as Glycogen
    - 40% as Endogenous Fats

→ Endogenous Fat transported in the form of VLDL.

→ FATS (exogenous / diet) transported in the form of chylomicrons.

Q A Person on fat free carbohydrate rich diet continues to grow obese.
Which Lipoprotein increased?

A VLDL

ATKIN'S DIET → Low calorie, Low carbohydrate diet

JUICE → Contains FRUCTOSE (most lipogenic)

THERMOGENIC EFFECT OF FOOD / SDA (Specific Dynamic Action)

- Energy required to digest, absorb, transport & metabolise
- Maximum for - Proteins > Carbohydrates > Fats

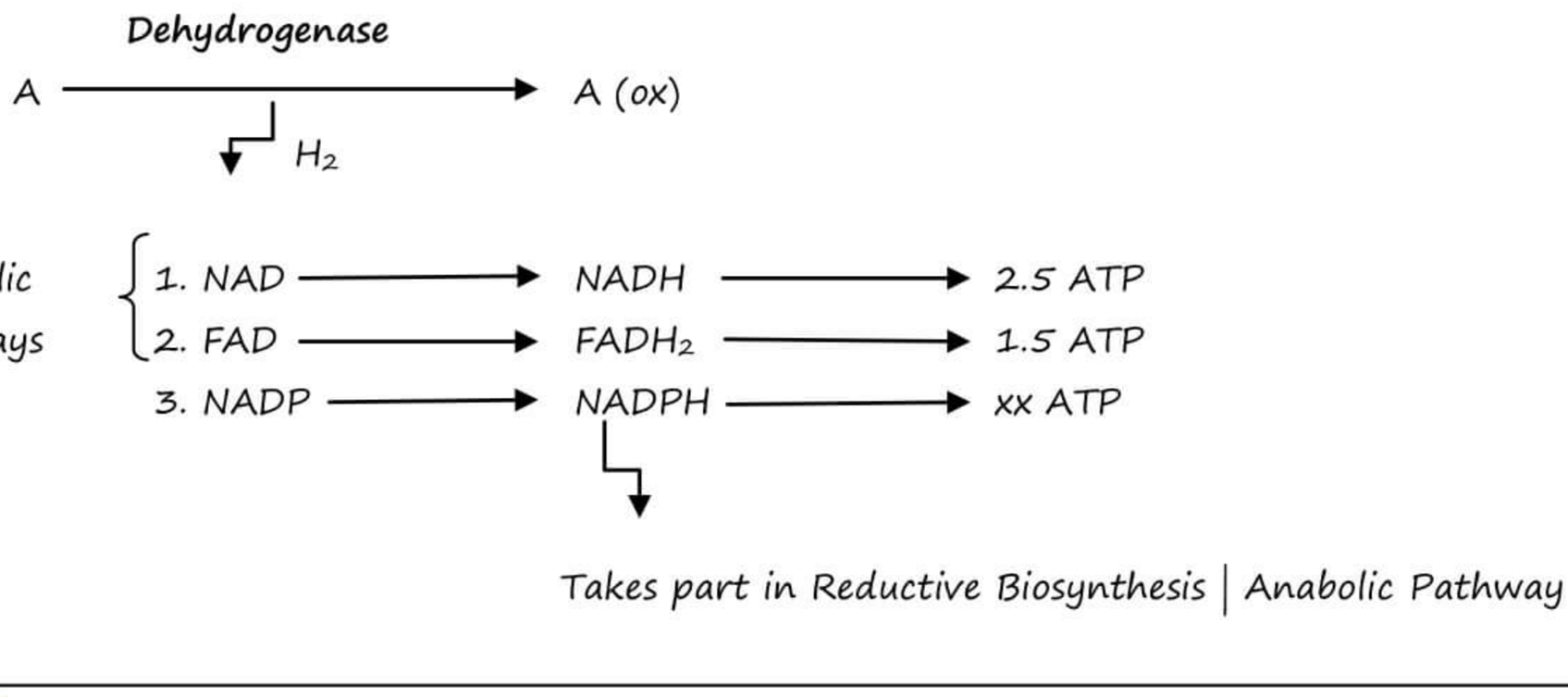
ENZYME

EC No. → Enzyme commission / Code Numbers

1. Oxido Reductase → Tranfer Hydride ions/ electrons.
2. Transferase → Molecular formula is changed
3. Hydrolase → Use H_2o to break
4. Lyase → Can make / break [do not require H_2O / ATP]
5. Isomerase → Molecular formula do not change
6. Ligase → Use ATP to make

$+ O_2$	→ Oxidation	e^-	→ H atom / H_2 / Reducing equivalent (added)
$+ H_2$ $+ e^-$	} Reduction	Proton	→ H^+ ion

DEHYDROGENASES



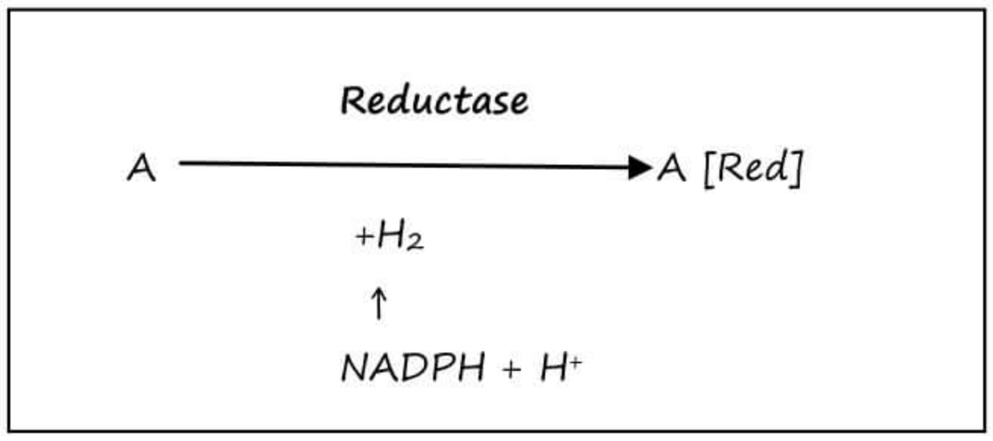
NADPH

PRODUCED FROM

1. HMP (major)
2. Malic Enzyme
3. Cytoplasmic Isocitrate Dehydrogenase (Not In TCA)

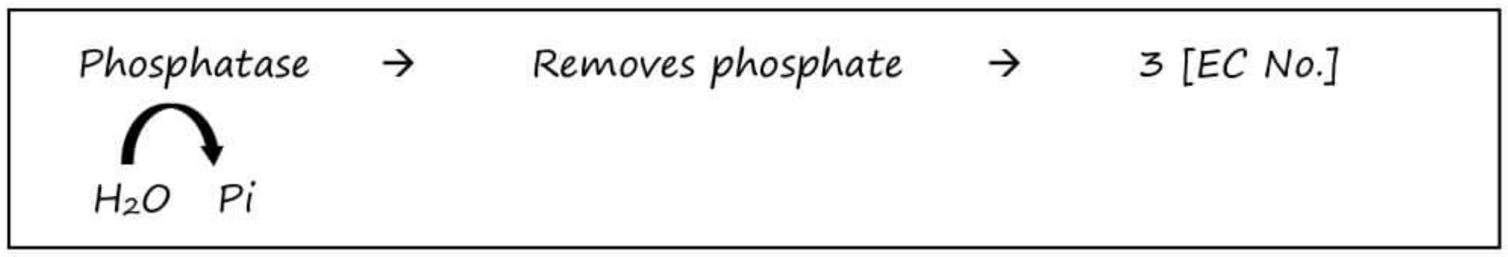
USED IN

➔ Reductive Biosynthesis / Anabolic pathway



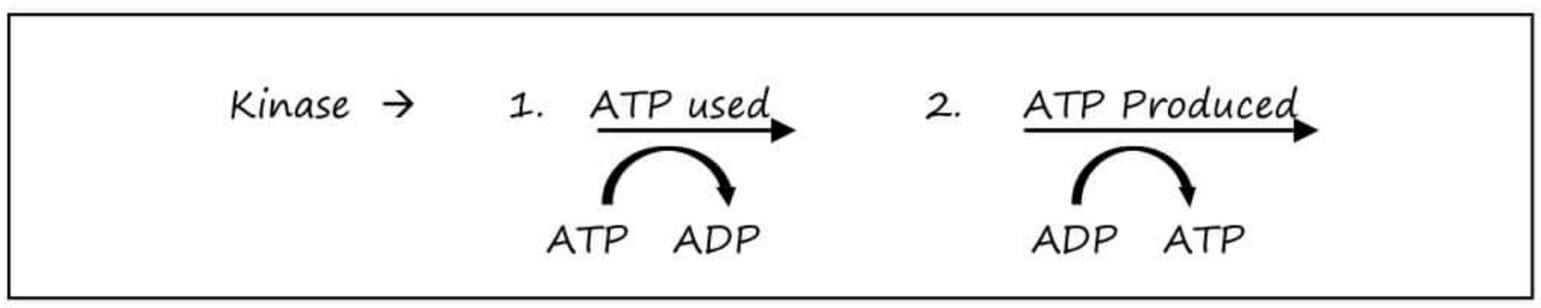
		EC No.
Dehydrogenase (Oxid)	→	1
Reductase (Red)	→	1
Kinase (Transfer phosphate)	→	2
Phosphorylase (Transfer phosphate)	→	2

- Organic Phosphate is transferred by Kinase
- Inorganic Phosphate is transferred by Phosphorylase



All Kinases, Phosphorylases & Carboxylases uses → Mg (co – factor)

But Pyruvate Kinase use → K > Mg



→ **SUBSTRATE LEVEL PHOSPHORYLATION**

Substrate —Kinase→ Product
Only 1 step

- Less ATP Production

→ **ETC**

- ATP also formed by this
- Oxidative Phosphorylation
- Most ATP produced by this

SYNTHESIS

➔ Done by

1. Synthase → no ATP Needed ; EC no. → 4 (Lyase)
2. Synthetase → ATP used EC no. → 6 (Ligase)

➔ All Synthases are Lyases

EXCEPT		**EC no.**
1. Nitric oxide Synthase	→	1
2. Glycogen Synthase	]	2
3. Citrate Synthase	]	
4. ATP Synthase	→	3

CARBOXYLATION – Addition of Carbon di Oxide

➔ Carried by CARBOXYLASE

- EC No. → 6
- Requires

A → ATP
B → Biotin [B_7]
C → CO_2 and Mg^{2+}

DECARBOXYLATION

➔ **TYPES**

1. Oxidative [EC no -1] → Requires Vitamin B1
2. Simple [EC no- 4] → Requires Vitamin B6

➔ Enzyme → DEHYDROGENASE [EC 1] in Oxidative Decarboxylation

Enzyme → LYASE [EC 4] in Simple Decarboxylation

➔ **Examples of Simple Decarboxylation**

1. Histidine to Histamine
2. Glutamate to GABA
3. Tryptophan to Tryptamine

Amino Acid ⟶ Amine
-acid

Amino Acid ⟶ Keto Acid
-NH2

→ NAD	→	requires B_3	-	COO^-
→ FAD	→	requires B_2	+	NH_3^+
→ CoA	→	requires B_5		

→ COOH	→	ionised to	COO^-	→ creates	-ve charge
NH_2	→	ionised to	NH^+	→ creates	+ve charge

CARBOHYDRATE CHEMISTRY

BASICS OF CARBOHYDRATE CHEMISTRY

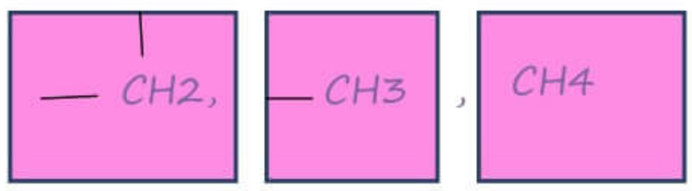
(CH_2O) n → Molecular/Chemical formula n→ no. of total carbons

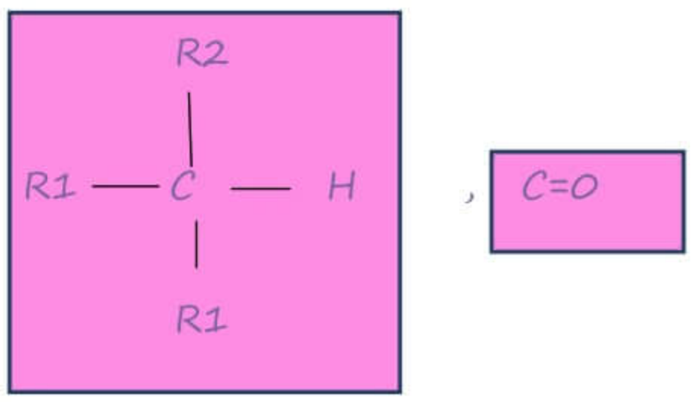
Isomers → 2^n n→no. of asymmetrical/chimeric carbons

SYMMETRIC CARBON

➔ Any 2/3/4 valencies are occupied by same atom/group of atoms

➔ Eg.





ASYMMETRIC CARBON

→ Whenever a compound has asymmetric carbon, that compound will show both structural & optical isomerism

→ Central carbon is asymmetric & shows both Structural & Optical isomerism

CARBOHYDRATES DEFINITION →Poly hydroxy Aldehyde or Ketones

POLYHYDROXY → Many OH groups

OH GROUP

1. Polar
2. Has tendency to bind phosphate
3. Suffix → 'ol'

   Eg. Alcohol

   Glycerol

   CHOLESTER 'OL' → Amphipathic (polar & non-polar components)

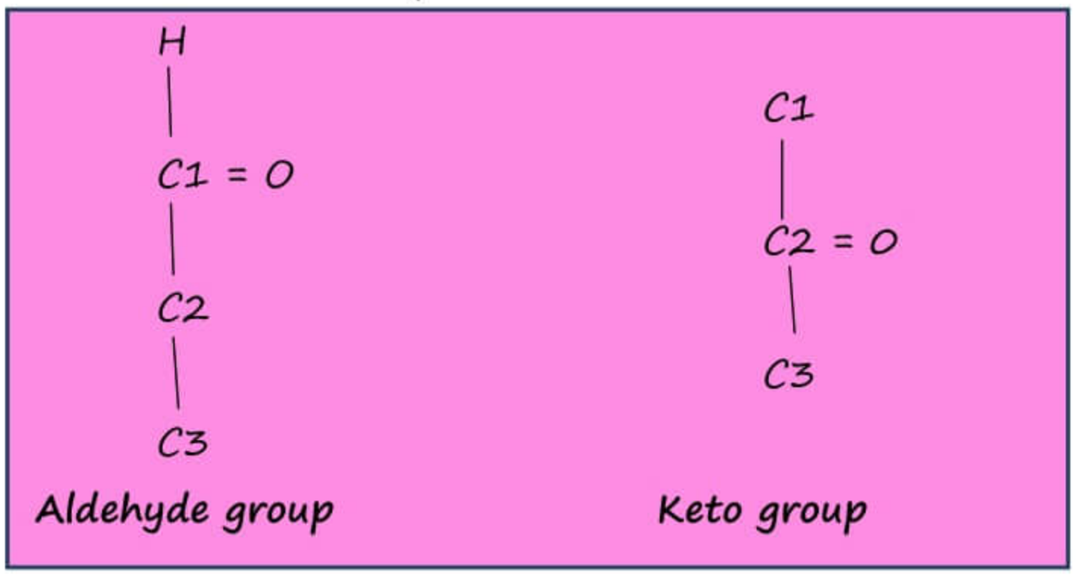
No. of OH groups are less than 1 the no. of carbon atoms

Ex: → 6C - 5 OH

→ 5C - 4 OH

ALDEHYDE OR KETONE

➔ Functional Groups



Aldehyde group is always present at C1

Keto group is always present at C2

Functional carbon is symmetric but only in linear configuration

ISOMERISM

(1) STRUCTURAL / STERIO ISOMERISM

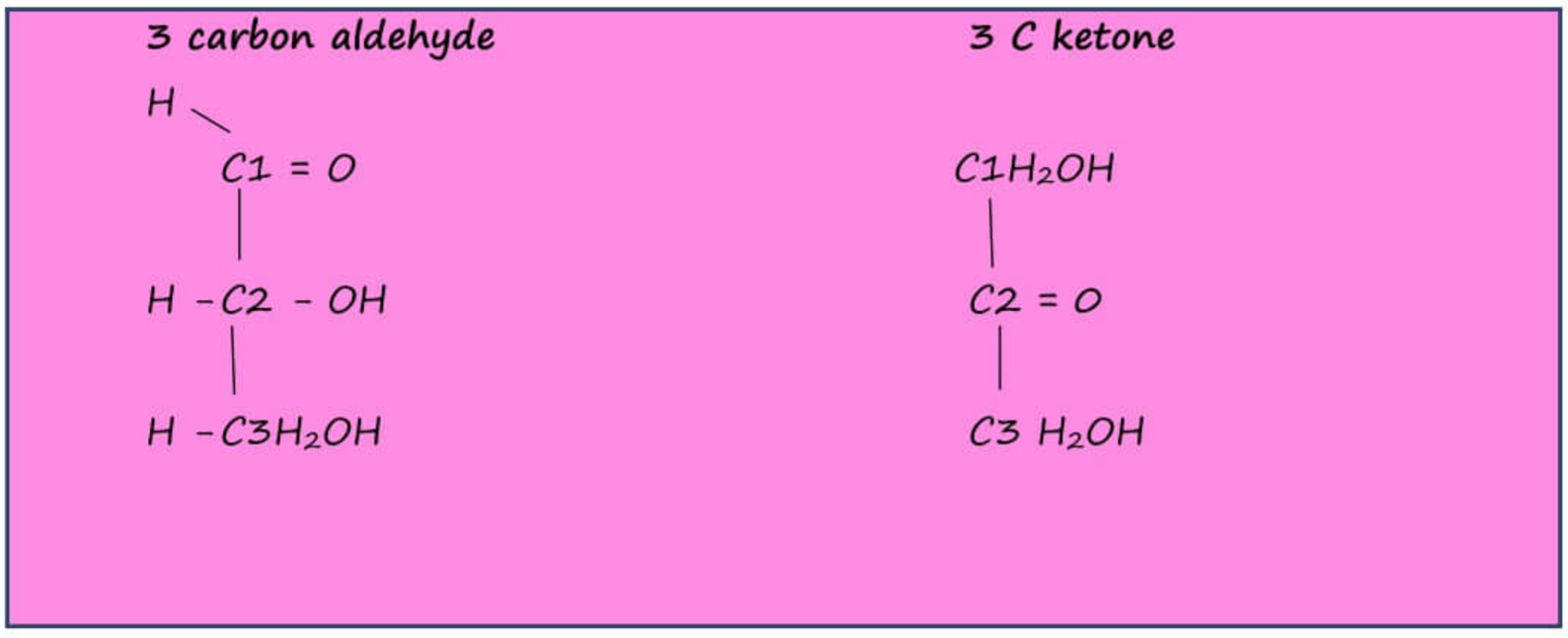
1. Functional
2. Enantiomerism
3. Epimerism
4. Anomerism

(II) OPTICAL ISOMERISM

ISOMER → Compounds having same molecular formula but different structure

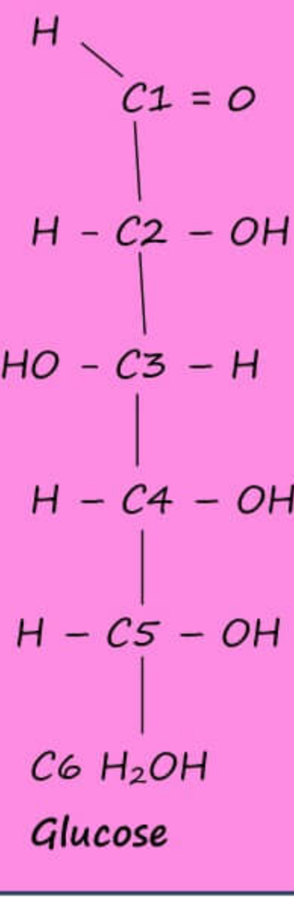
(1) STRUCTURAL / STERIO ISOMERISM

1. FUNCTIONAL ISOMERISM → Different Functional groups [aldehyde or keto]



Glyceraldehyde

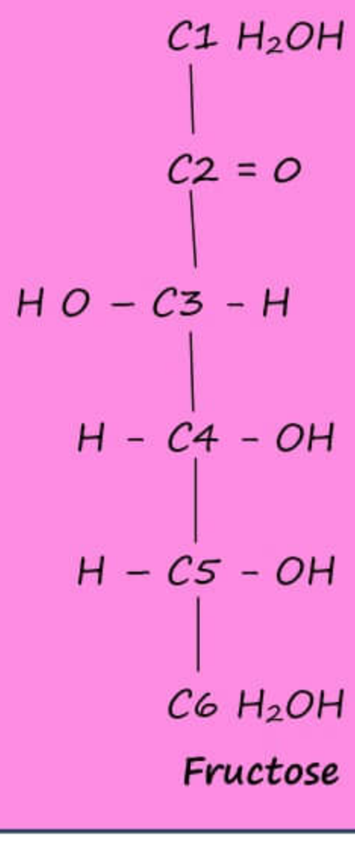
6C ALDEHYDE



Glucose

Dihydroxy Acetone [DHA]

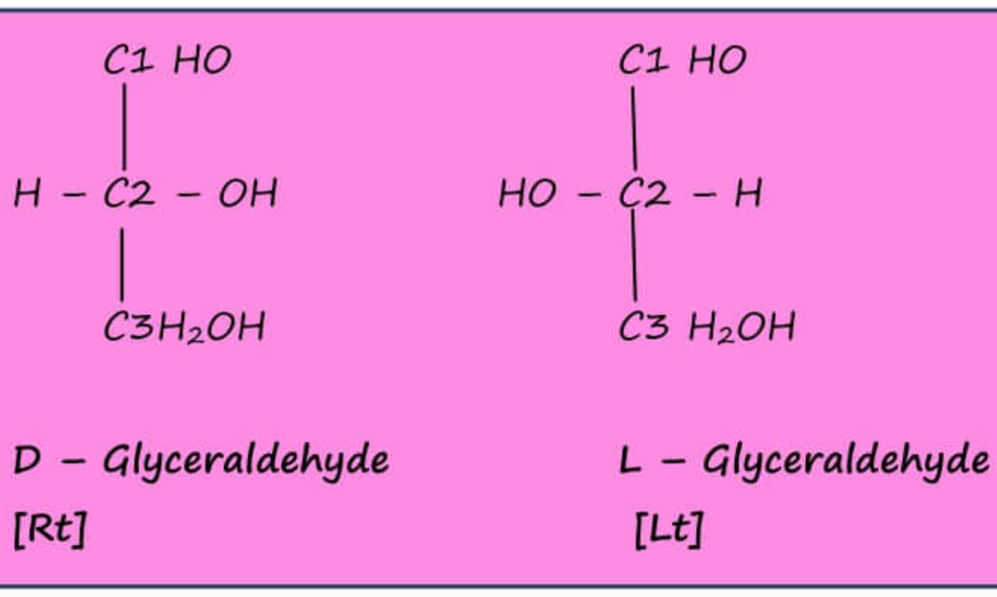
6C KETONE



Fructose

2. ENANTIOMERISM | D|L ISOMERISM | MIRROR IMAGES

→ Different H & OH orientation around the penultimate carbon | Reference | 2nd last carbon

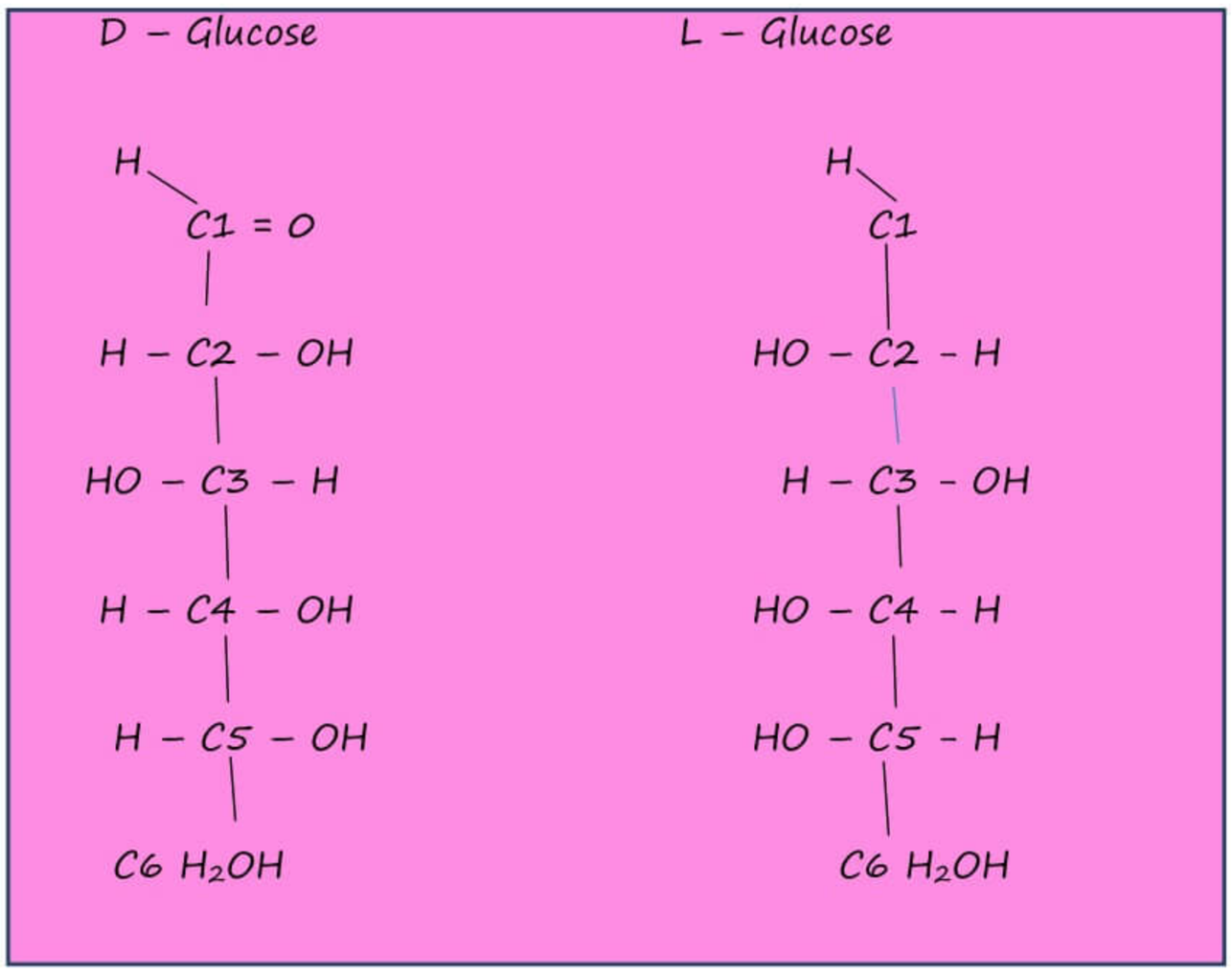


D - Glyceraldehyde

[Rt]

L - Glyceraldehyde

[Lt]



ABUNDANT FORMS IN

Carbohydrates → D forms
Amino Acids → L forms

➔ **ABUNDANT FORM IN CARBOHYDRATES → D**

Body → D
Cell → D
Plasma → D
Nature → D

➔ **ABUNDANT FORM IN AMINOACIDS → L**

In Proteins → L
Free AA → L or
→ D
Eg. D – Serine, D – Aspartate } Found in Brain

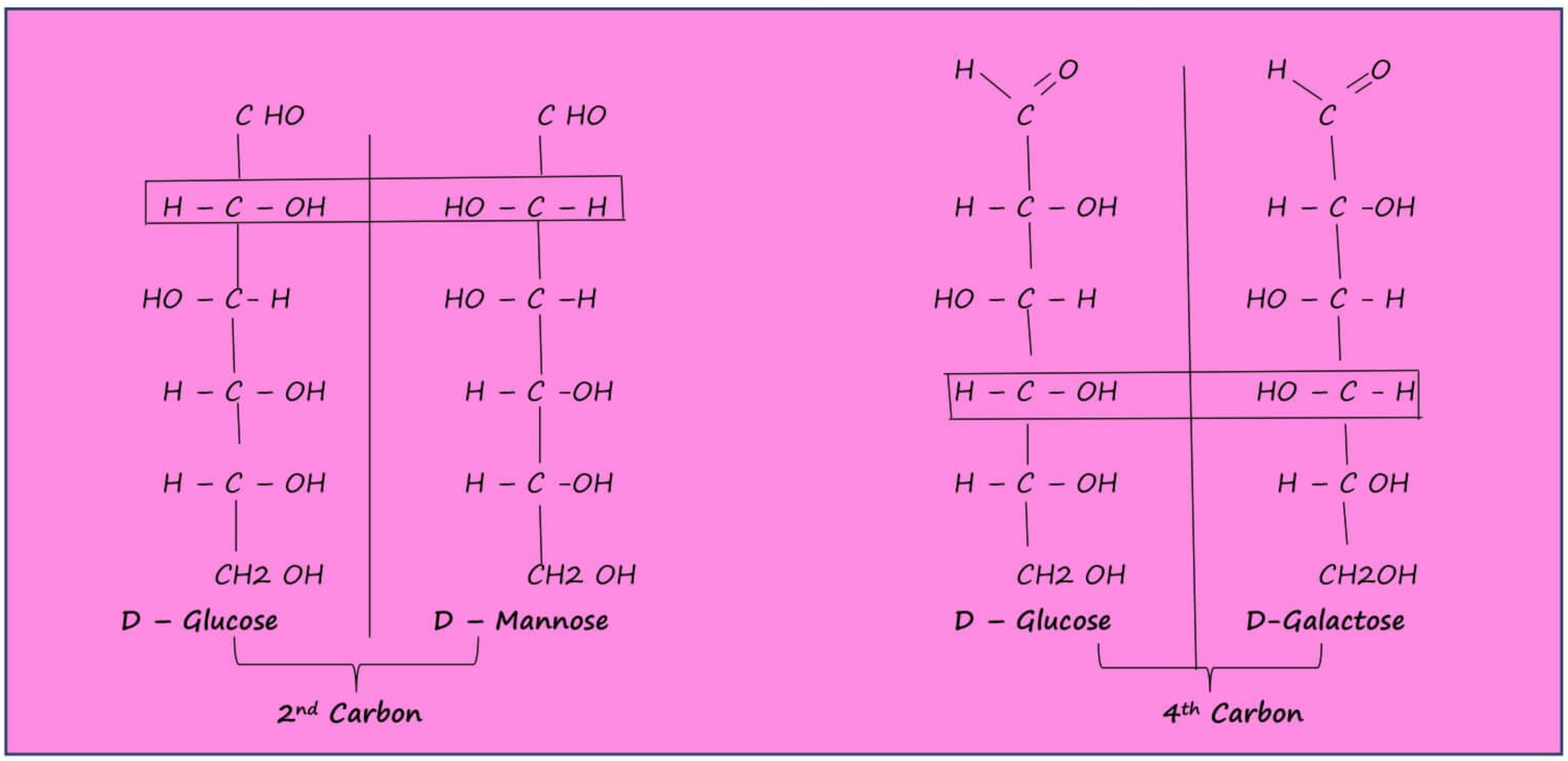
➔ Which form of amino acid is present in Body → D & L
➔ Which form of amino acid is abundant in body → L

➔ Synthesized AA → L Forms

Source of D -AA → Always Exogenous

3 EPIMERISM

→ Different H & OH orientation around only one carbon other than penultimate carbon



→ Mannose is epimer of glucose at C2

Galactose is epimer of glucose at C4

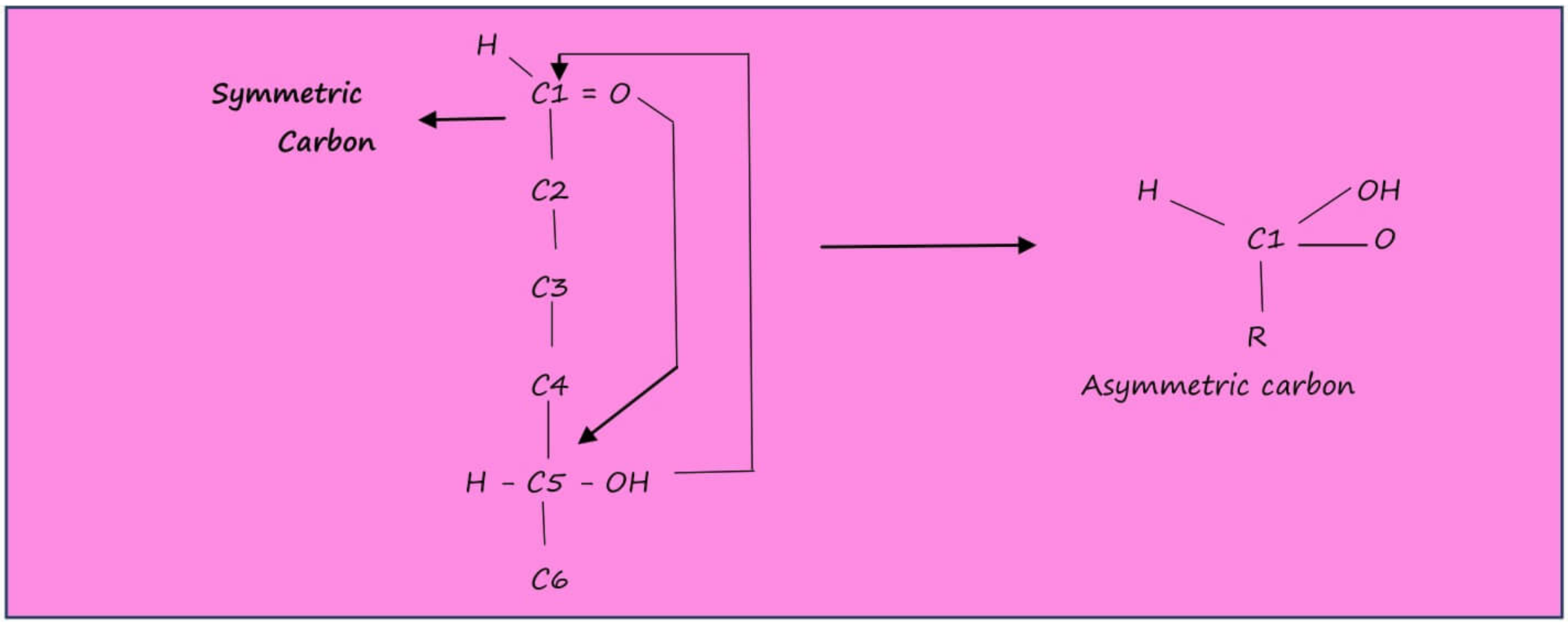
→ Mannose & Galactose are not epimers of each other

4. ANOMERISM

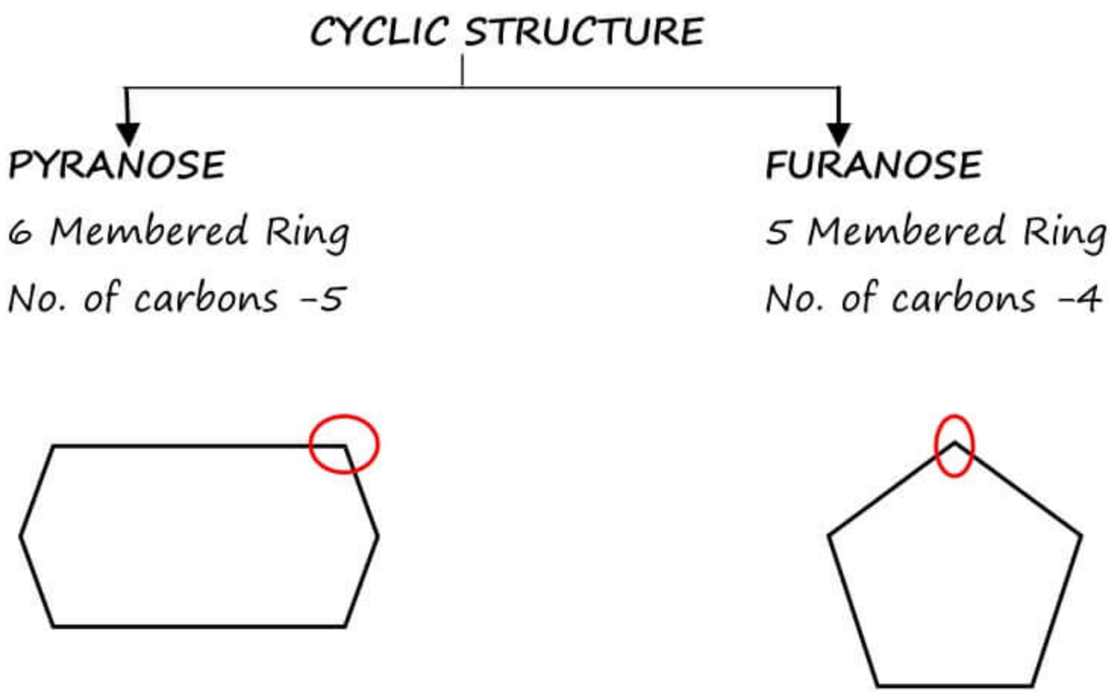
→ Linear Structures → Cyclic Structures

[Powder form] [Solution form]

→ The combining carbons are Functional carbons; Always combine with 2nd last carbon



→ Only for cyclic form

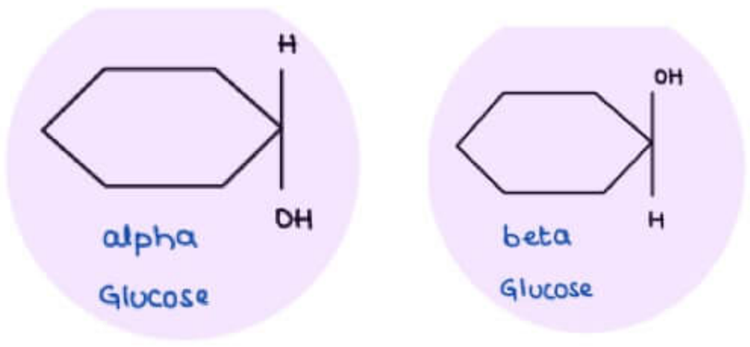


→ Glucose → 99% Pyranose, 1% Furanose
mainly Pyranose

→ Fructose → 99% Furanose, 1% Pyranose
mainly Furanose

→ Hexoses (6C) → Both Pyranose & Furanose exists
Pentoses (5C) → Only Furanose exits

TYPES



Functional → different functional groups
Enantiomerism → different H & OH Orientation at penultimate carbon
Epimerism → different H & OH Orientation only at 1 carbon
Anomerism → different H & OH orientation around functional carbon

II OPTICAL ISOMERISM

→ Same molecular formula but different optical properties

→

→ Glucose is always d (+)

→ Fructose is always l (-)

Levo rotatory power of Fructose > Dextro rotatory power of Glucose

→ RACEMIC MIXTURE → Equal d + l, Optically inactive

→ RACEMASE → Interconvert 2 isomers; D ⟷ L

→ Misnomer

CLASSIFICATION OF CARBOHYDRATES

	No. of Carbohydrates Units
MONOSACCHARIDES	1
DISACCHARIDES	2
OLIGOSACCHARIDES	3 – 10
POLYSACCHARIDES	> 10

MONOSACCHARIDES

→ No. of carbons → 3 – 9

3C	→	Aldehyde	Keto
4C	→	Erythrose	Erythrulose
5C	→	Ribose	Ribulose
6C	→	Glucose	Fructose
7C	→	Gluco heptulose	Sedoheptulose
8C	→	x	x
9C	→	Sialic Acid	

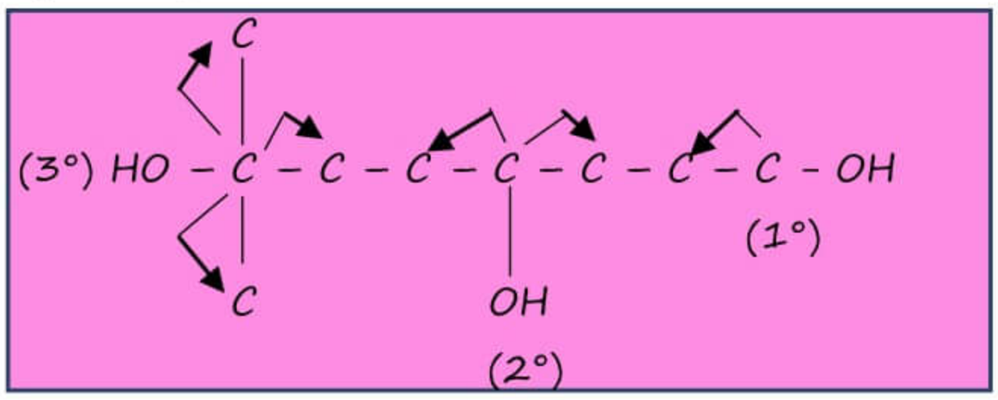
REACTIONS

1. OXIDATION

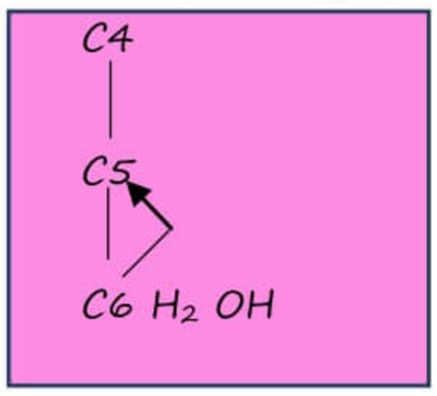
→ Forms Acids

→ If at C1	→	Aldonic	→	Gluconic
→ If at C6	→	Uronic	→	Glucuronic
→ If at C1 & C6	→	Saccharic Acid		

→ At primary alcohol (C6) → Uronic



→ In case of Glucose (Prototype)



2. REDUCTION

→ Forms Alcohols

→ Hygroscopic in nature

→ Causes cell swelling

→ Glucose	→	Sorbitol / polyol	→	snow flake cataract
→ Galactose	→	Galactitol / Dulcitol	→	oil drop cataract
→ Mannose	→	Mannitol	→	↓ ICT

DISACCHARIDES

→ 2 monosaccharides bound by glycosidic bond O——O

1.	Maltose	→	Glu + Glu	→	α(1 → 4)	→ Reducing sugar
2.	Isomaltose	→	Glu + Glu	→	α(1 → 6)	→ Reducing sugar
3.	Trehalose	→	Glu + Glu	→	α(1 -> 1)	→ Non - Reducing sugar
4.	Sucrose	→	Glu + Fruc	→	α(1 → 2)	→ Non - Reducing sugar
5.	Lactose	→	Gal + Glu	→	β (1 → 4)	→ Reducing sugar

ALL MONOSACCHARIDES ARE REDUCING SUGARS (Functional group is free)

TESTS

1. MOLISCH TEST
   - → General test given by all
   - → No. of carbons → ≥ 5

2. BENEDICT'S TEST → Given by reducing sugars
3. SELIWANOFF TEST → Distinguish b|w keto & Aldehyde sugar
   Positive in keto sugar

4. BARFOED'S TEST → Positive in monosaccharides
   → Distinguishes b|w mono & Disaccharides

5. GOD – POD TEST → Enzymatic test
   ↓ ↓ → Routinely done
   Glucose Peroxidase → Measures blood glucose
   Oxidase → GLUCOSE $\xrightarrow[C1]{GOD}$ Gluconic Acid + H_2O_2
   $\xrightarrow{POD}$ coloured Compound
   → Accurate

POLYSACCHARIDES

HOMO POLYSACCHARIDES → Made up of same carbohydrate units
→ Mostly Branched

HETERO POLYSACCHARIDES → Made up of different carbohydrates units
→ Mostly unbranched

HOMO

(1) Starch → Plants
   → Less branched
   Branching point comes after 24–30 glucose

(2) GLYCOGEN
   - → Present in animals
   - → More branched
     - Branching points comes after 8–12 glucose residues

SIMILARITIES

→ Made up of α glucose

→ Has α (1-4) bonds in straight chain

→ Has α (1-6) bonds in branch points

STARCH

→ Made up of

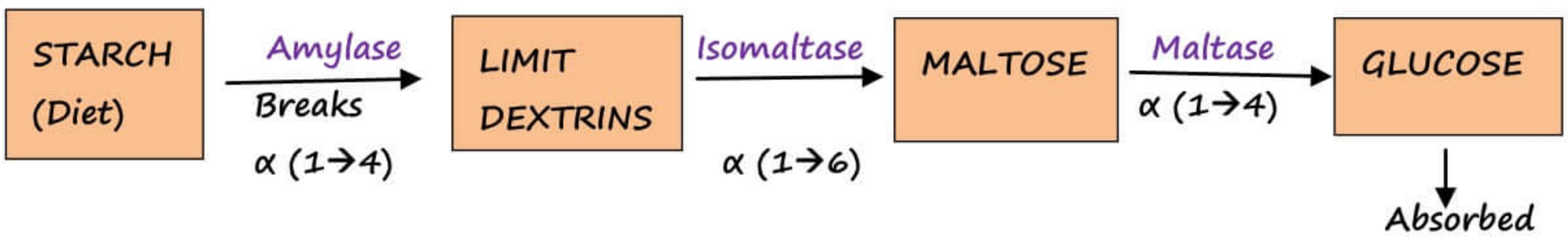
1. Amylose

- 20%
- Unbranched

2. Amylopectin

- 80%
- Branched

→ DIGESTION



3. DEXTRAN

→ Made up of α – glucose

→ Has α (1 → 4), α (1 → 6), α (1 → 2), α (1 → 3) bonds

→ High molecular weight structure

→ Highly branched structure

→ i/v (intravenously) Dextran is used as Plasma volume expander in hypovolemic shock

→ In Gel Filtration chromatography, Gel is dextran

→ Dental plaques → Are network of Dextrans

4. CELLULOSE

→ UNBRANCHED

→ Made up of β – Glucose

→ Acts as FIBRES in the diet

β bonds are not easily broken

FIBRES

→ TYPES

1. **INSOLUBLE**
   - → Cellulose
     Hemicellulose
   - → Excreted unchanged

2. **SOLUBLE**
   - → Pectins
     Gums
   - → Absorbs H_2O & converted to Gel form, which is excreted
   - → Better in preventing Constipation

5. INULIN

→ Made up of β Fructose

→ **USES**

→ Ideal for measuring GFR

→ PREBIOTIC (Food for Bacteria)

NORMAL CONSTITUENTS	
Carbohydrates	C, H, O
Fats	C, H, O
Amino acids	C, H, O, N, NH_2

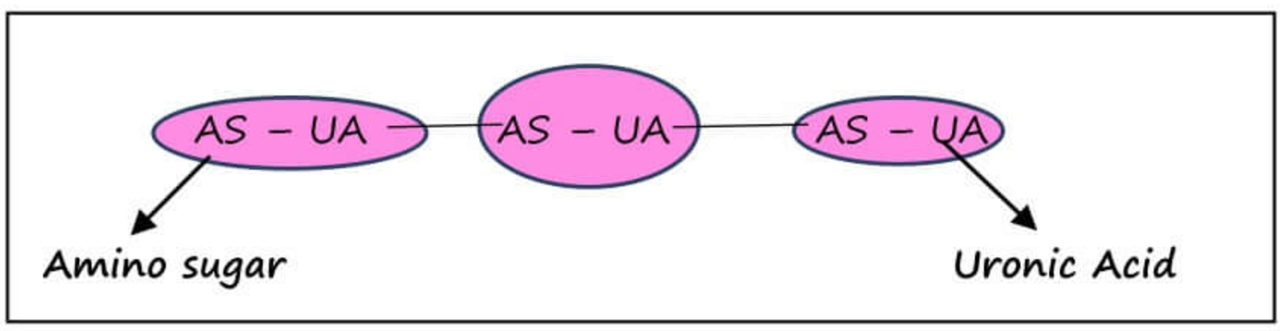
HETEROPOLYSACCHARIDS

➔ Aka GAGS → Glycosa Amino Glycans

➔ Aka Mucopolysaccharides → Present in mucus secretions (lubricant)

➔ Definition → Tandem repeats of AS – UA

→ Tandem Repeat → Repeated one after another



→ All GAGS combine with proteins to form PROTEOGLYCANS

PROTEO GLYCANS	GLYCO PROTEIN
• Carbohydrate >>> Protein • Ex: GAGS • Carbohydrate is always Heteropolysaccharide	• Protein >>> Carbohydrate • Ex: Collagen All plasma proteins → EXCEPT ALBUMIN (only protein) • Carbohydrate is never a poly-saccharide

→ Highly SULFATED

→ Contains more negative charge

SO_4^{2-}
COO^-
CH_3COO^-
} **Slimy & Slippery**

→ **EXAMPLES**

1. HYALURONIC ACID
   → Only GAG which is not Sulfated
   → Longest GAG
   → Located in Synovial Fluid & Vitreous humour
2. CHONDROITIN SULFATE → Present in Cartilage, Bone, Tendon
3. DERMATAN SULFATE → Found in Skin, Blood vessels, valves
4. KERATAN SULFATE
   → Only GAG without uronic acid
   → Present in cornea & connective tissue
   → Responsible for Transparency of cornea
5. HEPARIN → Released from mast cell & liver
6. HEPARAN SULFATE
   → Present on cell surfaces
   → Has a role in retinal cell – cell attachments

MUCOPOLYSACCHARIDOSIS

➔ LYSOSOMAL STORAGE DISEASE
   → Mucopolysaccharides accumulates in Lysosomes
➔ Autosomal Recessive
   → EXCEPT HUNTER DISEASE → X – Linked Recessive (♂)

GENERAL CLINICAL FEATURES

➔ COARSE FACIAL FEATURES
   → Depressed nasal bridge
   → Frontal bossing
➔ Copious nasal discharge
➔ Short stature (due to growth retardation)

- ➔ Clawing of hands
- ➔ Protuberant abdomen (due to Umbilical Hernia, Hepatosplenomegaly)
- ➔ Corneal Clouding
- ➔ Skeletal Features
    - → Dysostosis Multiplex due to defective bone formation
    - → Have bullet shaped middle phalanx
    - → Small irregular carpal bones
    - → Broad Proximal pointed short metacarpals

➔ SPECIFIC C|F

HEPARAN SULFATE (HS) → MR (Mental Retardation)
DERMATAN SULFATE (DS) → Atherosclerosis

OTHER FEATURES

REILLY BODY INCLUSIONS in Leukocytes

Presence of Mucopolysaccharides in Urine

TYPES	NAME OF DISEASE	ENZYME DEFICIENT	ACCUMULATES	CLINICAL FEATURES
I H	HURLER DISEASE	α – L – Iduronidase	DS + HS	Inguinal Hernia (+)
II	HUNTER DISEASE	Iduronate Sulfatase	DS + HS	No Corneal Clouding
I S	SCHEIER DISEASE	α – L – Iduronidase	DS	No Mental Retardation
VI	MAROTEAUX LAMY SYNDROME	Aryl Sulfatase B	DS	No Mental Retardation

ERT (Enzyme Replacement Therapy)

I → ALDURAZYME
II → ELAPRASE
VI → NAGLAZYME

MC Mucopolysaccharidosis ⟶ SANFILIPPO (III) > HUNTER (II) > HURLER (I)

MPS with no corneal clouding ⟶ HUNTER > SANFILIPPO

I-CELL DISEASE (Inclusion cell/ Inclusion Body Disease)

→ not a MPS

→ Cf are same & more severe

→ Lysosomal protein targeting disorder

→ Mucolipidosis

→ **LYSOSOMES**

→ Contains HYDROLASES

- Synthesized in RER
- Goes to Golgi apparatus where phosphorylation occurs & then transported to Lysosomes

Mannose is required on Hydrolases to reach lysosomes

↓

Mannose should be phosphorylated to MANNOSE- 6-Phosphate by enzyme N - Acetyl Glucosamine Phosphatase

↓

In I- cell Disease, N - Acetyl Glucosamine phosphatase is deficient

↓

Hydrolases do not reach lysosomes

↓

Inclusion Bodies formed in Lysosomes

→ **Dx** → Serum Lysosomal Enzymes ↑↑

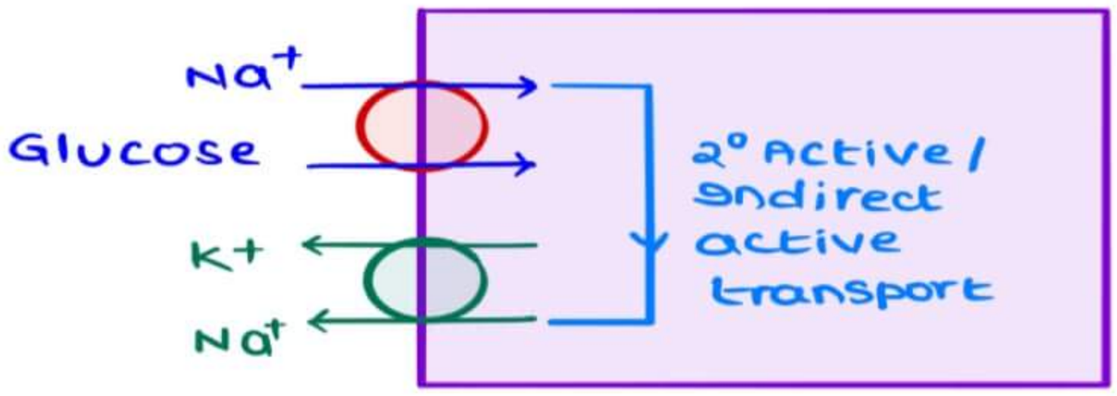
GLUCOSE TRANSPORT

FACILITATIVE TRANSPORT	ACTIVE TRANSPORT
GLUT & Na independent Down the concentration gradient Bidirectional - But after transport Glucose is Converted to Glucose – 6- Phosphate - Phosphorylation of Glucose is done for the entrapment of Glucose inside the cells	Na Glucose Symport / SGLT Na dependent Against concentration gradient Unidirectional Present only in few places - In Intestine - In Kidney

ACTIVE TRANSPORT / Na GLUCOSE SYMPORT

→ ATP UTILIZED BY Na GLUCOSE SYMPORT is '0'

- 2° / Indirect Active transport
- Na^+ thrown out of the cell by Na^+ K^+ Pump (using ATP)



FACILITATIVE TRANSPORT

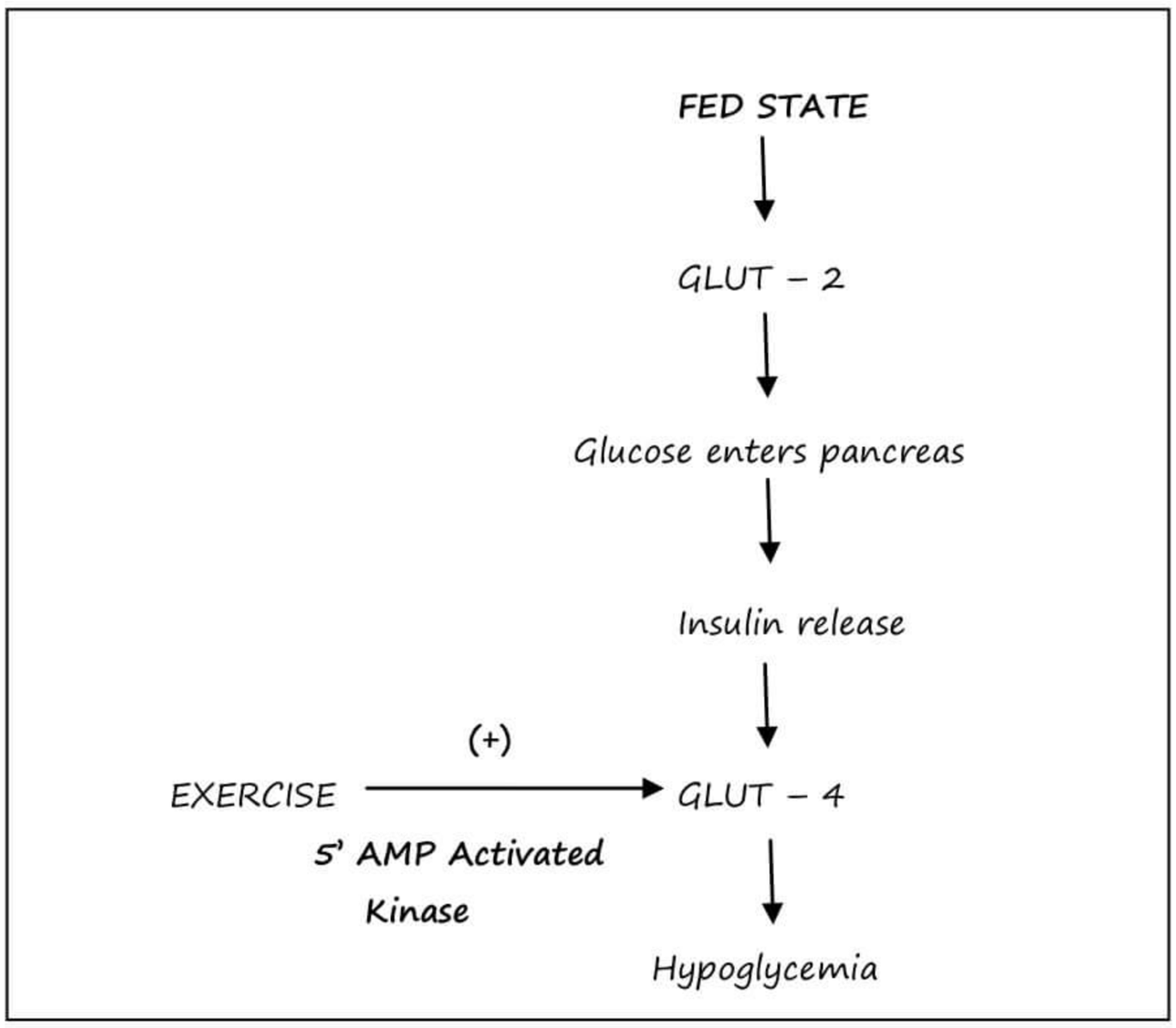
	LOCATION	FUNCTION
GLUT-1	Brain, Placenta, Kidney, RBC	Glucose uptake during fasting
GLUT-2	Liver, Pancreas, Intestine	Liver-glycogen formation pancreas-insulin secretion
GLUT-3	Brain (neuronal), placenta kidneys	Glucose uptake during fasting
GLUT-4	Skeletal muscles, cardiac muscles and adipose tissues	Insulin stimulated glucose uptake after meals
GLUT-5	Small intestine, testis (sperms)	Fructose transport
GLUT-6	WBC, spleen	Not known
GLUT-7	Liver endoplasmic reticulum	Glucose transporter in ER

AFFINITY α 1/Substrate Requirement

→ GLUTS 1 & 3 have high affinity
- Less substrate is required
- Active during fasting
- Do not depend on insulin

→ GLUTS 2 have low affinity
- More substrate isrequired
- Active during fed state
- Do not depend on insulin

→ GLUT 4 depends on Insulin



→ In Diabetic patient, dlt relative or absolute deficiency of Insulin, GLUT- 4 is not active & hypoglycemic state is not achieved.

→ Exercise can directly activate GLUT - 4

- With the help of enzyme 5' AMP activated kinase

GLUCOSE TRANSPORTATION IN INTESTINE

→ SGLT 1 present in Apical membrane

GLUT 2 present in Basolateral membrane

→ **Na^+ K^+ pump present in basolateral membrane**

- Maintains a low Na^+ concentration inside the cell
- Creates a electrochemical gradient that favours inward movement of Na^+
- K^+ is pumped into the cell by Na^+ K^+ pump, but also leaks out through K^+ channels
- Na^+ absorbed across the apical membrane through Na^+ channels dlt electrochemical gradient.

NET FLOW OF Na^+

From Lumen
↓
Across the Apical membrane
↓
Through the cell
↓
Across the Basolateral membrane
↓
Into Interstitial Fluid

→ Glucose transport across the apical membrane is coupled with Na^+ by Na^+ - Glucose Symport [SGLT -1]

→ It creates a Glucose concentration gradient across Basolateral Membrane

[as glucose concentration increases inside the cell]

→ Now, Glucose can go down its concentration gradient across the Basolateral membrane by GULT -2.

GLUCOSE TRANSPORT

KIDNEYS

GLUT 1 & 3 (High Affinity transporters)

SGLT – 1
High affinity (low capacity)
(10%) Reabsorption of glucose)

GLUT – 2 (Low Affinity Transporters)

SGLT – 2
Low affinity (High Capacity)
(90% Reabsorption of glucose)

SGLT–2: Main active transporter in kidneys

Mutations

SGLT – 1 → Glucose – Galactose Malabsorption
SGLT – 2 (Glucose kidneys) → Familial Renal Glycosuria or Glycosuria
GLUT – 2 → Fanconi Bickel Syndrome (Glycogen storage disease (XI)

GLUT – 2 is present in Intestine, Liver, Pancreas, Kidney.

Hepato Renal glycogen accumulates

- Growth Retardation
- Polyuria
- Polydipsia
- Fasting hypoglycemia
- Post prandial Hyperglycemia resembling diabetes.

BETA GALACTOSIDASE OR LACTASE

LACTOSE INTOLERANCE / LACTASE DEFICIENCY

(1°)
Enzyme deficient

(2°)
Loss of Intestinal brush border
Gastroenteritis
Autoimmune disease

Lactose —Lactase→ Galactose + Glucose

→ Present in intestinal brush border

→ Lactose is present in milk & milk products

C/F

→ Osmotic Diarrhoea

→ Distended Abdomen

→ Vomiting, Bloating

→ Flatulence

Treatment → Avoid dairy products, Lactose free milk, Lactase pills

LACTOSE → Gal + Glu

(Joined by β- bond, STRONG BOND)

β – Galactosidase (general term) → Any enzyme that can break a β Bond on the side of galactose

→ **Lactase is aka β-galactosidase**

KRABBE'S DISEASE

→ β Galactosyl ceramide accumulates

→ d/t β-Galactosyl ceramidase is deficient **(non- functional)**

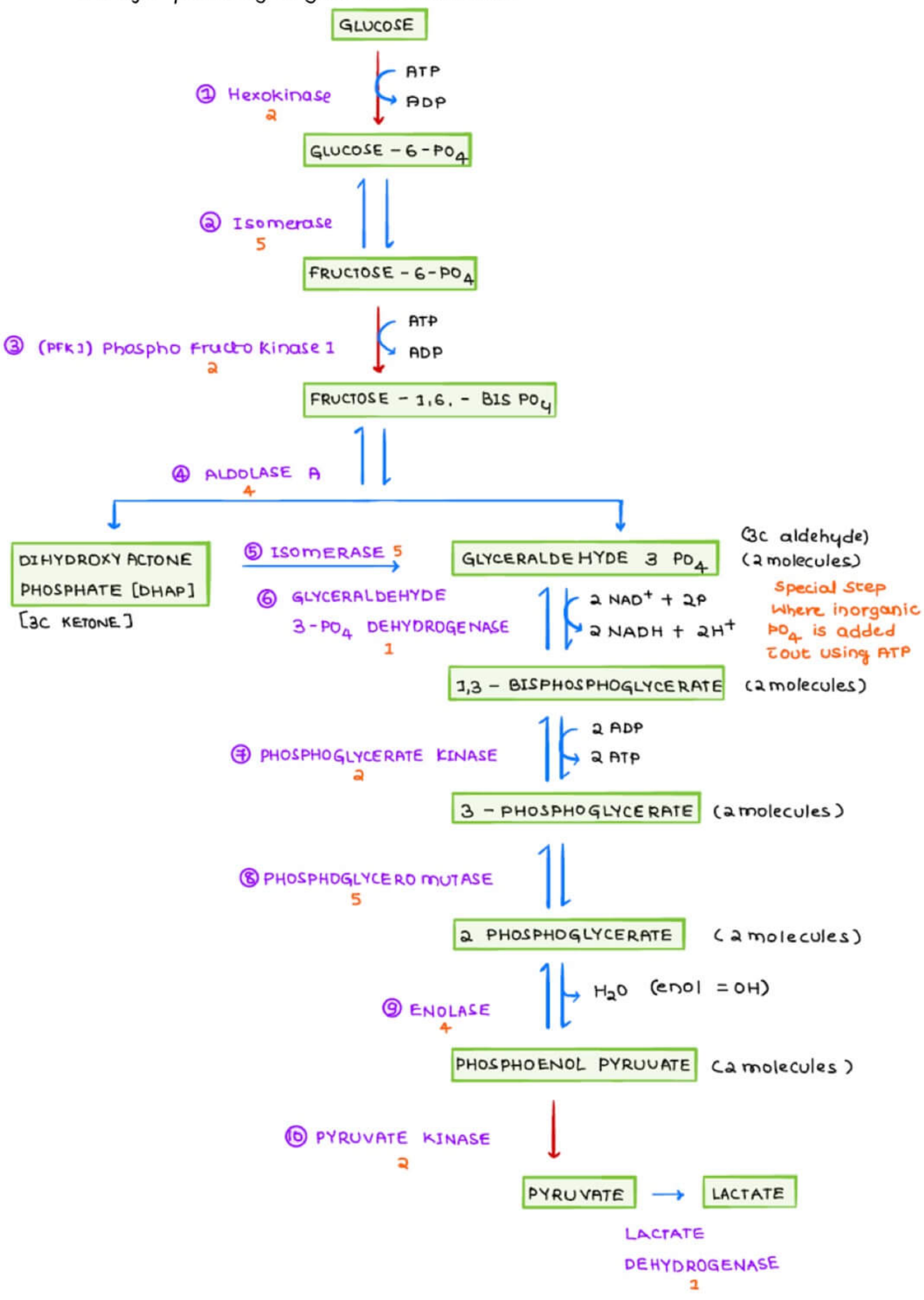
→ β- Galactosyl ceramidase is also known as β-galactosidase.

CARBOHYDRATE METABOLISM

GLYCOLYSIS

GLYCOLYSIS / EMBDEN MAYERHOF PATHWAY

→ Major pathway of glucose metabolism



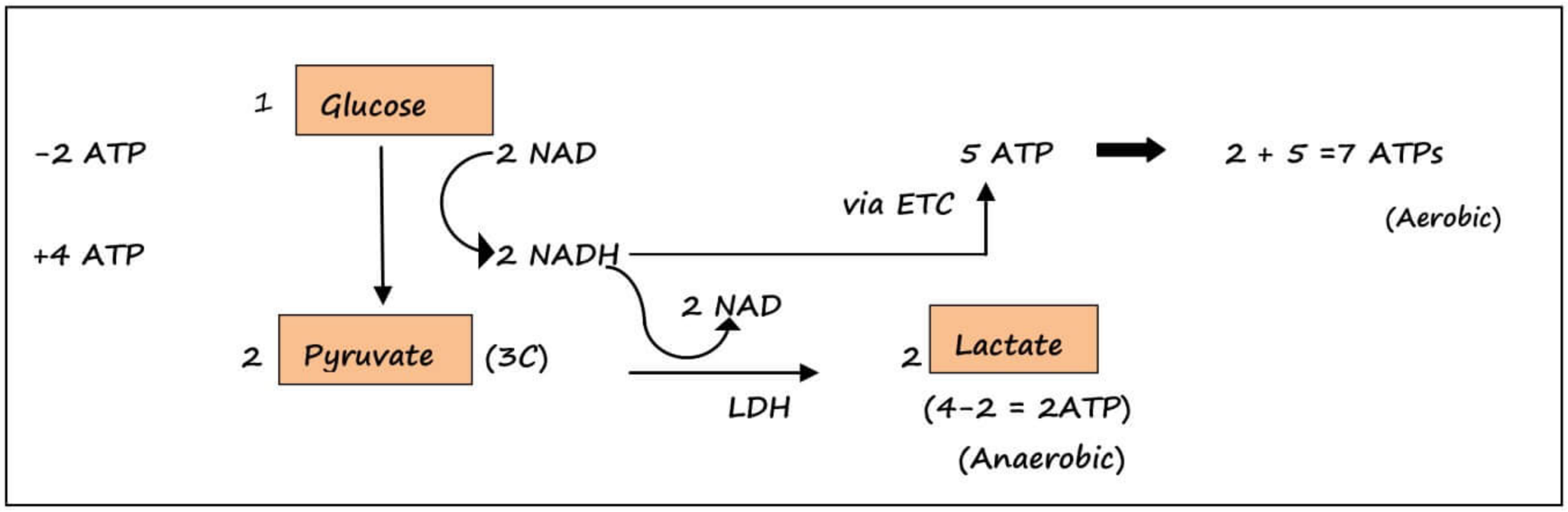
IRREVERSIBLE STEPS / REGULATORY STEPS	SUBSTRATE LEVEL PHOSPHORYLATION
1. Hexokinase	1. PG Kinase
2. PFK 1	2. Pyruvate Kinase
3. Pyruvate Kinase	

PYRUVATE KINASE

- ➔ Requires K > Mg
- ➔ 2nd mc human enzyme deficiency
  1st mc human enzyme deficiency → G6PD Deficiency of HMP pathway

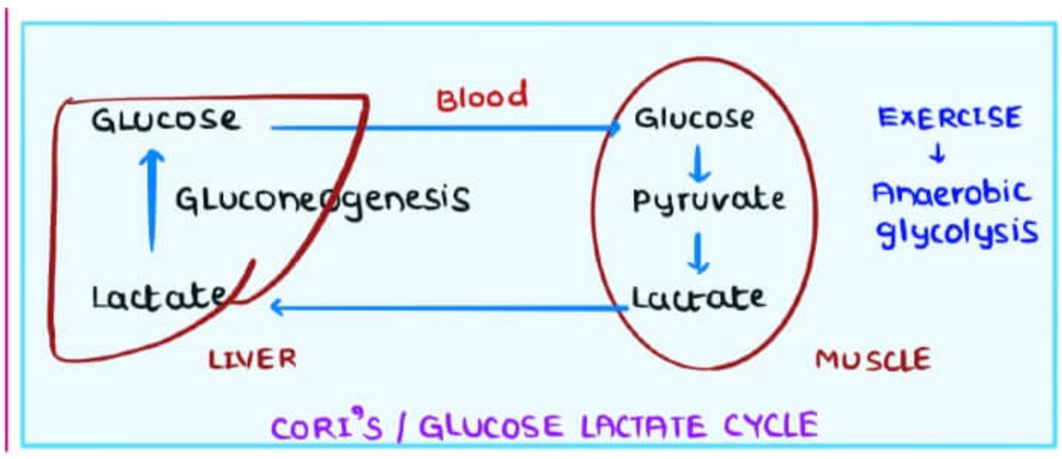
- ➔ Pyruvate Kinase & G6PD patients presents with Hemolysis
  - HEINZ BODIES are seen in G6PD deficiency

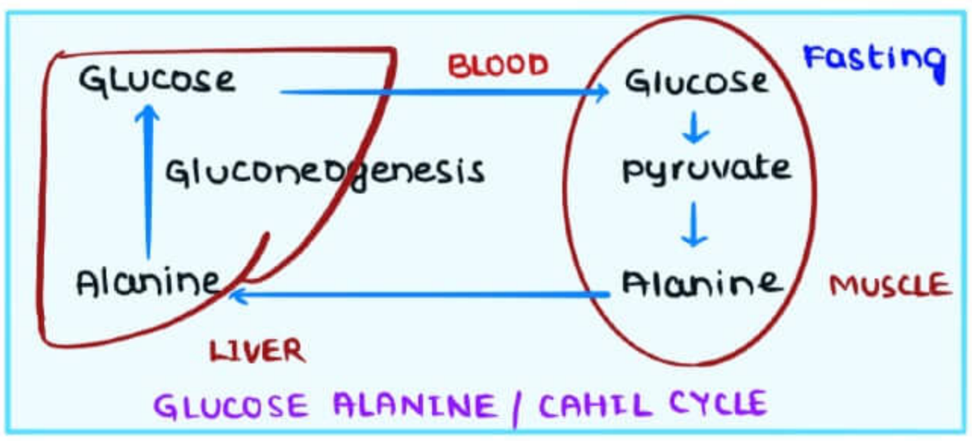
ENERGETICS



→ Purpose of extra step of anaerobic glycolysis is → NAD formation
- Regeneration of NAD for further use

→ In RBC, no. of ATPs formed by Aerobic Glycolysis → 2 ATP
→ Dead end of Glycolysis → Lactate





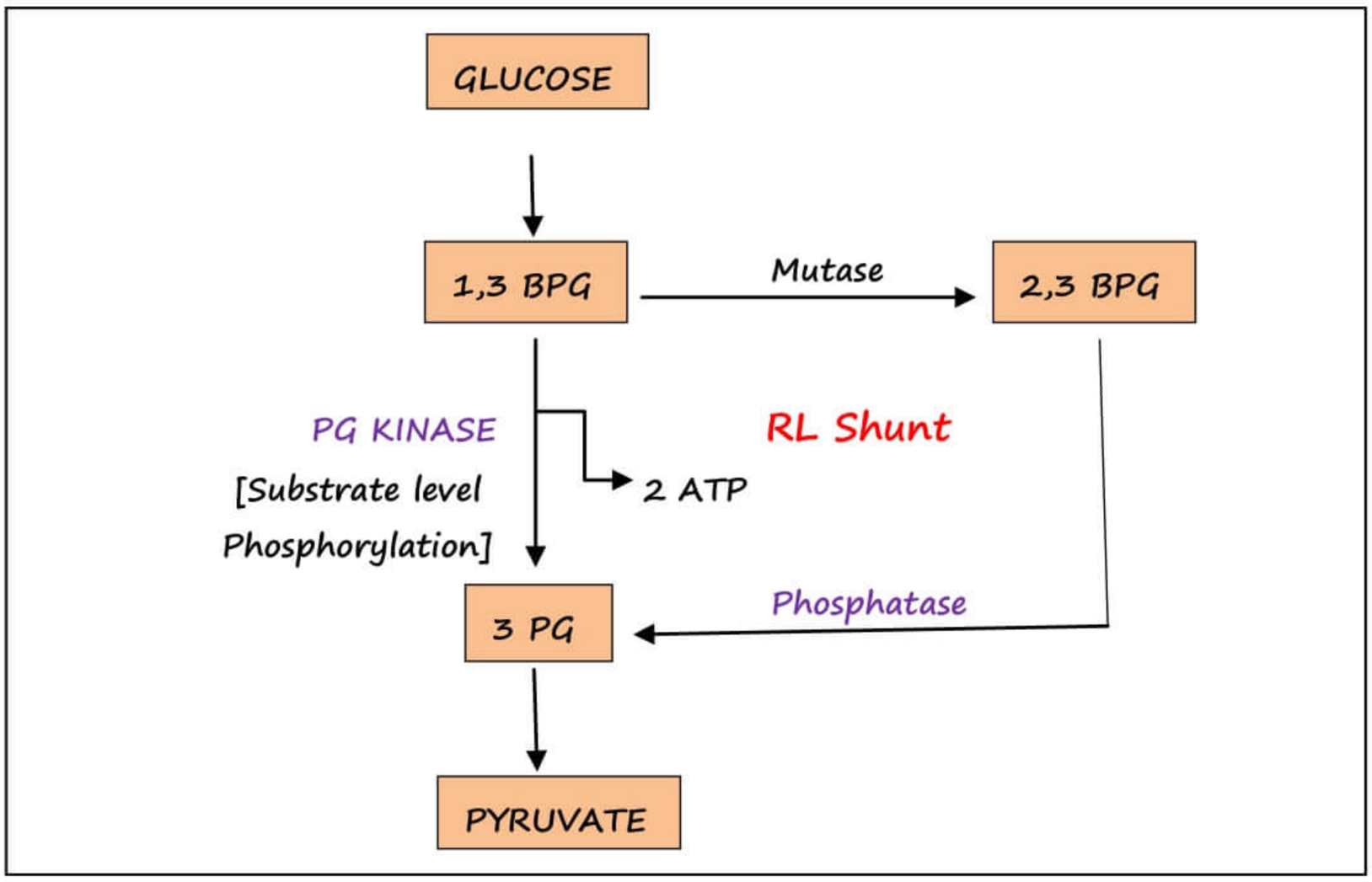
→ Most Glucogenic amino acid → Alanine (3c)

RL SHUNT / RAPAPORT LEUBERING SHUNT / CYCLE

→ In RL shunt, one Substrate Level Phosphorylation (in 1,3 BPG to 3 PG conversion) do not occur

→ Occurs only in RBC's

- **In RL Shunt, net ATP formed is zero**



FULL STORY OF RBC

GLUCOSE

90% Glucose	10% Glucose
Glycolysis	RL shunt
2 ATPs / Glucose	No ATP
	2, 3 BPG produced

→ 2, 3 BPG releases O_2 from Hb A ($\alpha_2 B_2$)

- 2,3 BPG binds to β- chain (will not affect any other Hb)

INHIBITORS

1. IODOACETATE → GLYCERALDEHYDE – 3 – P DEHYDROGENASE
2. ARSENITE → GLYCERALDEHYDE – 3 – P DEHYDROGENASE
3. Na FLOURIDE → ENOLASE

- Also used in Blood Glucose Estimation

4. OXAMATE → LACTATE DEHYDROGENASE

WITH ARSENITE INHIBITION

- Glycolysis continued & Pyruvate is formed
- No ATP formed

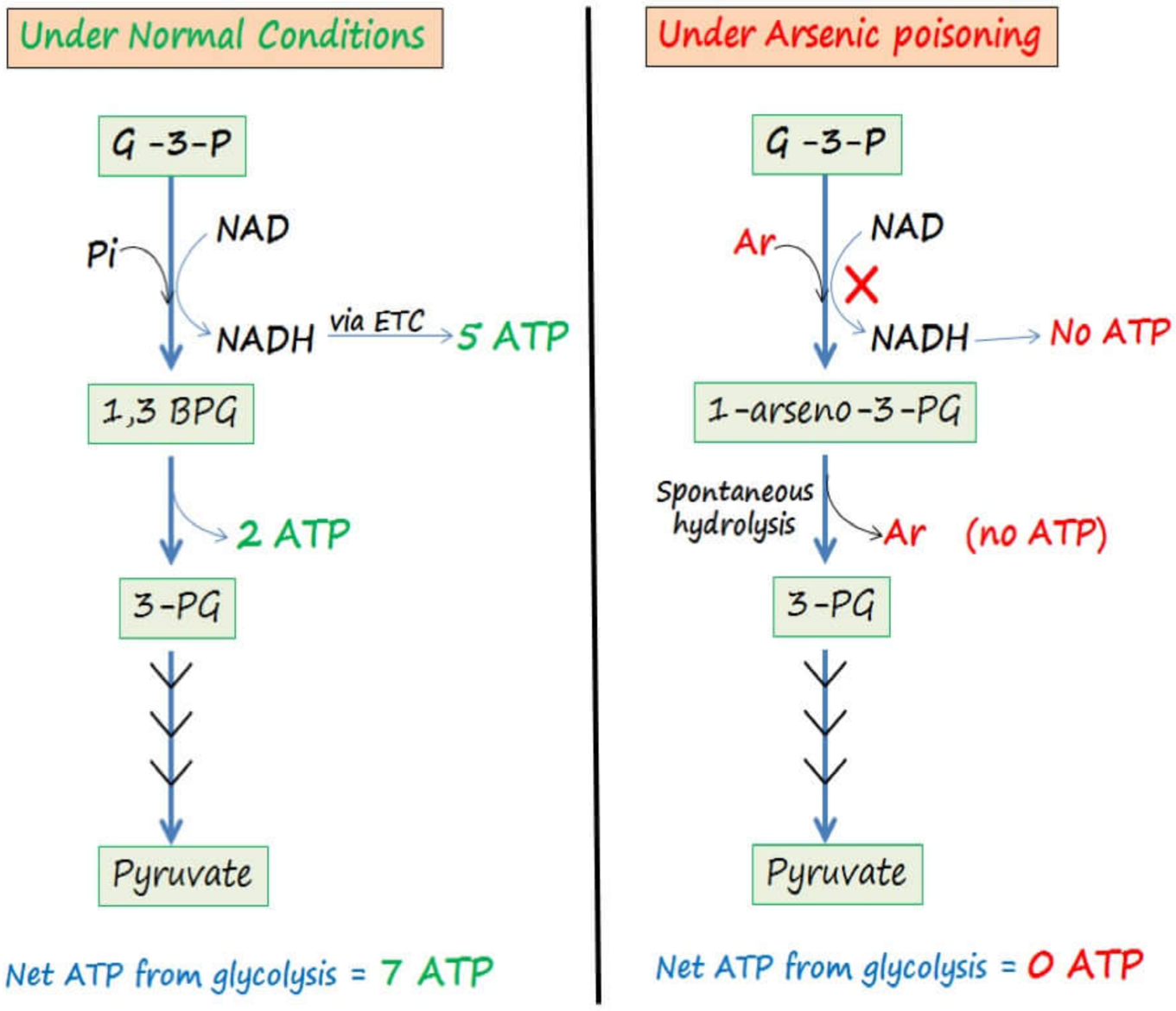
→ **Glycolysis occurs in cytoplasm**
→ **Link reaction, TCA & ETC occur in mitochondria**

ARSENIC POISONING

→ Acts as a poison by inhibiting ATP formation from glycolysis

→ Glycolysis pathway is not inhibited i.e. pyruvate is formed

→ but **no net ATP production** from glycolysis



Arsenic also inhibits enzymes:

- Pyruvate dehydrogenase of link reaction
- α-ketoglutarate dehydrogenase of TCA cycle

LINK REACTION

- ➔ Link b/w Glycolysis & TCA
- ➔ Activated by Insulin
- ➔ Occurs in Mitochondria
  - Pyruvate is a polar compound
  - Outer membrane channel present in outer mitochondrial membrane
  - Pyruvate enters the inner mitochondrial membrane through H^+ / Pyruvate Symporter

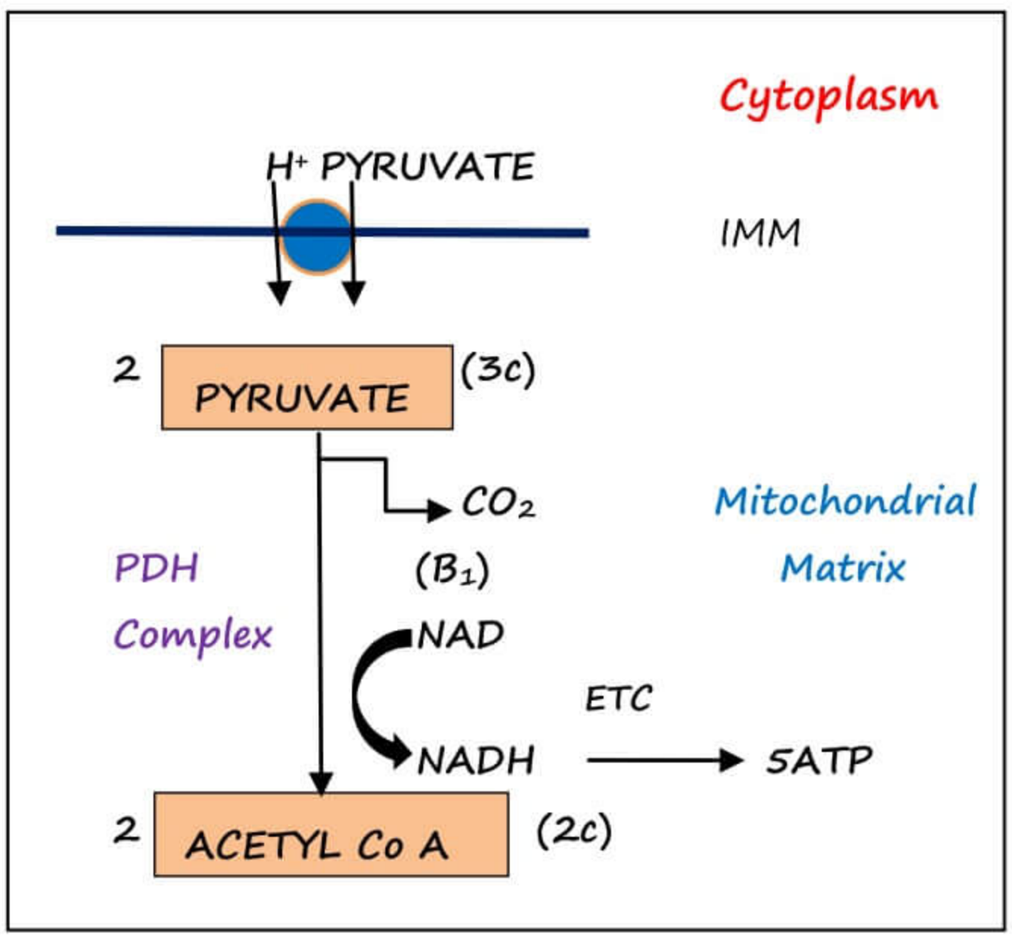
H^+ / Pyruvate Symporter

- Present in inner mitochondrial membrane
- Both H^+ & Pyruvate enters mitochondria through this

PYRUVATE DEHYDROGENASE COMPLEX [PDH COMPLEX]

- ➔ A multi enzyme complex a/w inner mitochondrial membrane
- ➔ Reaction occurs in matrix
- ➔ Consists of 3 enzymes
  - E_1 - Pyruvate dehydrogenase
  - E_2 - Dihydro lipoyl transacetylase
  - E_3 - Dihydro lipoyl dehydrogenase

5 coenzyme are required for link reaction & TCA: Lipoic acid, B_1, B_2, B_3, B_5



Compounds crossing IMM

- → Pyruvate
- → Malate
- → Aspartate

Compounds can't cross IMM

- → NADH
- → Oxaloacetate

LINK REACTION IS IRREVERSIBLE

- Carbohydrates can be converted into fats
- Fats cannot be converted to carbohydrates

EXCEPTIONS

1. Glycerol (comes from TG)
2. Propionic Acid (comes from odd chain FA)

→ Acetyl Co A is never Glucogenic

FATE OF PYRUVATE

→ PDH Complex Deficiency
Beri Beri (B1 Deficiency)
} **LACTIC ACIDOSIS**

REGULATION OF PDH

End product Inhibition

1. Acetyl Co A (in Fasting)
2. NADH

Covalent modification

1. Active in Dephosphorylated state
2. Done by Insulin

FATES OF ACETYL Co A

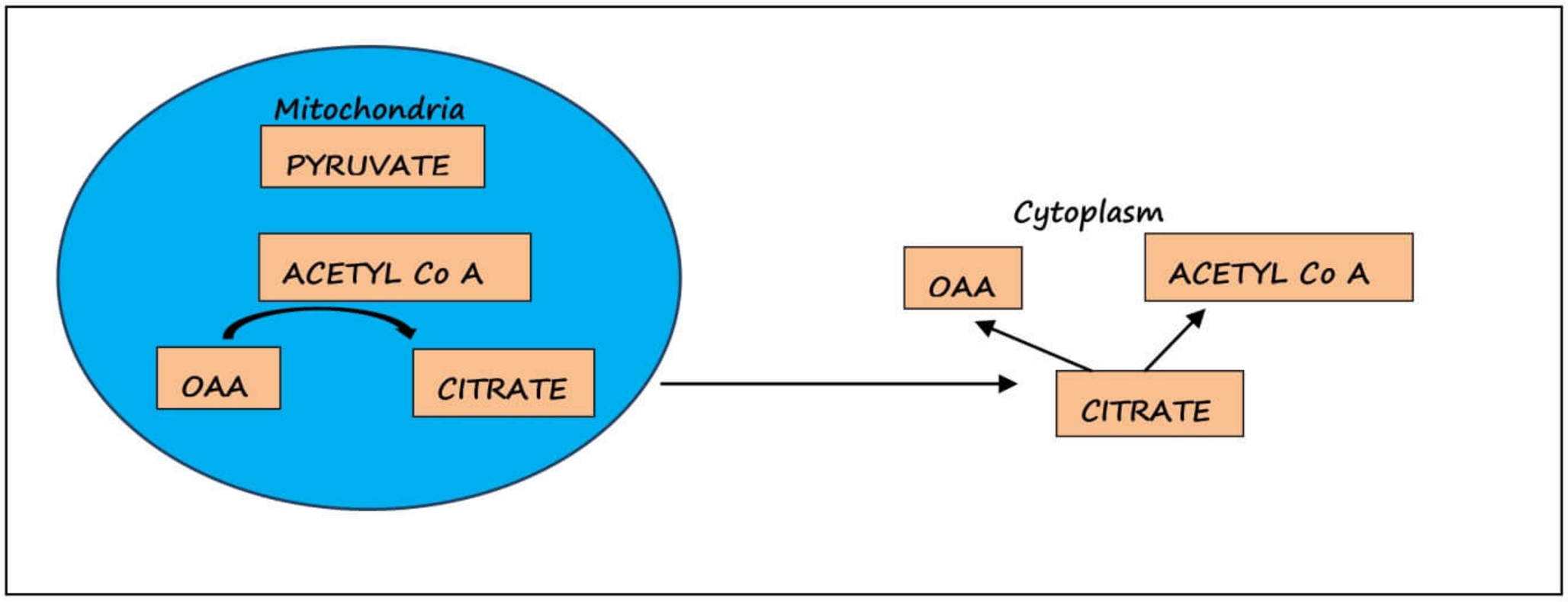
In Mitochondria

1. TCA
2. Ketone Body Synthesis
3. Activation of Gluconeogenesis

In Cytoplasm

1. Fatty Acid Synthesis
2. Cholesterol Synthesis

ENTRY OF ACETYL CoA IN CYTOPLASM IS BY CITRATE SHUTTLE



TCA CYCLE

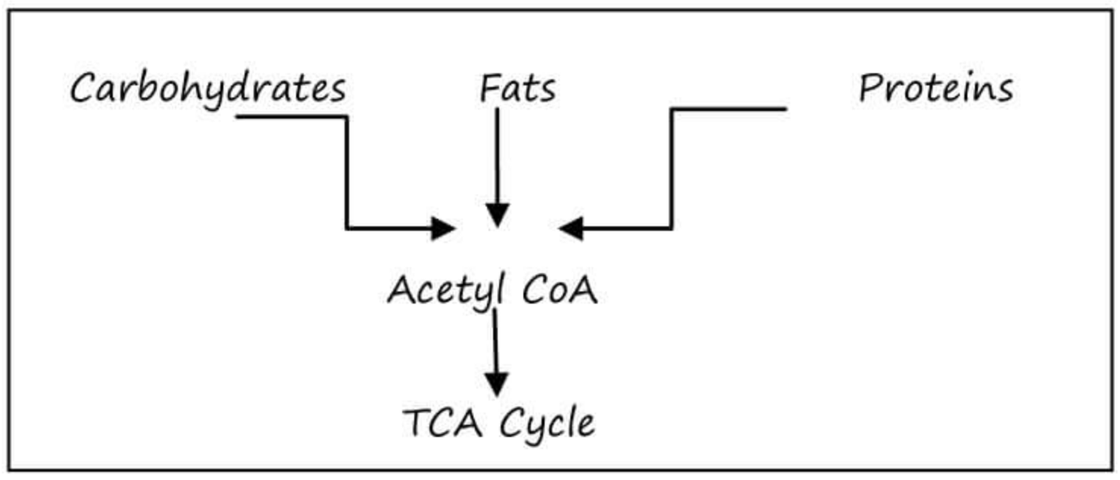
TCA CYCLE (Tri Carboxylic Acid Cycle)

COOH
COOH → citrate
COOH → Isocitrate

Kreb cycle	→	TCA CYCLE
Kreb Henseleit cycle	→	Urea cycle
Citric Acid cycle (COOH)	→	First compound formed is citrate (COO^-)

AMPHIBOLIC CYCLE [Anabolic & Catabolic]

➔ CATABOLIC ROLE



→ **ANABOLIC ROLE**

- TCA intermediates used for synthesis of Compounds
- Eg : Succinyl Co A → Haem

VITAL CYCLE → No enzyme deficiency is present

ANAPLEROTIC REACTIONS → Which replenish TCA Intermediates

PYRUVATE (3c) —(+ CO_2, Pyruvate Carboxylase)→ OXALOACETATE [4c]

Q **TCA activated by**

A Insulin

B Glucagon

C Both

D None

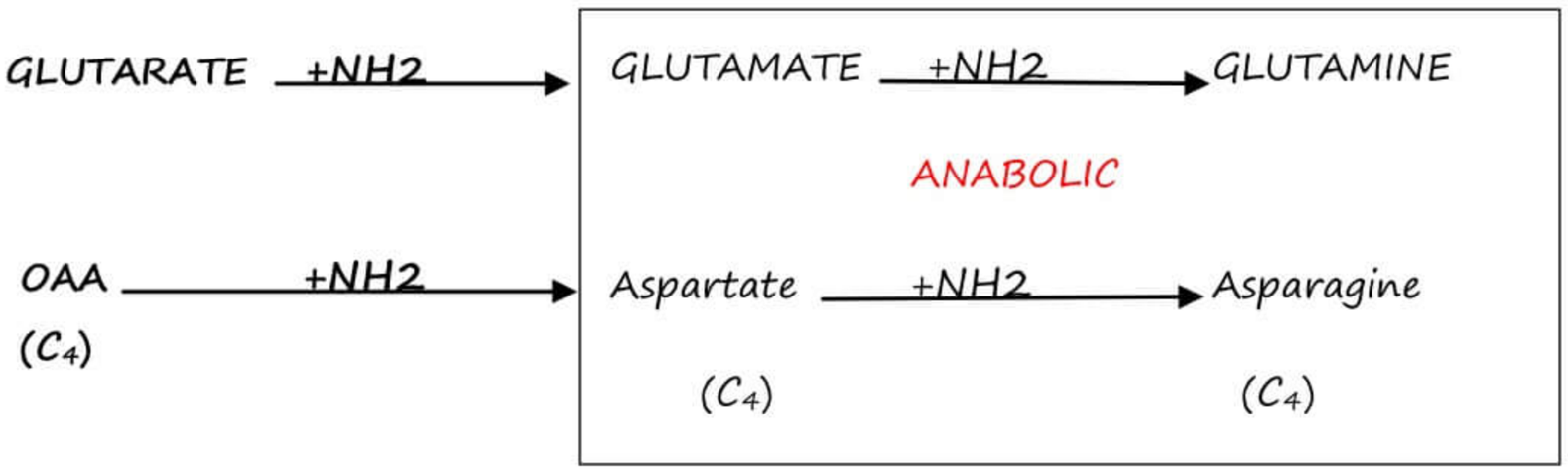
Ans d

* **TCA do not have hormonal control.**

TCA CYCLE IS CONTROLLED BY

1. Energy Status of the cell
2. Availability of Oxaloacetate [carrier / 1st substrate of TCA Cycle & also has catalytic role in TCA Cycle (recycled)]

TCA is Cycle, not a pathway



→ SUCCINATE / SUCCINYL Co A [4C]

→ MALONATE (3c) → Inhibitor of TCA Cycle

MALATE (4c) → Intermediate of TCA Cycle

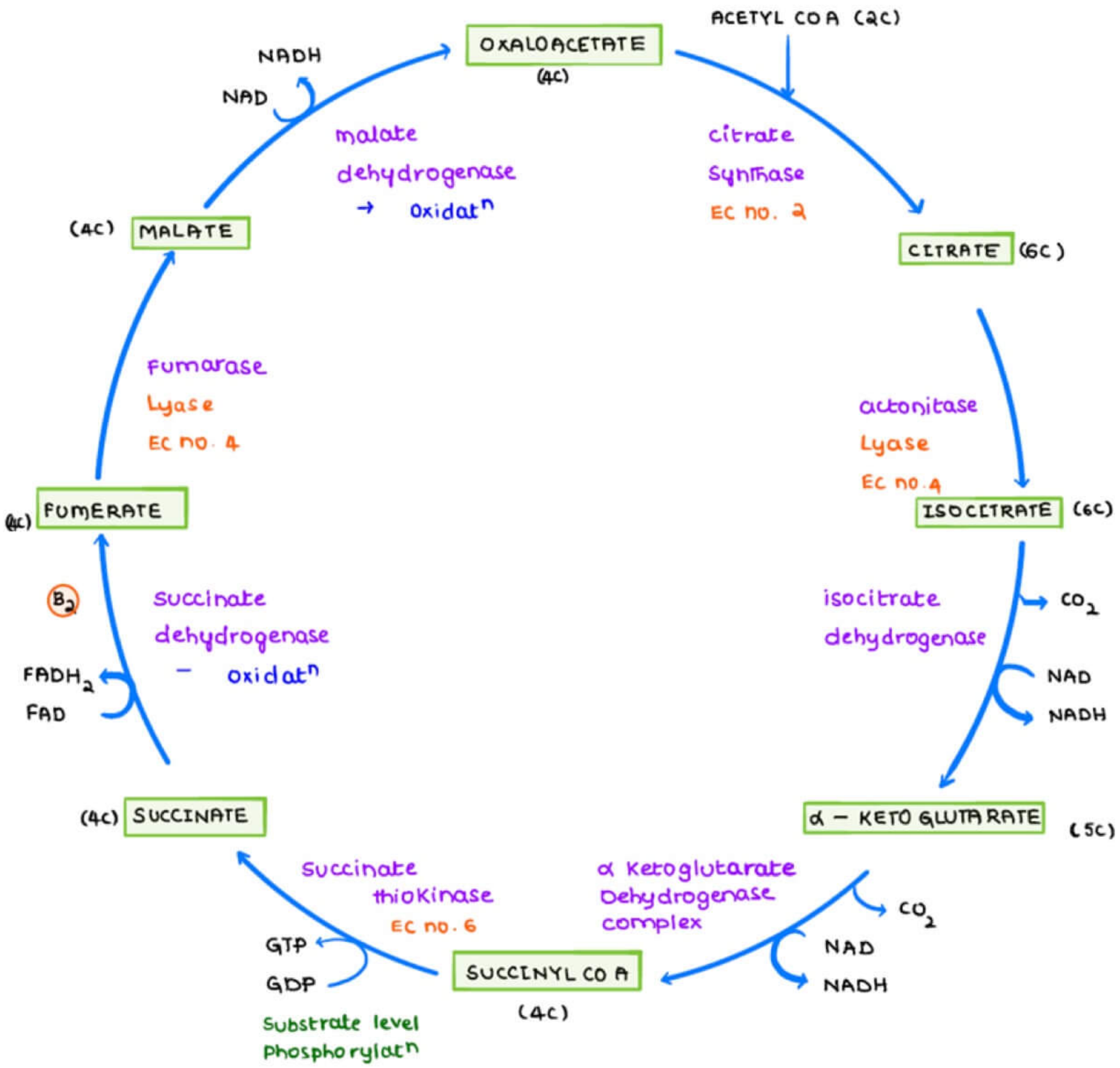
MALONATE / MALONYL Co A [3c]

Inhibitor of

1. TCA Cycle [Succinate Dehydrogenase]
2. ETC [Complex II]
3. Beta Oxidation of Fatty Acids [CPT I] [CPT → Carnitine Palmitoyl Transferase 1]

All enzymes are lying in mitochondrial matrix

EXCEPT Succinate Dehydrogenase → lies in inner mitochondrial membrane



→ Thiokinase produces → ATP

Thiokinase (in Liver, Kidney, and starvation) produces → GTP

→ Acetyl Co A is not the intermediate of TCA Cycle; other substances are intermediates of TCA Cycle

ENERGETICS OF TCA CYCLE

1. ACETYL Co A

– 3 NADH	→	7.5
1 $FADH_2$	→	1.5
1 ATP	→	1
		10 ATP

ENERGETICS OF COMPLETE BREAKDOWN OF GLUCOSE

1 GLUCOSE
↓ → 7 ATP
2 Pyruvic Acid
↓ → 5 ATP
2 Acetyl Co A
↓
TCA → 20 ATP

32 ATP/Glucose complete oxidation

ALPHA – KETO – GLUTARATE DEHYDROGENASE COMPLEX	PYRUVATE DEHYDROGENASE COMPLEX [PDC]
→ A multi enzyme complex	→ A multi enzyme complex
→ Requires 5 co – enzymes	→ Requires 5 co – enzymes
– Lipoic acid	– Lipoic acid
– TPP	– TPP
– FAD	– FAD
– NAD	– NAD
– Co – enzyme A	– Co – enzymes A
→ Not regulated by Phosphorylation & Dephosphorylation	→ Regulated by Phosphorylation & Dephosphorylation

TCA CYCLE OCCURS IN AEROBIC CONDITIONS & TCA CAN'T OCCUR IN ANAEROBIC CONDITIONS

In Anaerobic condition

- If TCA occurs, NADH & $FADH_2$ are produced from NAD, FAD
- But ETC can't operate as there is no O_2
- NADH & $FADH_2$ accumulated
- NAD & FAD depleted
- TCA Cycle stops

Rate Limiting Enzymes	Irreversible
1. Citrate synthase	– Citrate Synthase (A)
2. Alpha Keto Glutarate Dehydrogenase	– α KG – DH
3. Iso citrate Dehydrogenase	

DEHYDROGENESES

Oxidation	α KG Dehydrogenase		NADH
Decarboxylation	Iso citrate Dehydrogenase		
	Succinate Dehydrogenase	→	FADH2
Oxidation	Malate Dehydrogenase	→	NADH

KINASE → Thiokinase

PASTEUR'S WARBURG AND CRABTREE EFFECT

BASICS

➔ Aerobic glycolysis → 32 ATP produced by 1 glucose

➔ Anaerobic glycolysis → 2 ATP produced by 1 glucose

16 glucose to be used → 32 ATP

- Anaerobic glycolysis is wastage of Glucose

PASTEUR'S EFFECT

→ Occurs in normal cell

→ In the presence of O_2, Anaerobic glycolysis is inhibited

WARBURG'S EFFECT

→ Occurs in cancer cells

→ Paradox from normal

→ Even in the presence of O_2, glucose is converted to lactose

- **'KNOWN AS' AEROBIC GLYCOLYSIS with NO oxidative phosphorylation**
- 2 ATPs are formed
  - Uses large amount of glucose to meet energy requirement.
  - Responsible for Cachexia
  - Lactate is the dead end of Glycolysis

CRABTREE EFFECT

→ When O_2 supply is kept constant & glucose concentration is increased, then the O_2 consumption by cell falls

↑ Glycolysis

↓

Glycer aldehyde 3 – phosphatase activity increases, has high affinity for pi

↓

Competes with ETC for pi (inorganic phosphate)

↓

ETC stops

↓

O_2 consumption by cells falls

PASTEUR EFFECT	CRABREE EFFECT
→ In aerobic conditions, glucose consumption decreases Or → In the presence of O_2, anaerobic glucolysis is inhibited	→ If glucose increases, then O_2 consumption falls Or → When O_2 supply is kept constant & glucose concentration is increased, then the O_2 consumption by cell falls

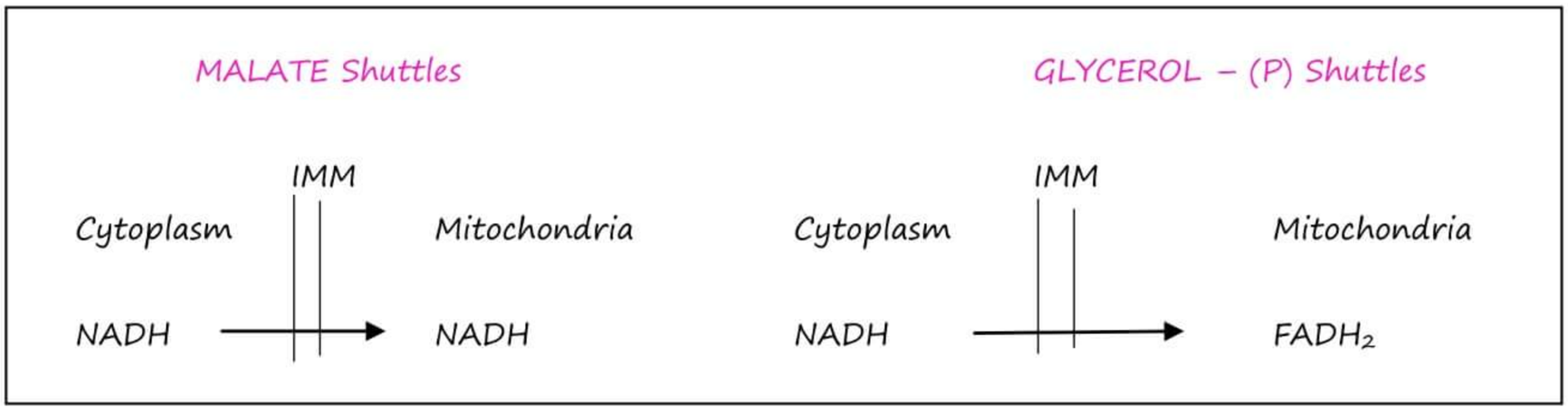
Warburg & Crabtree effect share common properties, but they are different

SHUTTLES

➔ NADH, Oxaloacetate } cannot cross IMM

Pyruvate, Malate, Aspartate } can cross IMM

SHUTTLES



Q Shuttle is required for

A **Glycolysis**

B Link Reaction

C TCA

D All

Ans a

Q If Aerobic Glycolysis uses Glycerol (P) shuttle, how many ATPs produced

A **5 ATPs**

MALATE SHUTTLE

[Liver & Heart]

Malate Dehydrogenase

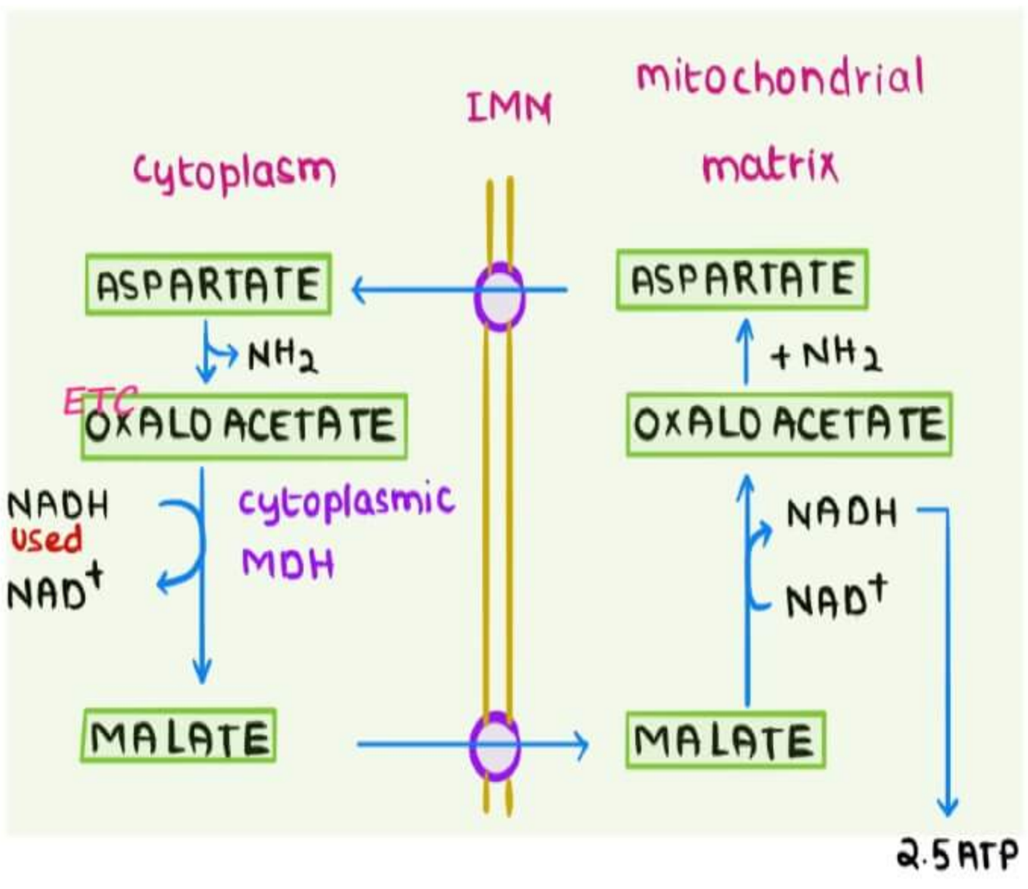
Aka Malate Aspartate Shuttle

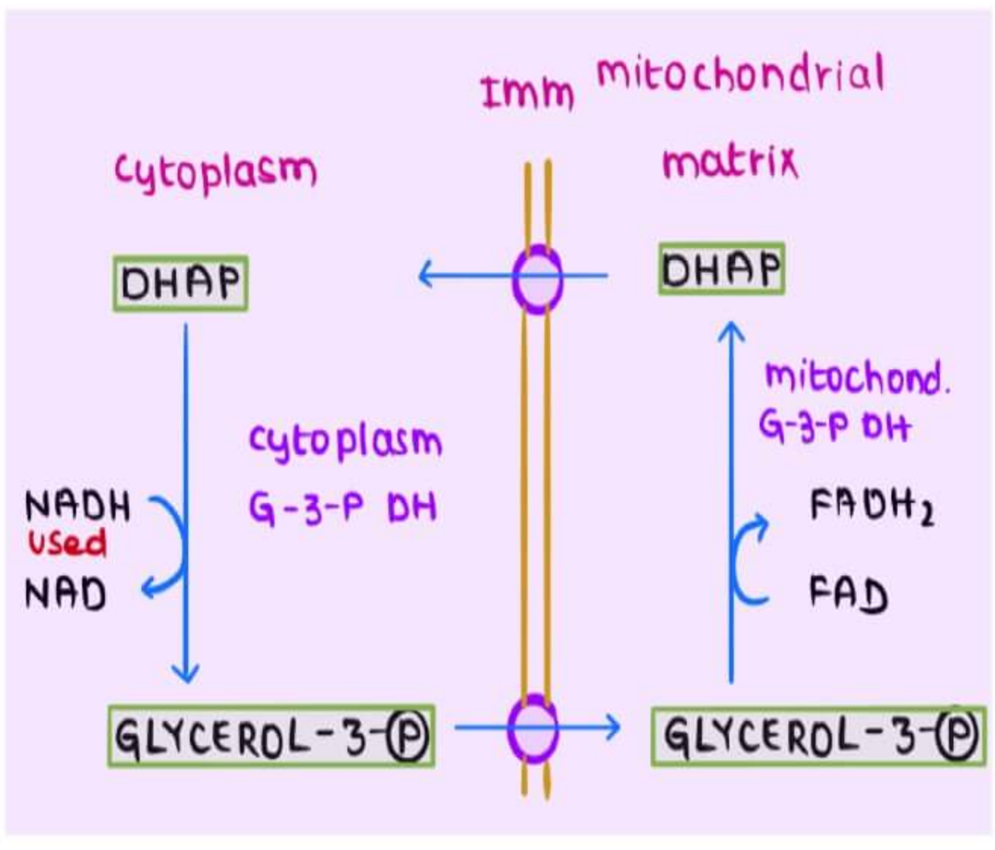
GLYCEROL – P SHUTTLE

[Skeletal Muscle & Brain]

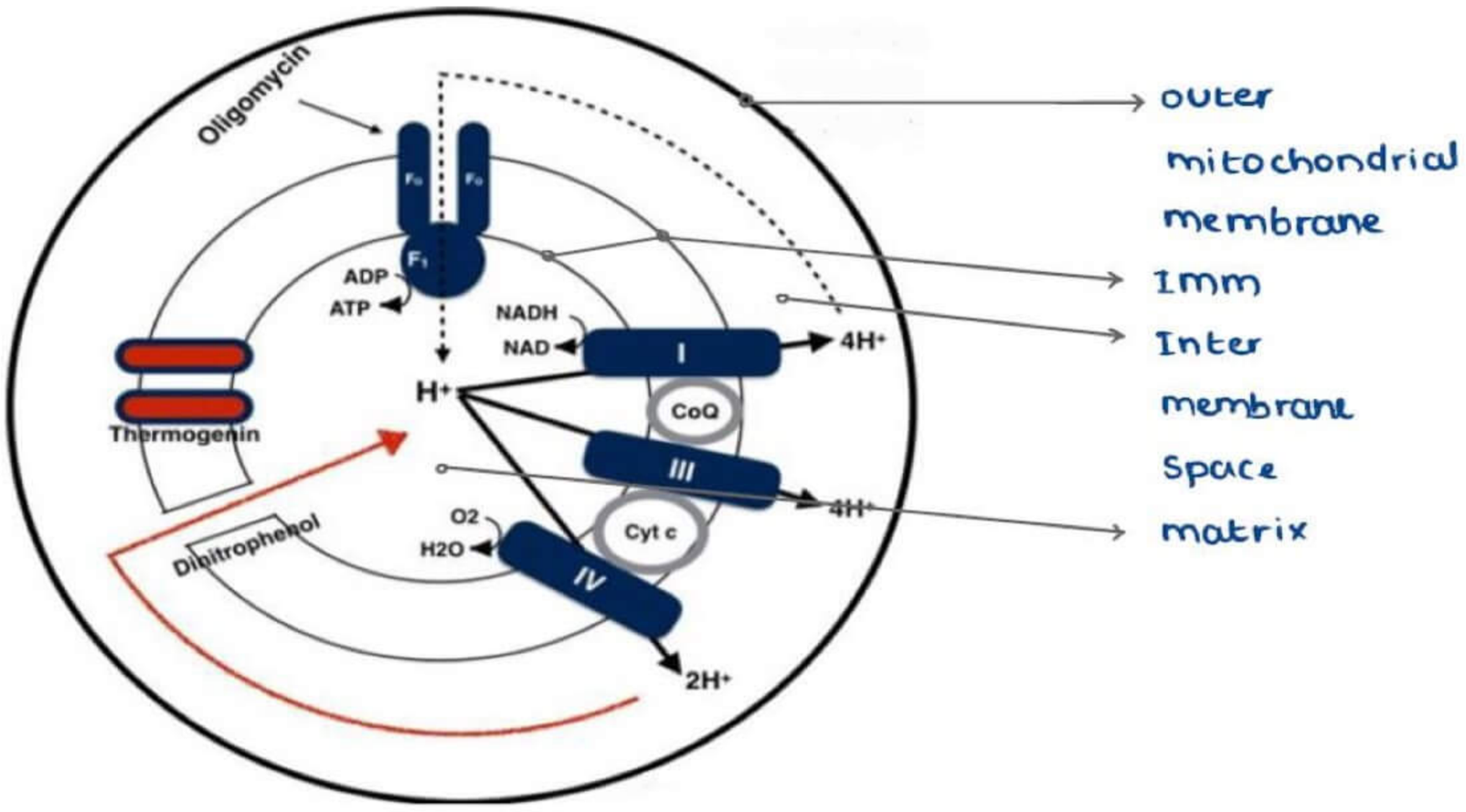
Glycerol – 3 – P Dehydrogenase

Less ATP but quick source of ATP produced





ETC (Electron Transport Chain)



1. NADH ⟶ NAD + H^+/e^-
2. **ELECTRON FLOW SEQUENCE**
    1. NADH
    2. COMPLES I
    3. CoQ
    4. COMPLEX III
    5. Cyt C
    6. COMPLE IV

3. Energy from the flow of e^-, used to throw e^- from matrix to intermembrane space by complexes

No. of protons thrown by different complexes

→ Complex I → 4

→ Complex III → 4

→ Complex IV → 2

10 H^+ / 1 NADH

→ The excess H^+ in intermembrane space creates osmotic gradient

- CHEMI OSMOTIC EFFECT

→ COMPLEX V
- Has 2 Portions
  1. **Fo**
     - Rolling gate
     - Proton Ion channel present on IMM
     - Attached to F_1
  2. F_1
     → Protruding towards the mitochondrial matrix
     → Has ATP Synthase activity
     → Can convert ADP → ATP

→ Excess H^+ from intermembrane space, enters the matrix via complex V
- When they cross Fo (rolling gate), mechanical energy is created
- This mechanical energy is transferred to F1 subunit and which in turn converts ADP ⟶ ATP

NAMES OF COMPLEXS

Complex I	→	NADH Co Q Reductase
Complex II	→	Succinate Co Q Reductase
Complex III	→	Cytochrome C Reductase
Complex IV	→	Cytochrome C Oxidase (Prosthetic group - Cu - Can't be separated)

COMPONENTS

1. PROTEIN COMPLEXES: Complex I to IV
2. MOBILE e - CARRIERS

- Co Q | Ubiquinone (only non-protein member)
- Cytochrome c→ Peripheral membrane protein

REDOX POTENTIAL

→ Every Successive Substance have ↑ affinity for e^-
→ NADH → Least redox potential
O_2 → Highest redox potential

NADH

1 ATP	←	complex I	→	4 H^+
1 ATP	←	complex III	→	4 H^+
0.5 ATP	←	complex IV	→	2 H^+

10 H^+ = 2.5 ATP

$FADH_2$

Complex II	→	'0' ATP
Complex III	→	1 ATP
Complex IV	→	0.5 ATP

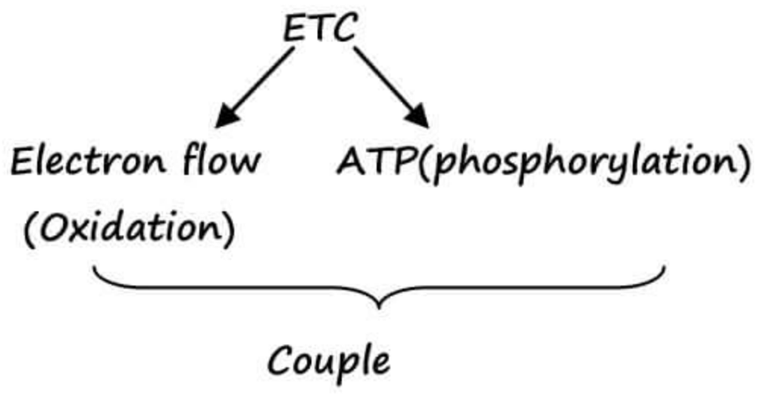
1.5 ATP

Name of complex II → Succinate Co Q Reductase

→ Do not give any H^+

→ Do not form ATP

COUPLING → Always together



UNCOUPLING

→ Oxidation occurs

→ Phosphorylation do not occur

➔ UNCOUPLERS → Substances which creates a hole in the IMM. Ex:

1. DRUGS

   Dinitrophenol

2. NATURAL / PHYSIOLOGICAL UNCOUPLERS

1. **THERMOGENIN**
   - Protein present in brown fat
   - Kind of proton Ion channel in IMM
   - Responsible for non-shivering thermogenesis
2. **THYROXINE**

OLIGOMYCIN

- ➔ Inhibitor of complex V
- ➔ Not an uncoupler
- ➔ It ligates F_o gate and closes it
- ➔ Inhibits both oxidation & Phosphorylation

ADP – ATP TRANSLOCASE

- ➔ Present in IMM
- ➔ Has 2 surfaces → Bigger & smaller
- ➔ Transfer the substances by FLIP- FLOP MECHANISM
  - Takes ADP IN
    Throws ATP OUT (for Anaerobic Reactions)
- ➔ ATRACTILOSIDE → Inhibitor of ADP – ATP TRANSLOCASE

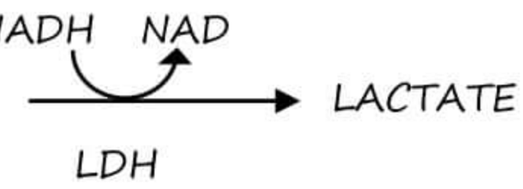
ADP to ATP conversion inhibited by → OLIGOMYCIN
ADP to ATP transfer inhibited by → ATRACTYLOSIDE

INHIBITORS of COMPLEX I to IV

I → Rotenone, Phenobarbitone
II → Malonate (3c)
III → Phenformin (Oral Hypoglycemic)
IV → CO, CN, H_2S

PHENFORMIN

- ➔ Inhibits ETC
  - ↑↑ NADH



→ Causes HYPERURICEMIA
- Lactate competes with uric acid for excretion from Kidney

EXCESS ALCOHOL → ↑↑ NADH → HYPERURICEMIA

GLUCONEOGENESIS

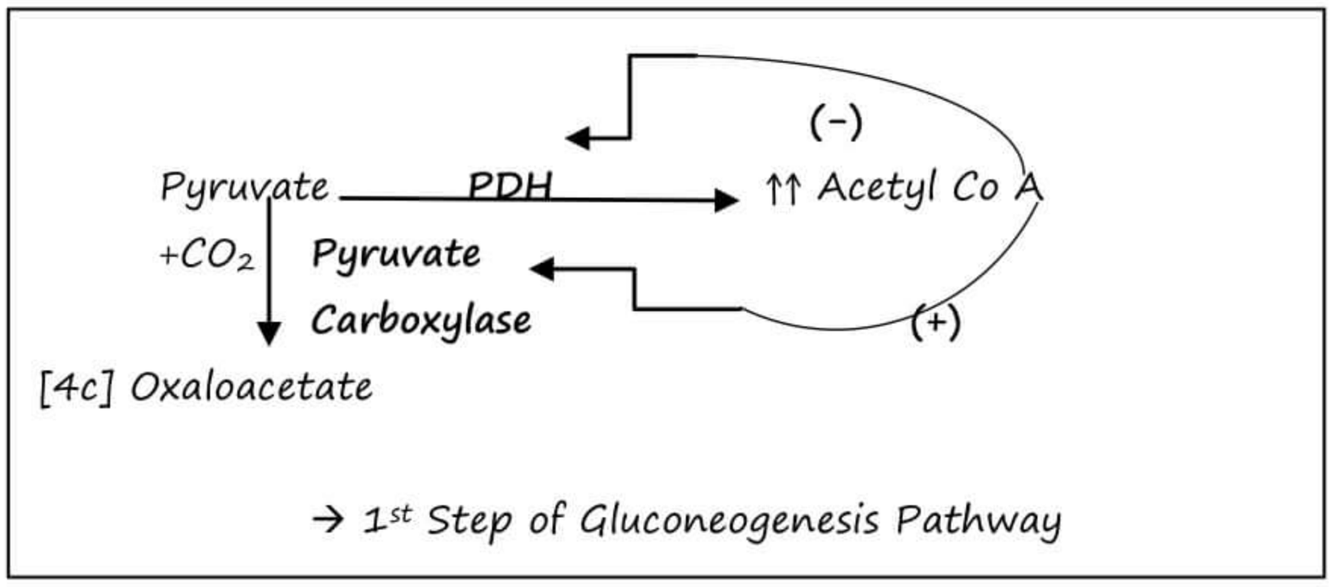
IN FED STATE,

Insulin activates PDH complex

Pyruvate → Acetyl CoA → Fats

IN FASTING

ß Oxidation of FA ⟶ Acetyl Co A



→ 1st Step of Gluconeogenesis Pathway

→ Occurs in both mitochondria & cytoplasm

→ Any pathway occurring both in mitochondria & cytoplasm, will first start from mitochondria

→ Occurs In Liver & Kidney

→ As it occurs in Fasting state, Enzyme will be active in Phosphorylated state,

- All enzymes activated by GLUCAGON
  Inhibited by INSULIN

RULE of 2 for GLUCONEOGENESIS

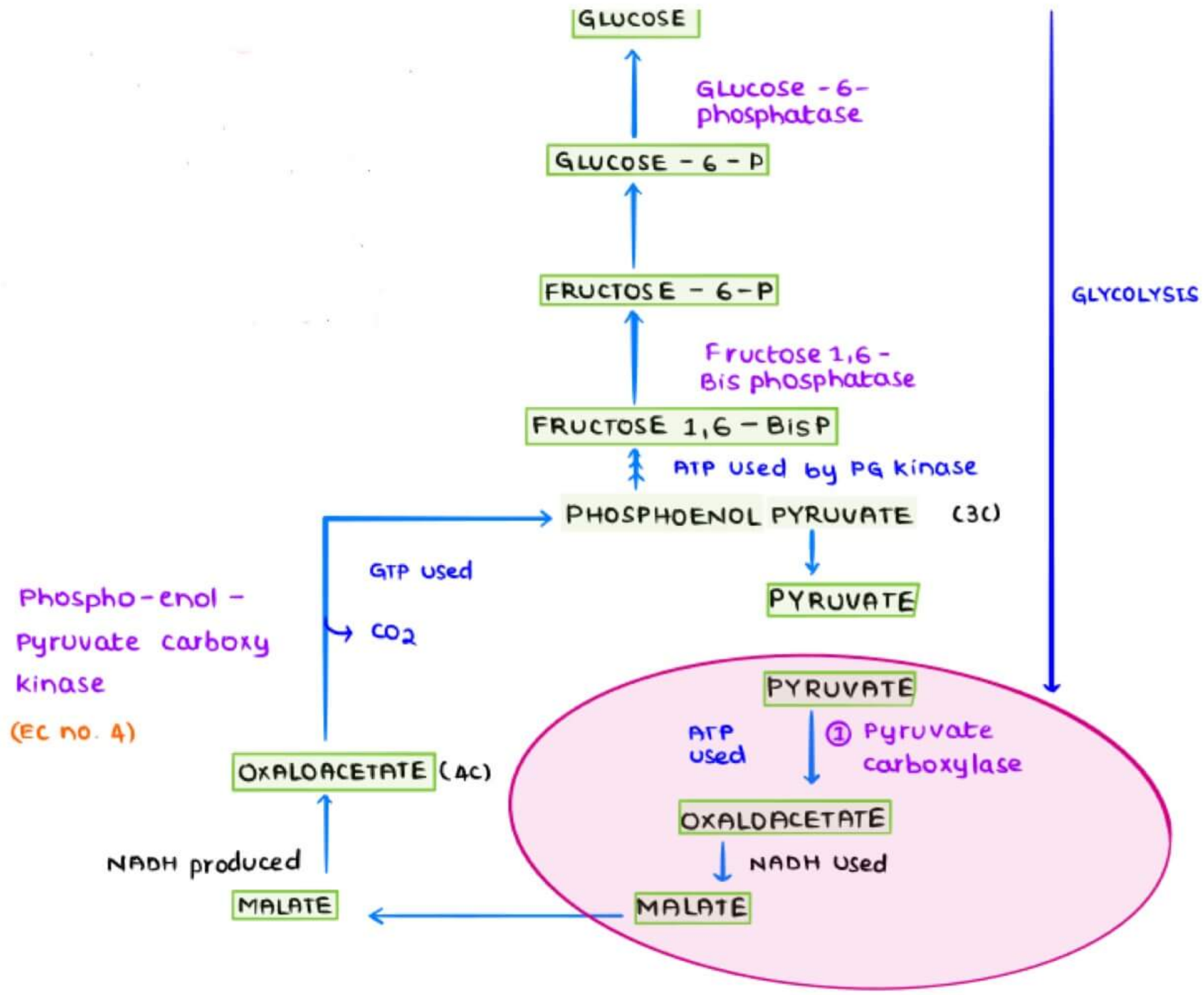
→ Occurs in 2 compartments

→ Occurs in 2 organs

→ Occurs in 2 situations

1. Fasting / starvation
2. Diabetes

GLYCOLYSIS	GLUCONEOGENESIS
Pyruvate Kinase	Pyruvate Carboxylase PEPCK (Phosphoenol Pyruvate Carboxy Kinase)
PFK - 1	Fructose 1, 6 Bisphosphatase
Hexo Kinase	Glucose – 6 - Phosphatase



SEQUENCE OF COMPARTMENTS WHERE GLUCONEOGENESIS REACTIONS OCCURS

1st STEP	→	Mitochondria
NEXT MANY STEPS	→	Cytoplasm
LAST STEP	→	Endoplasmic reticulum

LAST STEP

→ GLUCOSE – 6 – PHOSPHATE **Glucose – 6 – Phosphatase** GLUCOSE

→ Occurs in Endoplasmic Reticulum

TRANSPORTERS / CHANNELS

T1 → For the entry of Glucose – 6 – P in ER

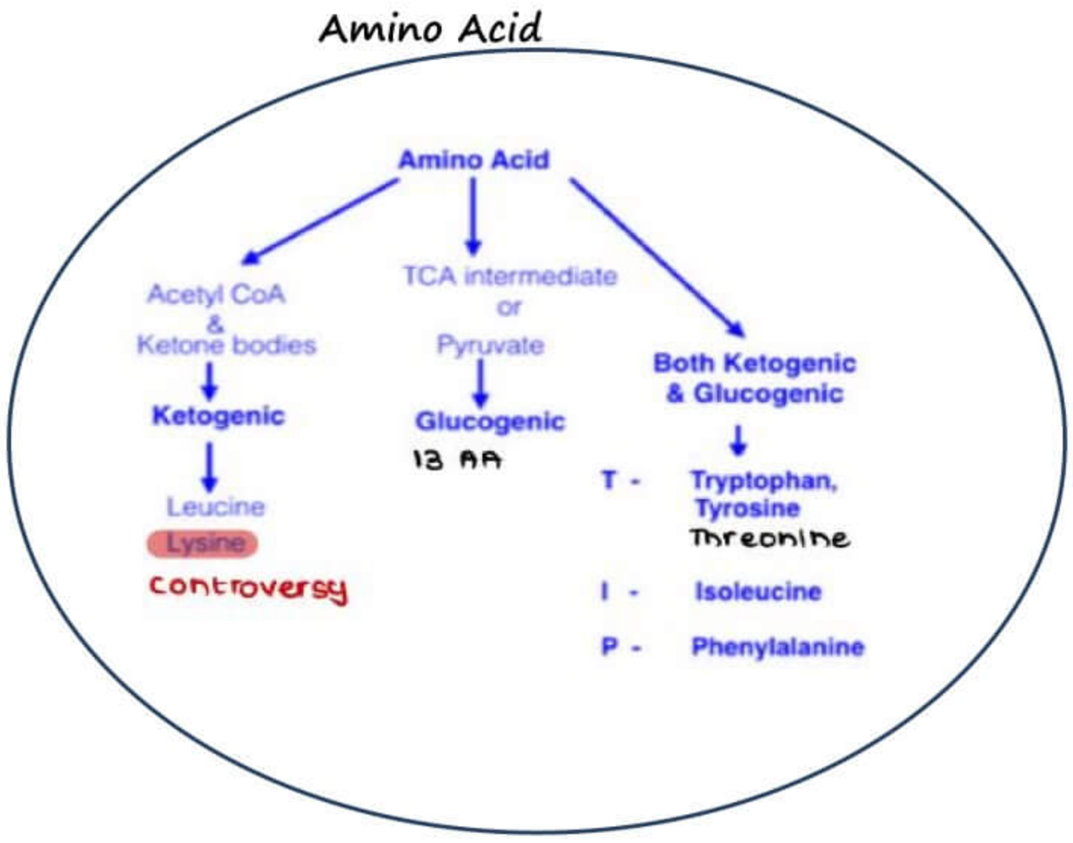
T2 → For the exit of Glucose from ER

T3 → For the exit of Pi

All these transporters are active during Fasting state only.

SUBSTRATES (mostly 3c)

1. Pyruvate (3c)
2. Lactate (3c)
3. Glycerol (3c)
4. Propionic acid (3c)
5. Glucogenic amino acids
6. Both Ketogenic & Glucogenic amino acids
7. Any TCA Intermediate



Ketogenic AA → 2 + 5 = 7

Glucogenic AA → 13 + 5 = 18

ENERGETICS

Pyruvate carboxylase	→	ATP	x 2	→	4 ATP	Used
PEPCK	→	GTP			2 GTP	
PG Kinase	→	ATP				

→ 6 High energy phosphates used to make Glucose from 2 molecules of Pyruvate

→ 6 ATPs used to make Glucose form 2 molecules of Lactate
→ 6 ATPs used to make Glucose form 2 molecules of Alanine

GLYCOGEN

→ Occurs in Liver & Muscle
- By weight glycogen is more in → Liver
  By % glycogen is more in → Muscle

→ GLYCOGENESIS → Synthesis → Occurs in cytoplasm
→ GLYCOGENOLYSIS → Breakdown → Occurs in cytoplasm
- **Both Rate Limiting enzymes belong to TRANSFERASES (EC No.2)**

`

→ STORED IN			END PRODUCT
LIVER	→	Used to Maintain Blood Glucose	→ Glucose
MUSCLE	→	Used for Muscle contraction	→ Glucose – 6 – (P)

Q If Muscle glycogen used for anaerobic glycolysis, then how many ATPs obtained
A 3 ATPs

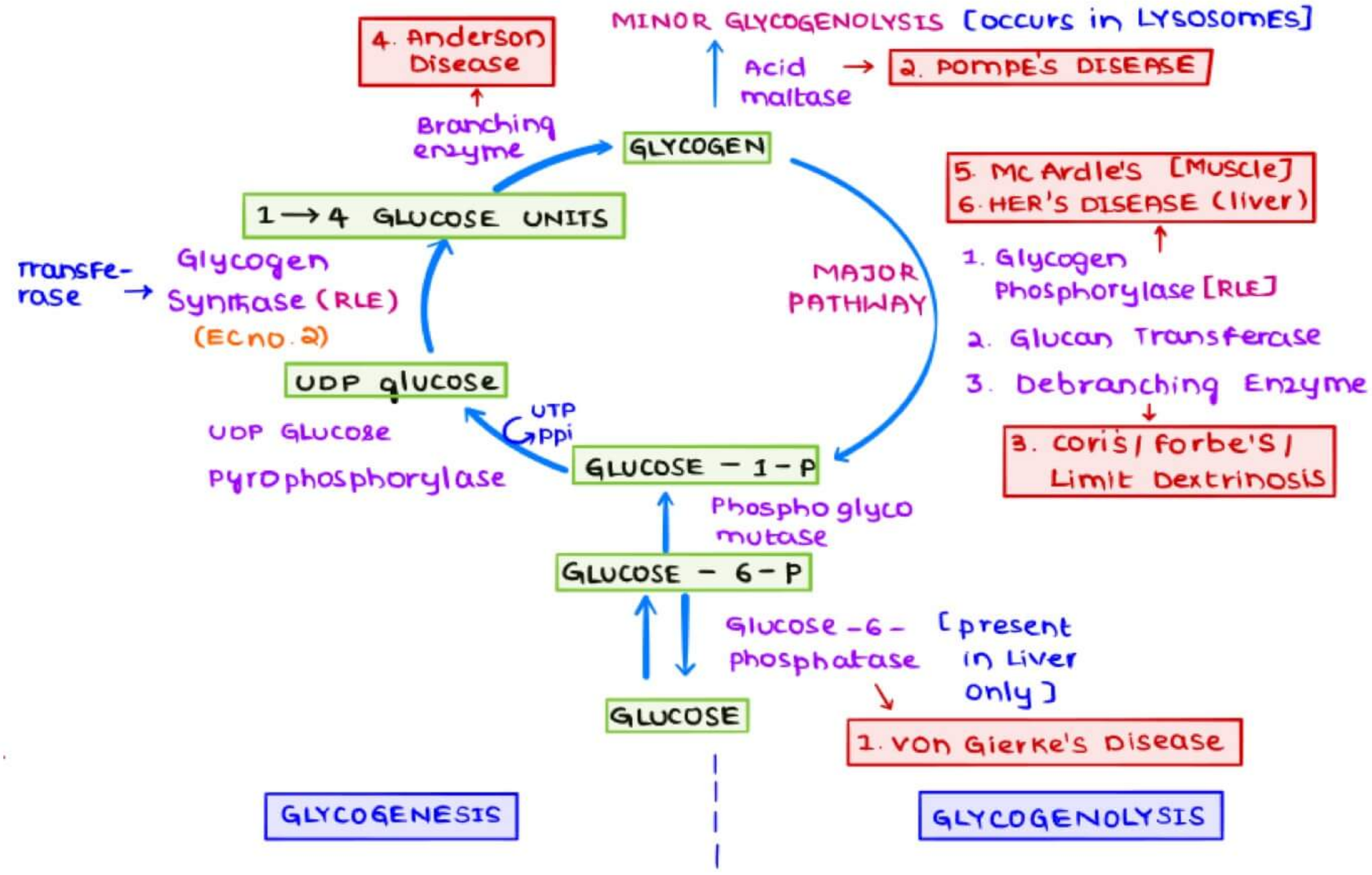
ANAEROBIC
IN GLYCOLYSIS, ATP consumed at
1. Hexokinase
2. PFK – 1

- Net ATPs → 4 – 2 = 2

In Muscle Glycogen Metabolism,
- Glucose–6–(P) is the end product which undergoes glycolysis. So, only 1 ATP consumed at PFK – 1 Reaction
- Net ATPs → 4 – 1 = 3

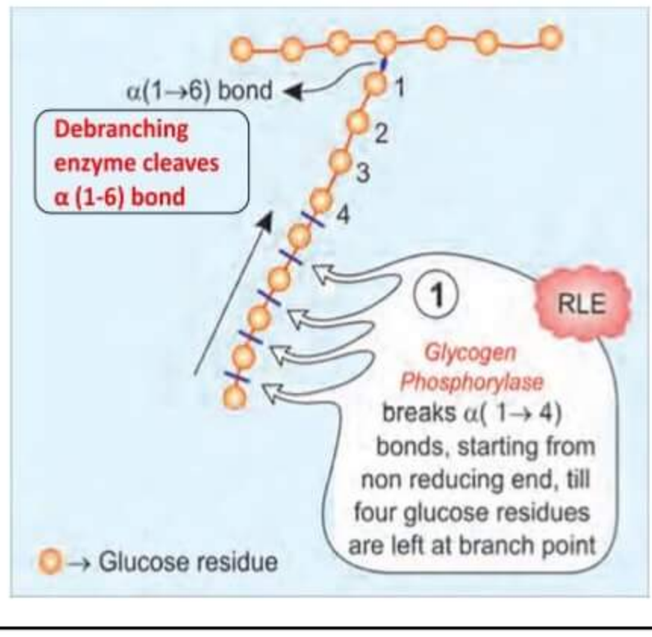
→ PRIMER OF GLYCOGEN SYNTHESIS → GLYCOGENIN [protein]
→ UDP – GLUCOSE → Activated glucose
→ GLYCOGEN SYNTHASE transfers glucose from UDP – Glucose to Glycogenin
- Acts as Transferase (EC no.2)

POMPE'S DISEASE
→ dlt Deficiency of Acid Maltase
→ Only Glycogen Storage disease which is a Lysosomal Storage Disease



MAJOR PATHWAY OF GLYCOGENOLYSIS

1. **GLYCOGEN PHOSPHORYLASE**
   - → Transfers pi, uses PLP (B6)
   - → Breaks α (1 → 4) bonds from one end
   - → It breaks the bonds until 4 α (1 → 4) bonds are left at branch point
   - → End product is → Glucose – 1 – P (90%)



2. **GLUCAN TRANSFERASE**
   - → Transfers 3 residues to neighbouring straight chain

3. **DEBRANCHING ENZYMES**
   - → Breaks α (1 → 6) bond
   - → End product → Glucose (10%)

→ Glucagon Transferase & Debranching enzymes has Bifunctional Activity
(Same protein with 2 enzymatic activity)
→ Common step for both Glycolysis & Gluconeogenesis → Hexokinase / Glucokinase step

GLYCOGEN STORAGE DISEASES

MAIN FEATURES

LIVER GLYCOGEN STORAGE DISORDERS → Hypoglycemia

MUSCLE GLYCOGEN STORAGE DISORDERS → Muscle Cramps & Exercise Intolerance

TYPE	DISEASES	ENZYME DEFICIENT	AFFECTED ORGAN
I	VON GIERKE'S [mc]	GLUCOSE – 6 – (p)ase	Liver
II	POMPE'S [Lysosomal disease]	Acid maltase	Liver, Muscle, Brain
III	CORI'S / LIMIT DEXTRINOSIS	De branching	Liver, muscle, Brain
IV	ANDERSON / AMYLOPECTINOSIS	Branching	Liver, Muscle, Brain
V	Mc ARDLE'S	Muscle Phosphorylase	Muscle
VI	HER'S	Liver Phosphorylase	Liver

→ Patients of Mc Ardle's Disease **don't** have increased Lactate levels after exercise.

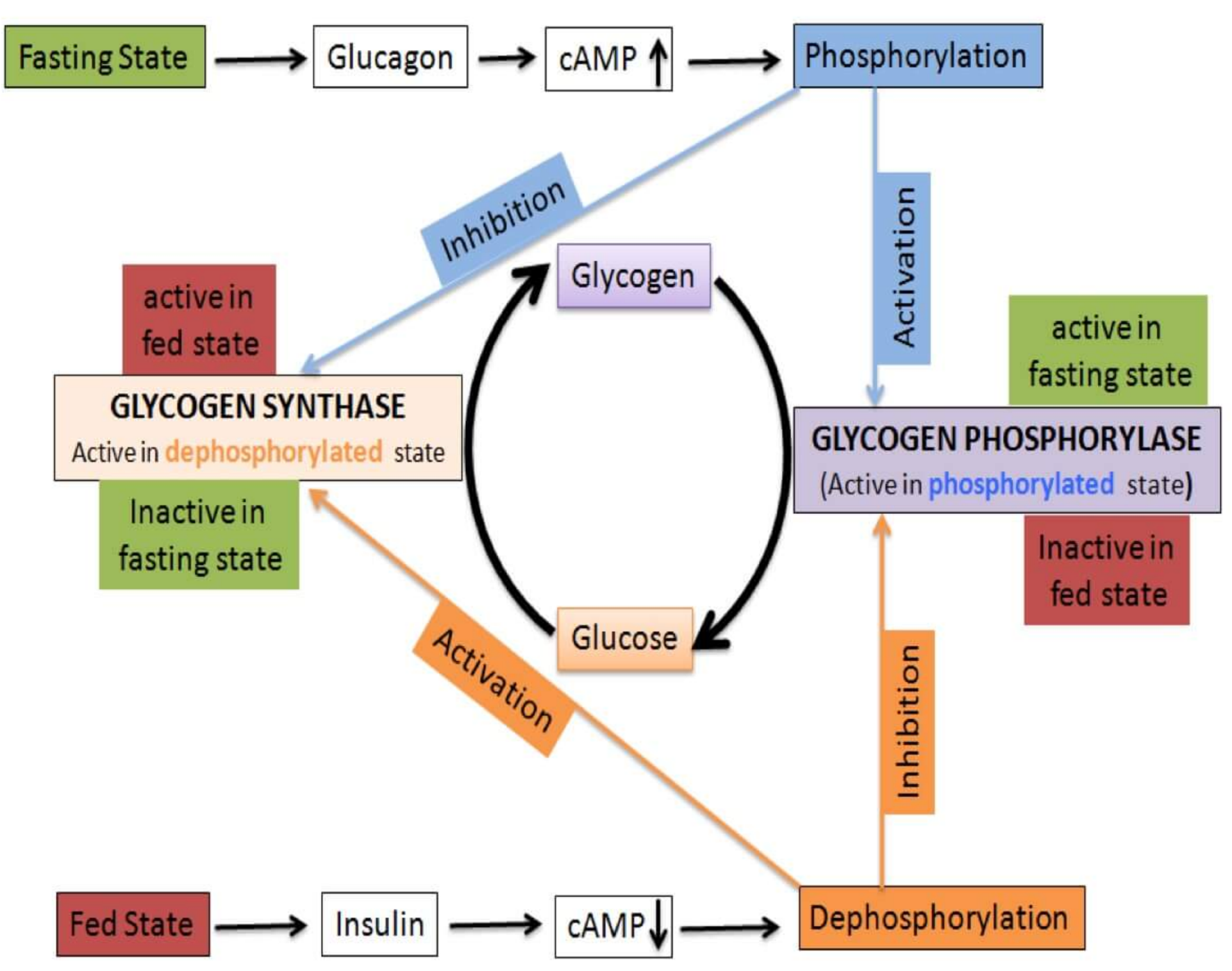
TYPE I	Type VI
VON GIERKE'S [mc]	HER'S
GLUCOSE – 6 – (P)ase Deficient	Glycogen Phosphorylase Deficient
No Glycogenolysis	No Glycogenolysis
No Gluconeogenesis	Gluconeogenesis occur
Severe Hypoglycemia	Mild Hypoglycemia
Ketosis	No Ketosis

VON GIERKE'S DISEASES – CLINICAL FEATURES

1. Severe Hypoglycemia (most important)
2. Ketosis
3. Hepatomegaly
4. Hyper Triglyceridemia
5. Lactic Acidosis
6. Hyper Uricemia
7. Enlarged Kidneys

FED SATE → INSULIN → [Dephosphorylation] → (+) → GLYCOGEN SYNTHASE

FASTING → GLUCAGON → (+)Phosp → GLYCOGEN PHOSPHORYLATION



GLYCOGEN PHOSPHORYLASE

→ Rate Limiting Enzyme

→ Catabolic Enzyme

-

(b) Inactive —**Phosphorylation**→ (a) Active

ACTIVATORS	INHIBITORS
[Livers & Muscles] cAMP, GLUCAGON, Epinephrine, Nor- Epinephrine → activated by phosphorylation	[Livers & Muscles] Phosphatase, Insulin → Inhibited by Dephosphorylation
Ca^{2+} + Calmodulin → Directly Activates	Glucose, Glucose – 6 – P, ATP → Product Inhibition
5' AMP → Mechanism unknown	Fructose – 1 – P

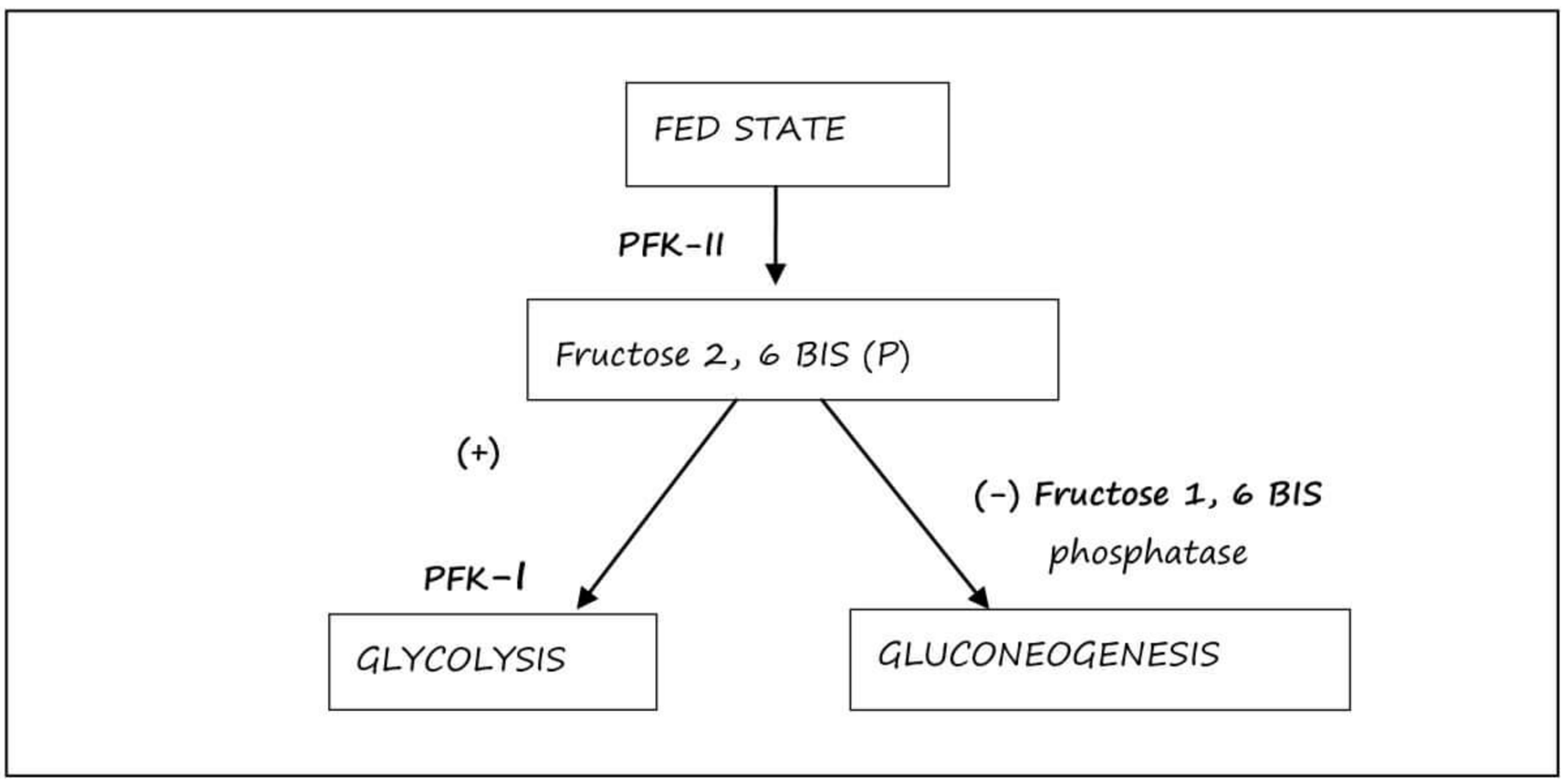
FRUCTOSE – 1 – P

→ Accumulated in Liver in Heriditary Fructose Intolerance

- Presented WITH Hypoglycemia

RECIPROCAL REGULATION AND TIGAR

RECIPROCAL REGULATION



REGULATOR [ACTIVATOR / INHIBITOR] IS MORE IMPORTANT THAN SUBSTRATE

Ques: No acetyl CoA , lots of pyruvate. Gluconeogenesis occurs or not

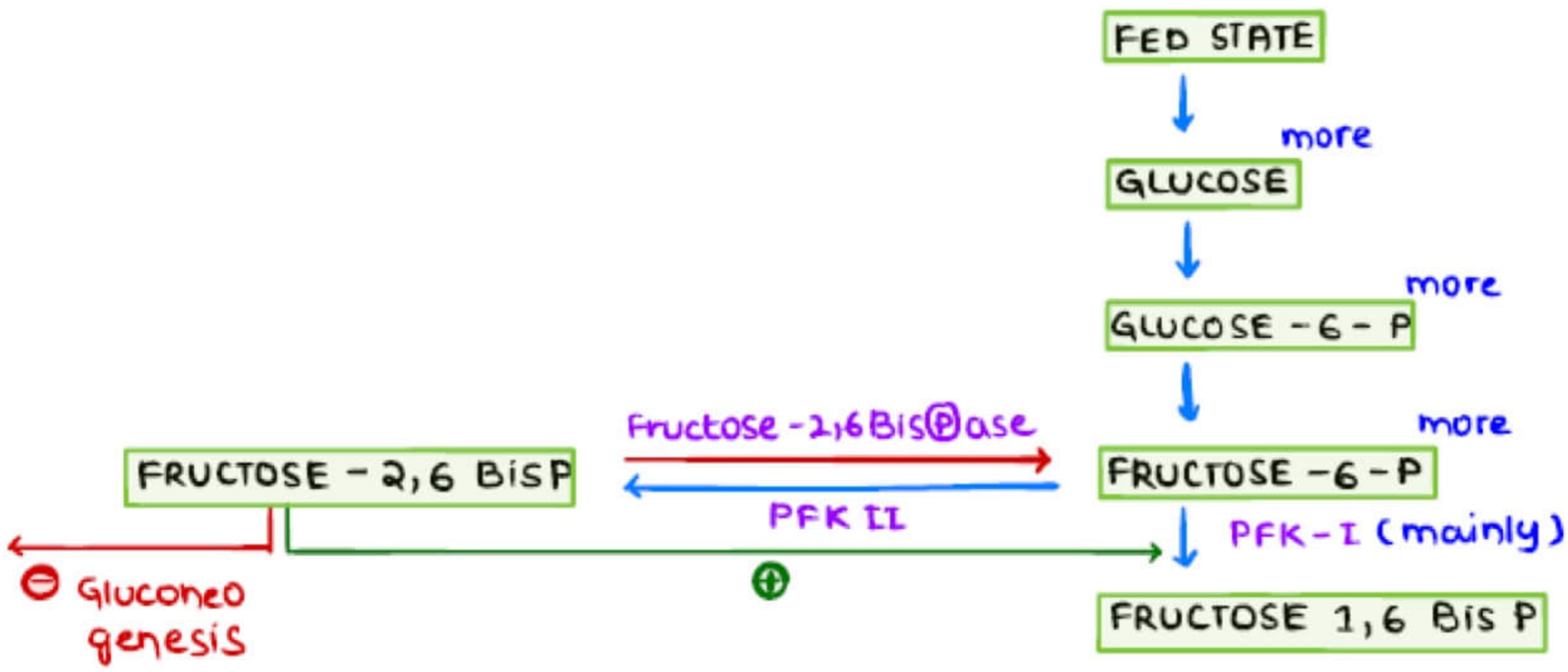
Ans . Acetyl CoA is activator & pyruvate is substrate

→ No Gluconeogenesis occurs

Ques: 1 molecule of Fructose 2,6 Bis (P) & 1000 molecules of Glycerol. Gluconeogenesis occurs or not

Ans. Fructose 2,6 Bis (P) is inhibitor & Glycerol is substrate

→ No Gluconeogenesis occurs



→ **Fructose - 2,6 Bis phosphatase**

- converts fructose - 2,6 Bisphosphate to fructose - 6 – phosphate
- active in cancer cells

→ Fructose - 1, 6 Bisphosphate → Glycolysis

Fructose - 1, 6 Bis Phosphatase → Gluconeogenesis

Fructose - 2, 6 Bisphosphate →Reciprocal Regulation

Fructose - 2, 6 Bis Phosphatase →Cancerous mutation (not in normal cells)

Ques: If Fructose 2,6 Bis (P)ase is active, what will happen to Glycolysis

(Ans) Fructose 2,6 BIS (P) —Fructose 2.6 Bis (P)ase→ Fructose 6 Phosphate

↓ Decreased

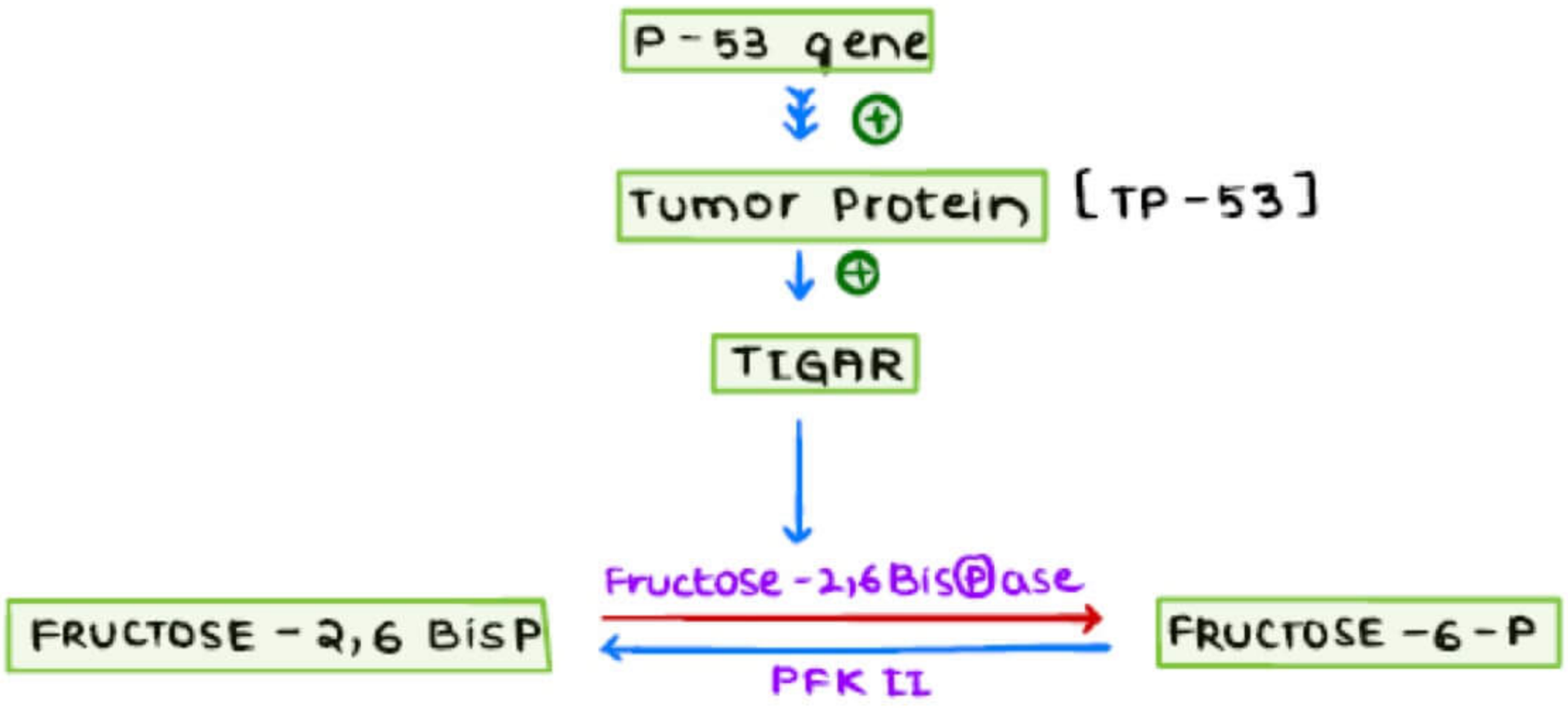
↓ Activation of PFK – 1 decreased

↓ ↓ Glycolysis

↓ Increased

TIGAR [TP-53 Induced Glycolysis & Apoptosis Regulator]

→ IN CASE OF CANCEROUS MUTATION,



→ Activation of TIGAR leads to

1. Decreased Glycolysis
   → Replication do not occur
   → Repair process begins
   → If Repair process do not begin for any reason,
      - Cell is killed by → Apoptosis (2nd function)

HMP [HEXOSE MONOPHOSPHATE PATHWAY]

→ Minor Pathway for the oxidation of Glucose

→ Glucose – 6 – P is the starting Material

→ aka PENTOSE PHOSPHATE PATHWAY

→ Ribose – 5 – P is Synthesized

→ NADPH also synthesized here

→ Anabolic pathway

→ Activated by Insulin

→ Inhibited by glucagon

→ Occurs in cytoplasm

2 PHASES

PHASE I	PHASE II
→ Oxidative Phase	→ Non – Oxidative Phase
→ NADPH is formed	→ Ribose – 5 – P is formed
→ Irreversible	→ Reversible

PATHWAYS WHERE ATPs ARE NOT PRDOCUED

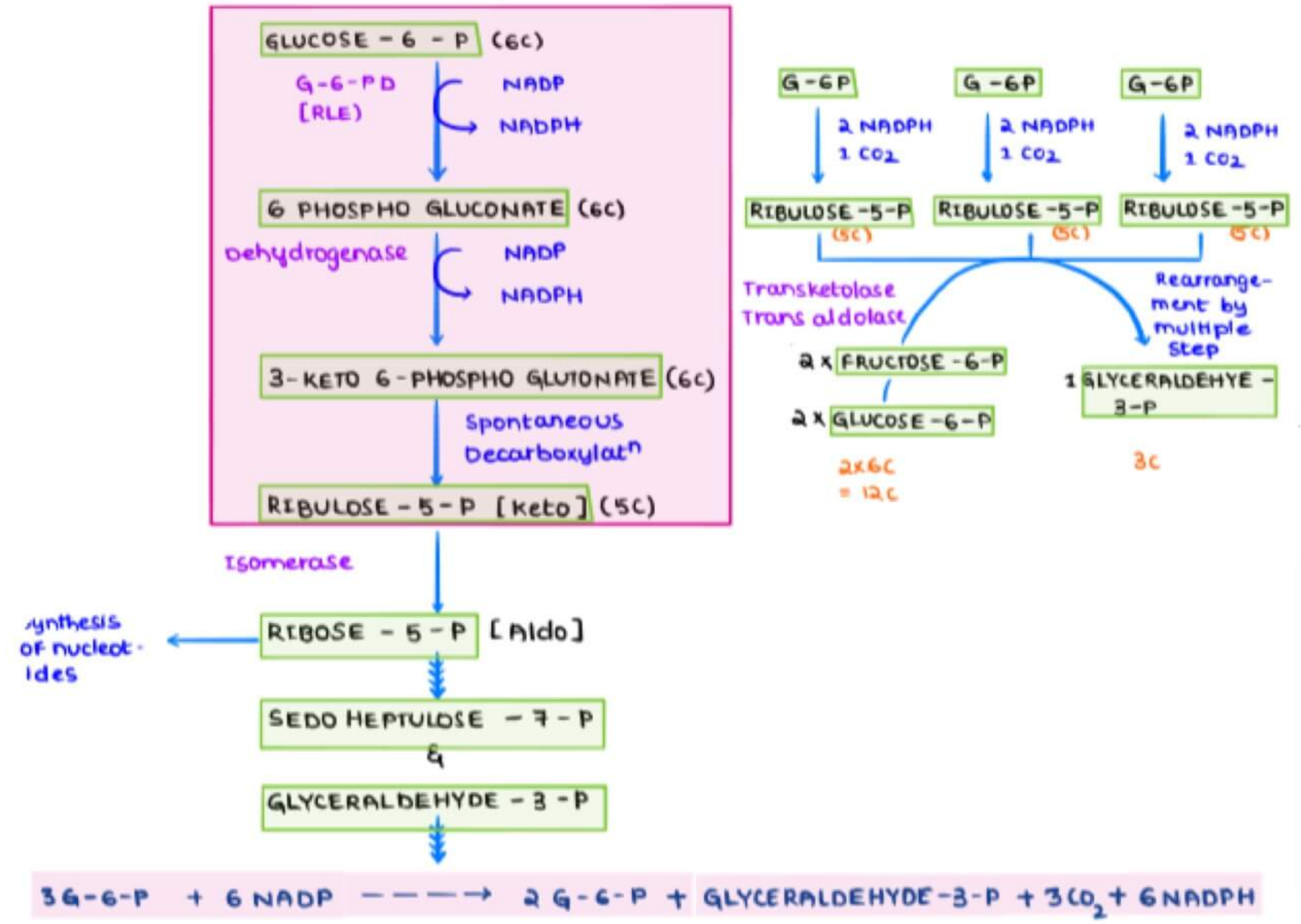
→ RL Shunt

→ α – Oxidation

→ Oxidation of very long chain Fatty Acids

→ Arsenic Acid poisoning in Glycolysis

→ Uronic Acid pathway



→ *Glyceraldehyde – 3 – P is intermediate as well as end product of Glycolysis*

→ *Glucose – 6 – P is substrate as well as product of this reaction*

- *HMP is a cycle, not a pathway*

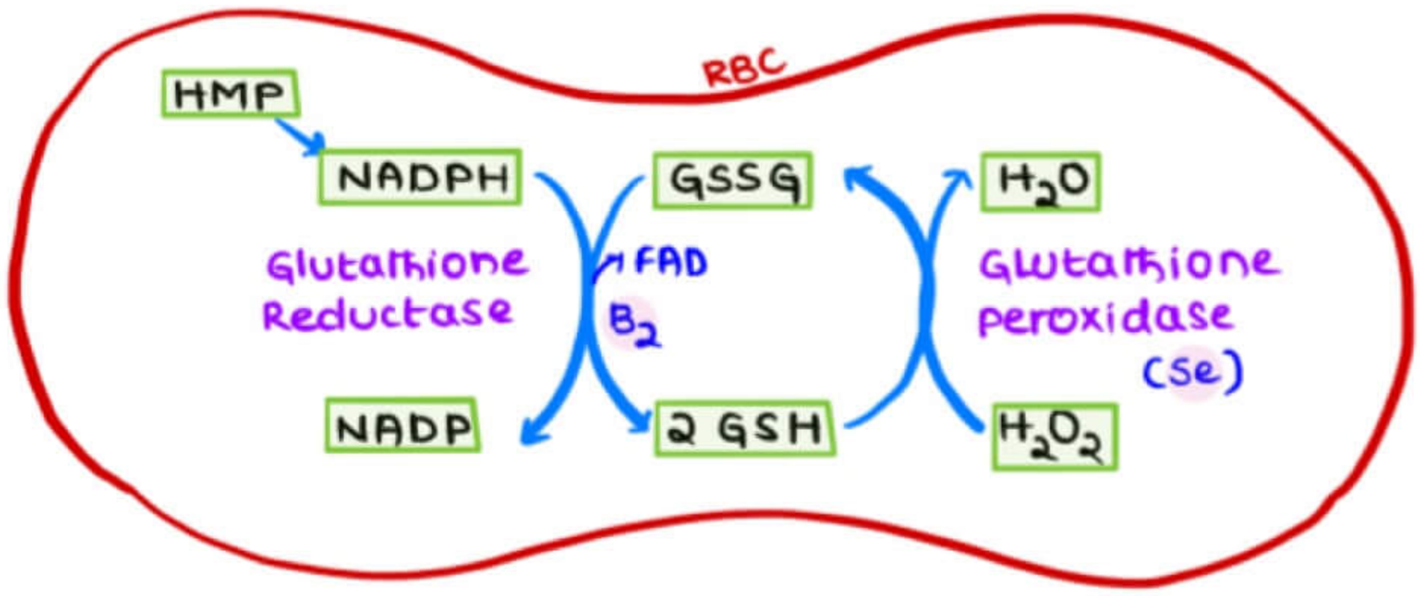
SITES OF HMP	NEVER THE SITES FOR HMP
→ Liver, Adipose tissue → Lactating mammary gland → Adrenal cortex → Gonads → Placenta → RBCs	→ Non Lactating Mammary Glands → Skin **LOW HMP ACTIVITY IN** → Skeletal muscles

ROLE OF HMP IN RBC

HMP → NADPH → REDUCED GLUTATHIONE

GLUTATHIONE

- Tripeptide
  - → 2 Peptide bonds
  - → 3 Amino Acids
    - GLUTA → Glutamate
    - THI → Sulfur containing AA – cysteine (has SH group)
    - ONE → Glycine



- HMP PAHSE II – transketolase - require vitamin B1 and Mg
- Marker of Vitamin B1 deficiency – RBC TRANSKETOLASE ACTIVITY
- Marker for Vitamin B2 deficiency – RBC GLUTATHIONE REDUCATSE ACTIVITY

→ G-6-PD Deficiency

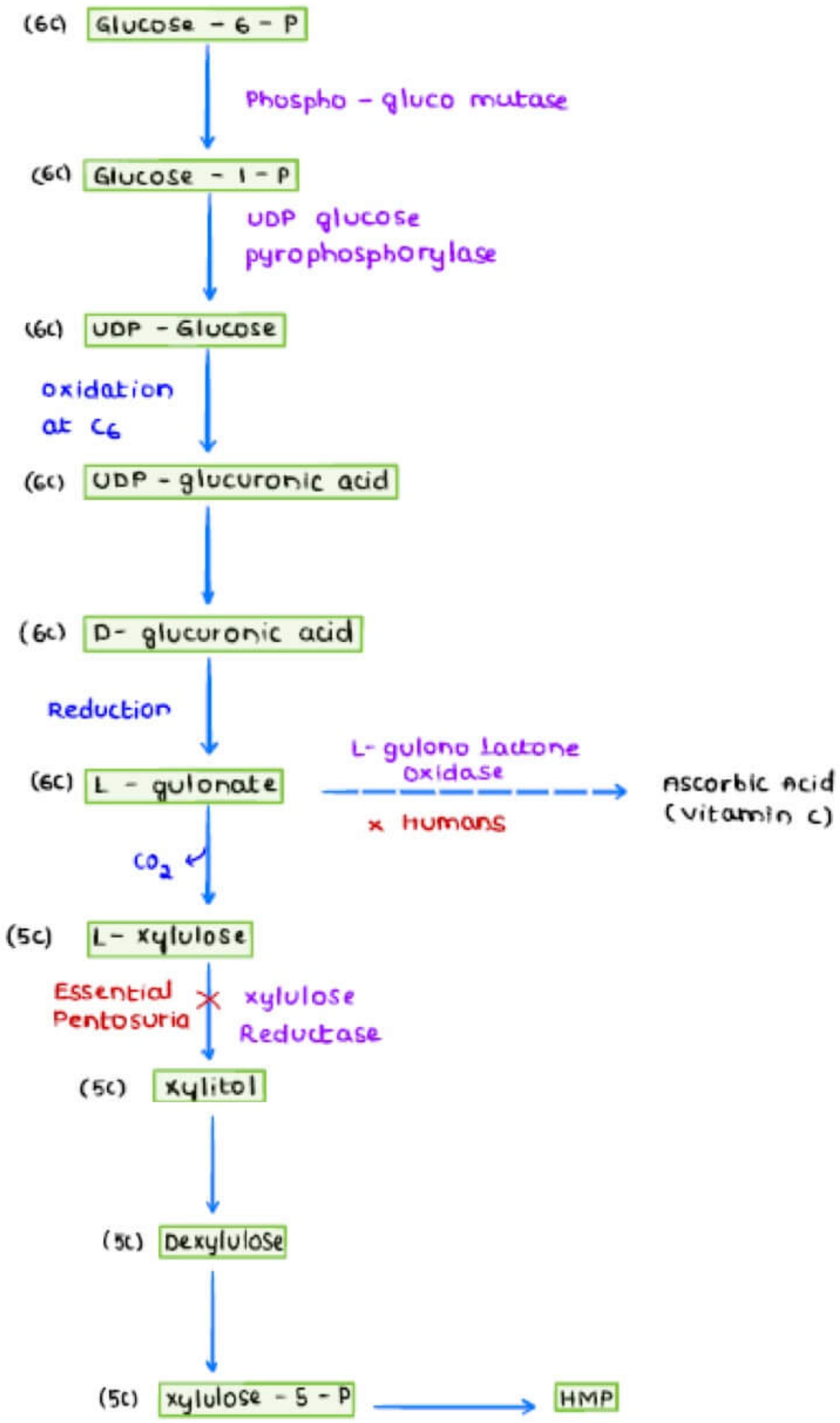
Defect of any enzyme in HMP
Defect of any enzyme in Glycolysis
} can lead to Hemolytic anemia

URONIC ACID PATHWAY

(Minor Pathway for oxidation of Glucose)

Similarities b/w Uronic Acid Pathway & HMP Pathway

1. Minor pathways
2. Starts with Glucose -6-P
3. No ATP formed
4. SITE – cytoplasm



UDP Glucuronic Acid Used For

- GAGs synthesis
- Detoxification phase II reaction of xenobiotics (act as conjugating agents)
- Conjugation of Bilirubin in Liver.

Uses of Uronic Acid Pathway

1. Vitamin C synthesis (not in humans)
2. Glucuronic acid synthesis
3. Pentoses synthesis

Essential Pentosuria

- Inherited disorder
- One of the component of GARROD's TETRAD
- d/t deficiency of Xylulose Reductase
- **L-xylulose** gets accumulated & excreted in urine
    - Monosaccharide – reducing substance
    - Benedict's test is positive +
    - Glucose oxidase test strip is negative -
    - Benign condition, but should be differentiated from diabetes
- **Pentosuria**
    - Can also occur in normal situations like consumption of large amount of fruits.

GARRODS TETRADS

4 diseases

1. C → Cystinuria
2. A → Alkaptonuria
3. A → Albinism
4. P → Essential Pentosuria

I. **Cystinuria** → Defect in dibasic a –a transporter .

In urine of these patients 4 aa will be released

C → Cystinuria

O → Ornithine

L → Lysine

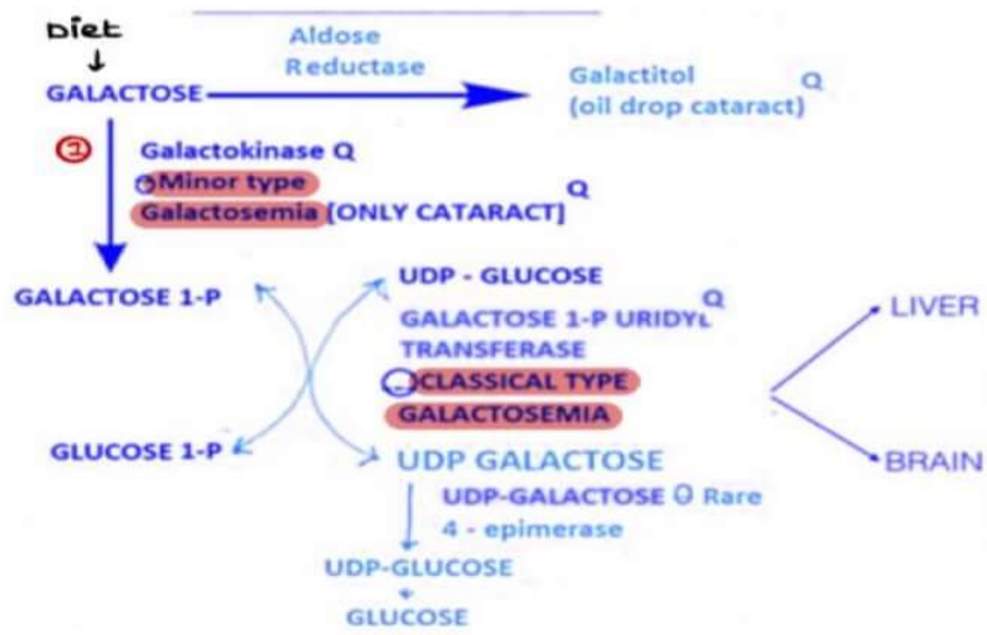
A → Arginine

II. **Alkaptonuria** → Defective Homogentisate Dioxygenase

III. **Albinism** → Tyrosinase

IV. **Pentosuria** → Xylulose Reductase

GALACTOSE & FRUCTOSE METABOLISM



1. MINOR GALACTOSEMIA

→ d/t Galactokinase deficiency

→ Only Cataract occurs (oil drop)

→ Aldose reductase can reduce any aldose sugars

2. CLASSICAL TYPE GALACTOSEMIA

→ dlt Galactose 1- P Uridyl Transferase deficiency

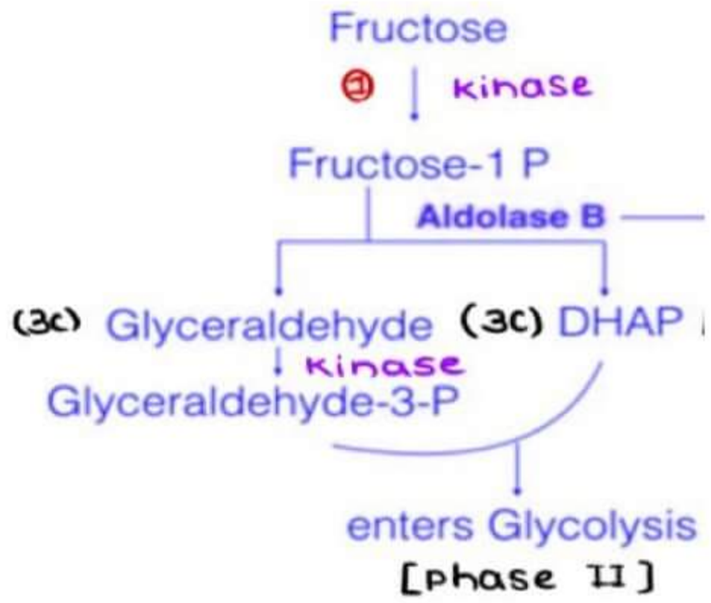
→ Liver (Hepatosplenomegaly, Jaundice) & Brain (MR) affected

→ Oil drop cataract also seen

→ Present in 1st week of birth

→ In developed countries, screening is done for Galactosemias.

FRUCTOSE METABOLISM



→ Energetics of Fructose & Glucose are same

- Fructose after Aerobic Glycolysis gives 7 ATPs

- Fructose after complete breakdown gives 32 ATPs

HEREDITARY FRUCTOSE INTOLERANCE

→ dlt deficiency of Aldolase B

- Fructose -1 P accumulates in
  - → Liver → causes hepatosplenomegaly, jaundice, hypoglycemia
  - → Kidney → Kidney failure occurs if not treated

FRUCTOSE is the most rapidly metabolized monosaccharide

→ Because it by pass PFK-1 Step

→ Fructose → Pyruvate → Acetyl Co A → Fats

→ FRUCTOSE IS THE MOST LIPOGENIC SUGAR

SORBITOL PATHWAY

→ All monosaccharides on reduction forms Alcohols

Ex:

Glucose	Aldose Reductase →	Sorbitol	
- 6 C		- 6 C	
- 50 H		- 60 H	→ POLYOL PATHWAY

GLUCOSE —(**Aldose Reductase**; NADPH → NADP)→ SORBITOL —(**Sorbitol Dehydrogenase**; NAD → NADH)→ FRUCTOSE

-

Synthesized in body

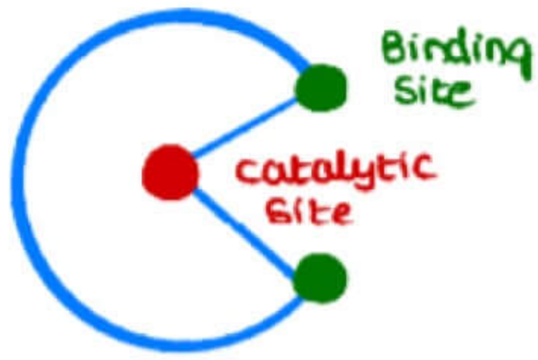
Aldose Reductase	→	Present in almost all cells of the body
Sorbitol Dehydrogenase	→	Present in few cells of the body

ENZYMES

ENZYME BASICS & SERINE PROTEASES

→ All enzymes are proteins

Except RIBOZYME [RNA acts as enzyme]



→ ACTIVE SITE = BINDING SITE + CATALYTIC SITE

CHYMOTRYPSIN

→ Protein breaking enzyme

→ Aspartate & Histidine are binding the substrate

→ Serine is responsible for cutting the substrate

- SERINE PROTEASE

SERINE PROTEASES

→ All have same CATALYTIC TRIAD

- Histidine
- Aspartate
- Serine

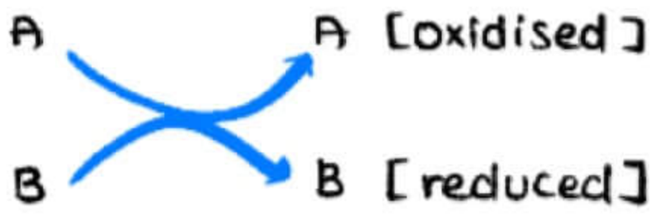
→ EXAMPLES

- Trypsin
- Elastase
- Plasmin
- Thrombin
- complement proteins
- clotting factors X & XI
- PSA (Prostate Specific Antigen)

→ Have role in Tumor metastasis

BI – BI REACTION

→ 2 Substrate, 2 Product Reaction



→ TYPES

1. Ordered → Mostly dehydrogenases
2. Random → Mostly Kinases
3. Ping Pong Reaction
   a. Serine proteases
   b. Amino Transferases
      - SGOT
      - SGPT

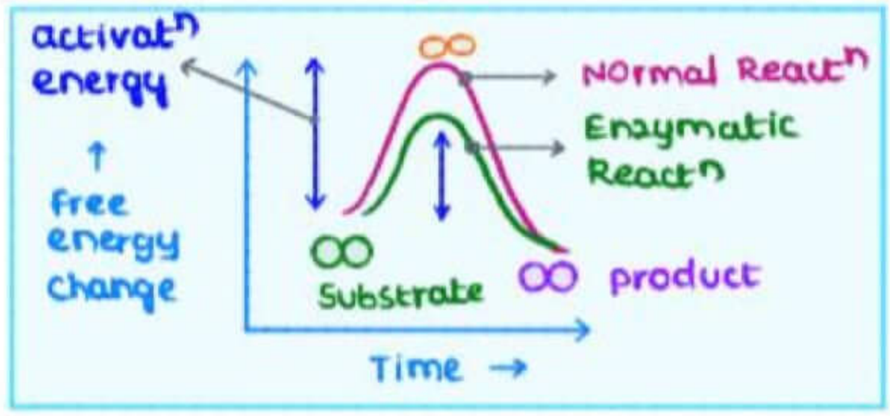
SITE SPECIFIC / DIRECTED MUTAGENESIS

→ Is tried to increase the velocity or activity of Serine Proteases
→ Site Specific mutation is used in order to get desired effect
→ Most of the times results are not positive

Q Oligonucleotide with single base change is used in → Site directed mutagenesis
Q Single mutation is detected by → RFLP

PROPERTIES OF ENZYMES

→ Not used in the reaction
→ ↑ Rate of reaction
→ Decrease the time of reaction
→ ↓ Activation energy
→ Do not change the equilibrium of reaction
→ Do not change the free energy of Substrate / product



COFACTORS & PROSTHETIC GROUPS

ACTIVE ENZYME = HOLO ENZYME = PROTEIN = APO ENZYME + NON-PROTEIN

NON-PROTEIN: Co FACTOR or PROSTHETIC GROUP

- Can be separated from protein
- Can't be separated from protein

- Can be separated from protein:
  - ORGANIC → Aka COENZYME
  - INORGANIC → - metal → Aka METAL ACTIVATED ENZYME

- Can't be separated from protein:
  - INORGANIC → - metal mostly → aka METALLOENZYME

→ All coenzymes are cofactors
→ Metals are cofactors but not co enzymes

CO ENZYMES

1. **Lipoic acids**
2. **Vitamins**

- All water-soluble vitamins act as coenzymes **(B-complex & Vit C)**
- Only fat soluble vitamin acting as coenzyme → **Vit K (carboxylation)**

3. **Nucelotides**

- NAD
- NADP
- FAD
- FMN

Lipoic acid is a coenzyme but not a vitamin

NUCLEIC ACIDS

SAM [S – Adenosyl Methionine]	→	Methyl donor
PAPS [Phospho Adenosyl Phospho Sulfate]	→	Sulfate donor

→ These are nucleotides but not co-enzymes

Mg REQUIRED FOR

→ Kinases
→ Phosphorylases
→ Carboxylases

Cu REQUIRED FOR

➔ **Oxidases**

- Cyt c Oxidase
- Tyrosinase
- Ascorbic Acid Oxidase
- Amino Acid Oxidase
- Lysyl Oxidase
- Cytoplasmic SOD [Super Oxide dismutase]

- Mitochondrial SOD requires Manganese

- Xanthine Oxidase
  Sulfite Oxidase

} Do not require copper, these require Molybdenum

ISOENZYMES

→ Physically distinct form of enzymes, they catalyse same biochemical reaction

→ SAME COMPONENTS

Reaction

Enzyme

Species

- Ex: LDH – 1

LDH – 2

- ALLOENZYME → If species is different

DIFFERENCE IN ISOENZYMES

→ Structure

- Genes
- Km, Vmax
- Electrophoretic mobility

LACTATE DEHYDROGENASE [LDH]

TYPES	MONOMERS		LOCATION
1	H H H H	→	Heart
2	H H H M	→	Functional plasma enzyme [Blood (WBC)]
3	H H M M	→	Lungs (mainly), Pancreas, Spleen, Kidney
4	H M M M		Muscles, Liver
5	M M M M		

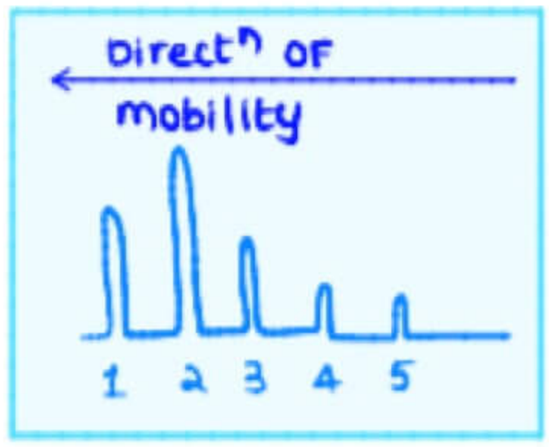
Q Predominant form of LDH in Liver → LDH – 5 (4 & 5 both are present)

→ **In normal persons,** LDH 2 > LDH 1

In MI , LDH 1 > LDH 2 → Flipped Ratio

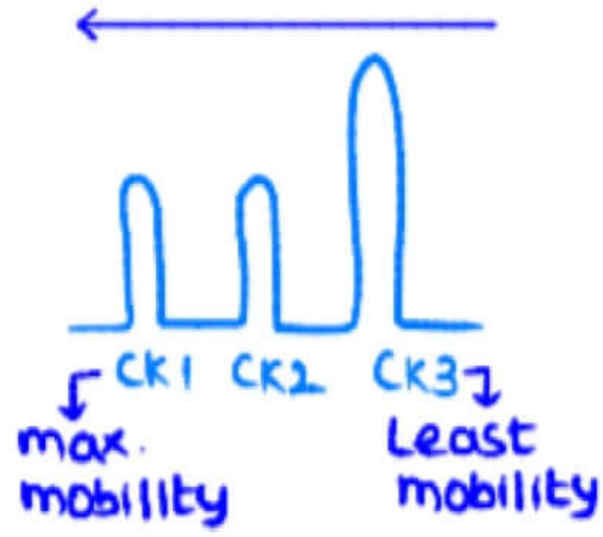
ELECTROPHORETIC MOBILITY

LDH 1 → Has max. Electrophoretic mobility
LDH 5 → Least Electrophoretic mobility



CREATININE KINASE (CK) ENZYME

TYPES	MONOMERS	LOCATION
CK – 1	BB	Brain
CK – 2	MB	Heart (raised in MI)
CK – 3	MM	Muscle



ENZYMES AND PROTEINS RAISED IN MI

Enzymes
- CK – 2 / CK– MB → 4 – 6 hrs
- AST / SGOT → 6 – 8 hrs
- LDH – 1 → 8 – 10 hrs

Proteins
- Myoglobin → 2 – 6 hrs [earliest marker, but nonspecific
- Troponin T & I → 3 – 6 hrs

TROPONINS [Regulatory proteins in cardiac muscle]

Troponin C → Calcium binding
Troponin I → Actin Myosin inhibitory ATPase [most specific]
Troponin T → Tropomyosin binding

BNP [Brain Natriuretic Peptide] → Marker for cardiac failure
IMA [Ischemia Modified Albumin] → New cardiac biomarker

HEXOKINASE

TYPES

I

II → Most abundant

III

IV → Glucokinase

HEXOKINASE	GLUCOKINASE
→ Phosphorylates all hexoses	→ Phosphorylation of Glucose only. (same properties like GLUT-2)
→ Present in all cells	→ Present in Liver & Pancreas.
→ High Affinity,	→ Low Affinity,
→ less Substrate required	→ more substrate required.
→ Low Km	→ High Km
→ Low V max	→ High V max
→ Feedback inhibition from the product (G6P)	→ Regulated by Insulin

ENZYME CLASSIFICATION

ENZYME

EC No. → Enzyme commission / code Numbers

1. Oxido reductase → Transfer electrons or hydrogen atoms
2. Transferase → Molecular formula is changed
3. Hydrolase → Use H_2O to break
4. Lyase → Can make/ break [do not require H_2O / ATP]
5. Isomerase → Molecular formula do not change
6. Ligase → Use ATP to make

CLASSIFICATION	Distinguishing Feature
1. Oxidoreductase	
Oxidases	Use O_2 as an electron acceptor
Dehydrogenase	Use molecules other than O_2 as electron acceptor (NAD, FAD, NADP) → Oxidative decarboxylases
Peroxidases	Use H_2O_2 as an electron acceptor
Oxygenases	Incorporate O_2 into the substrate
2. Transferases	
Methyltransferase	Transfer one carbon units
Aminotransferase	Transfer amino groups
Kinases	Transfer phosphate from ATP
Phosphorylase	Transfer phosphate from Pi
3. Hydrolases	
Phosphatase	Remove phosphate from a substrate
All digestive enzymes	
4. Lyases	
Synthases	Link 2 molecules without using ATP
Aldolase	Produce aldehydes via elimination reactions
Decarboxylase	Produce CO_2 via elimination reactions (simple only)
Hydratase	Add or remove water (do not break bond)
5. Isomerases	
Racemase	Interconvert L & D stereoisomers
Mutase	Transfer groups b/w atoms within a molecule
Epimerase	Interconvert epimers
6. Ligase	
Synthetase	Link 2 molecules via an ATP-dependent reaction
Carboxylase	Use CO2 as a substrate

EXAMPLES

Oxygenases

1. Monooxygenases [o]

→ RH ⟶ ROH

- aka Hydroxylases
- Ex: 1. Phenyl alanine Hydroxylase
  [Phenyl alanine to Tyrosine]
  2. Cyt P_{450} [Hydroxylation of Steroid hormones]
  3. NOS [Nitric Oxide Synthase] [EC no.1]

- Most hydroxylases are monooxygenases
  But Prolyl & Lysyl Hydroxylase are Dioxygenases
- Aka mixed function oxidases
- O_2 is added
- One atom given to substrate
  Other given to H_2 → H_2O

2. Dioxygenases [O-O]

→ Homogentisate Dioxygenase

- Deficiency causes Alkaptonuria

→ Whenever a macromolecule is synthesized, H_2O is removed
Whenever a macromolecule is broken down, H_2O is added → Hydrolases

HYDROLASES – EXAMPLES

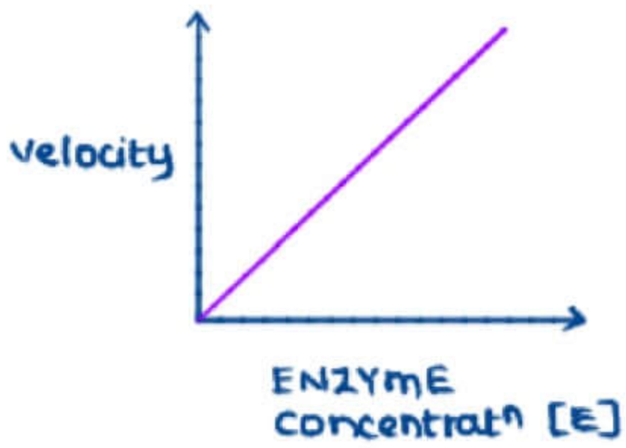
1. Carbohydrates breaking
   - Amylase
   - Lactase
   - Sucrase
   - Maltase
   - Isomaltase
2. Protein Breaking
   - Protease
   - Peptidase
   - Arginase
   - Urease
3. Lipid Breaking
   - Lipase
   - Esterase
   - Phospholipase
4. Nucleic Acid Breaking
   - Nuclease
   - Exonuclease
   - Endonuclease
   - Restriction endonuclease

HYDRATASE

Enolase	→	Glycolysis
Aconitase, Fumarase	→	TCA CYCLE
P E P C K	→	Gluconeogenesis

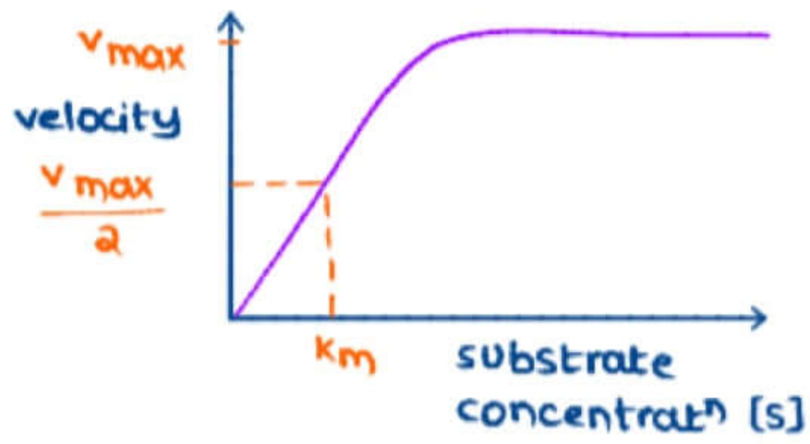
ENZYME KINETICS

VELOCITY VS ENZYME CONCENTRATION [E]→ LINEAR GRAPH



V α E

VELOCITY VS SUBSTRATE CONCENTRATION [S]



Rectangular hyperbola

aka MICHAELIS MENTON GRAPH

→ Initially as substrate concentration increases, velocity increases proportionally, after that it reaches a saturation point from which the velocity remains constant → **RECTANGULAR HYPERBOLA**

MICHAELIS MENTEN GRAPH

MICHAELIS MENTEN CONSTANT [K_M]

→ Substrate concentration at which velocity of reaction is half of V max

→ K_m can't be equal to Vmax/2

→ K_m is a constant

$$K_m \quad \alpha \quad \frac{1}{\text{Affinity}}$$

KLUTZ Formula = Affinity $\alpha \frac{1}{[S]}$

→ K_m is Signature of Enzyme

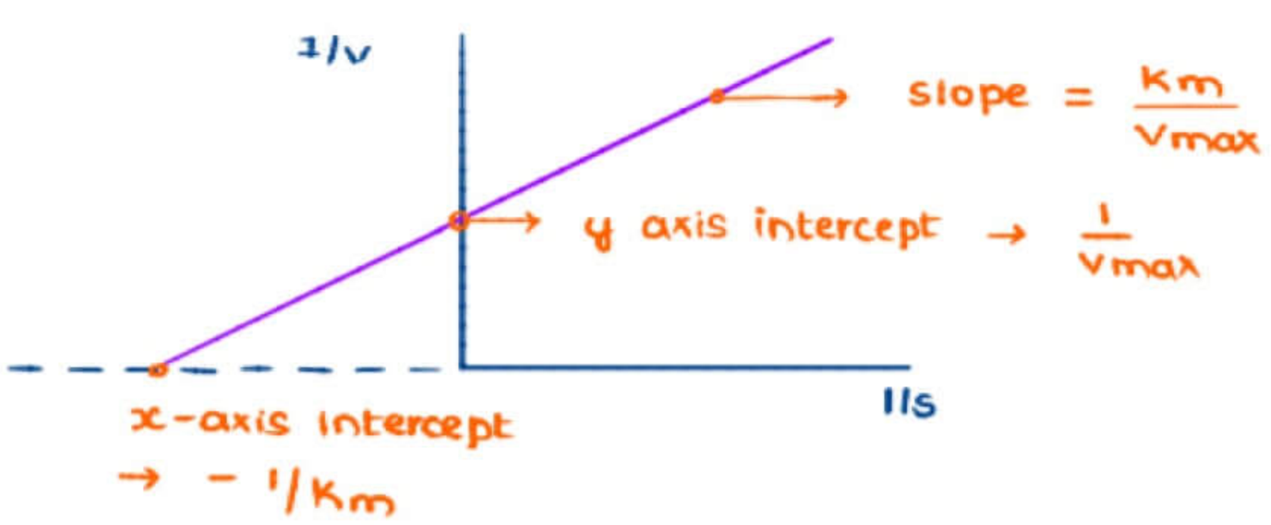
1. It is constant value for each enzyme
2. It is different value for each enzyme

- Ex: LDH 1 & 2 have different K_m values

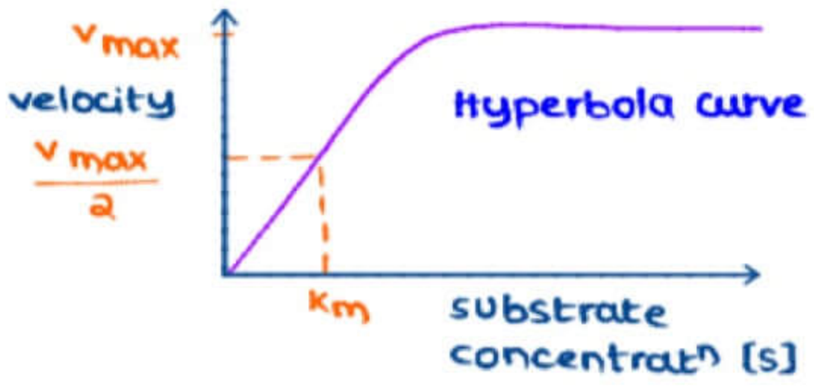
In initial portion of graph, when substrate concentration is less than Km,
FIRST ORDER KINETICS are followed → $V \propto [S]$

In later portion of graph, when substrate concentration is more than Km,
ZERO ORDER KINETICS are followed → Velocity is independent of [S]

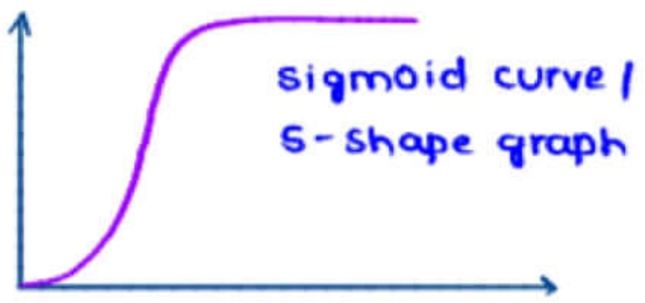
DOUBLE RECIPROCAL CURVE / LINEWEAVER BURK GRAPH [PLOT]

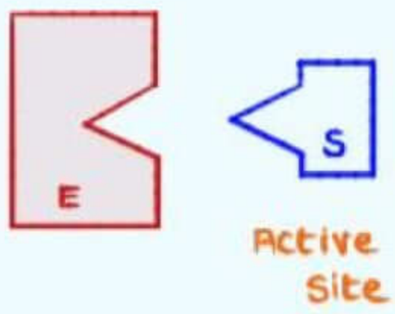
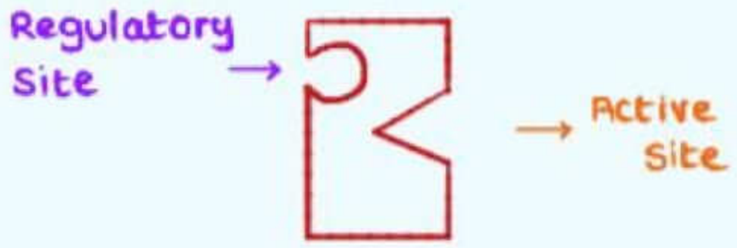


FOR SIMPLE ENZYMES



FOR ALLOSTERIC ENZYMES



SIMPLE ENZYMES	ALLOSTERIC | REGULATORY ENZYMES
→ most  → Substrate binds at active site & Reaction occurs	→ Few  → Regulator is more important than Substrate ↳ only in the presence of activator, reaction occurs ↳ in the presence of inhibitor, reaction can't occurs even if the abundant presence of substrate

REGULATOR → Inhibitor binds to regulatory site, induce changes at activation site & inhibits reaction;

Activator binds to Activator site, induce changes at activator site & reaction occurs

ALLOSTERIC | REGULATORY ENZYMES

→ Multi subunit enzymes

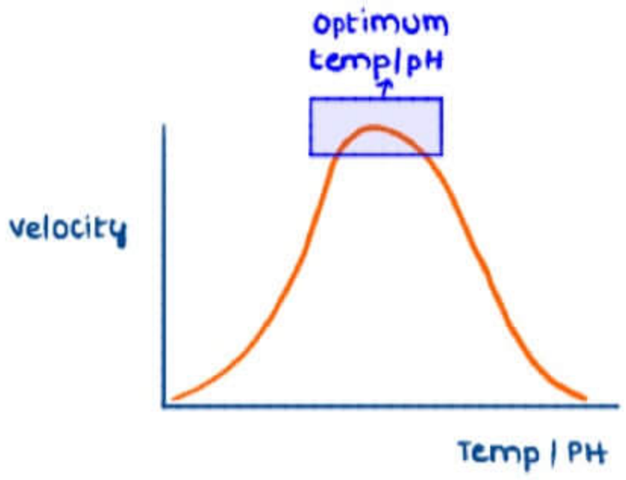
→ Shows co operativity

- One substrate binding to enzyme increases the affinity for other substrates

→ O_2 DISSOCIATION

1. Hb	2. Myoglobin
→ Has 4 chains	→ Only 1 chain
→ Shows cooperativity	→ Doesn't show
→ Sigmoidal curve	→ Rectangular Hyperbola

VELOCITY VS TEMP / pH



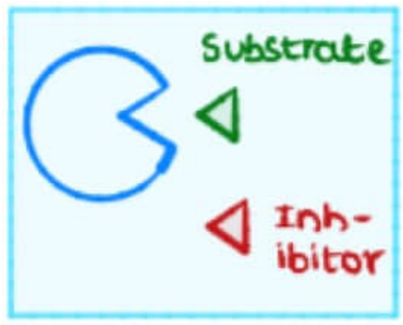
Bell Shaped

→ At extremes of temperature / pressure, proteins are denatured & velocity is negligible.

Velocity is maximum at optimum temperature & pH only.

ENZYME INHIBITORS

1. Competitive
2. Non-competitive
3. Uncompetitive
4. Allosteric
5. Feedback inhibition
6. Suicidal inhibition



COMPETITIVE INHIBITION

→ Inhibitor resembles substrate in structure. Inhibitor binds at active site.

→ K_m (Michaelis constant) does not depend upon change in enzyme and substrate concentration.

$K_m \propto 1 / \text{affinity}$

→ K_m defines affinity between a particular enzyme substrate pair.

→ But in competitive inhibition the affinity b/w enzyme and substrate decreases because now enzyme has affinity for both substrate and inhibitor.

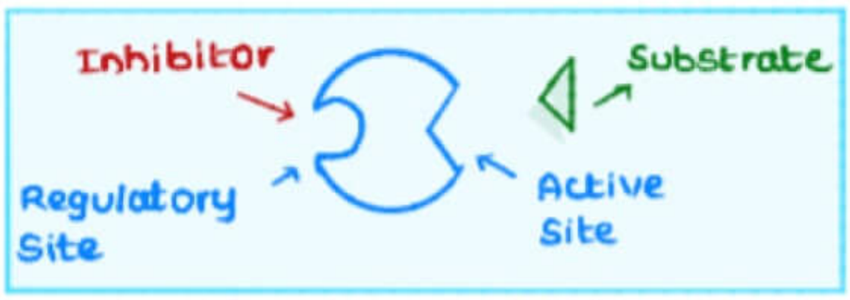
→ Affinity decreases so K_m increases

→ Inhibitor resembles substrate

→ V_{max} remains same but K_m increases.

NON – COMPETITIVE INHIBITION

- Substrate do not resemble inhibitor in structure
- Inhibitor binds at regulatory or allosteric site. When inhibitor binds at regulatory site, it changes the shape of active site, so that substrate cannot bind.



- Vmax is lowered

  K_m is same

  Affinity is same

➔ Inhibitor can bind with ES complex, and does not change the substrate affinity for the enzyme.

➔ This inhibition is mostly irreversible.

Competitive Inhibitors

Arsenate → Glyceraldehyde – 3 - PDH } **Glycolysis**

Oxamate → Lactate DH

Malonate (3C) → Succinate DH } **TCA**

Fluorocitrate → Aconitase

Sulfonamide (anti – bacterial) → PABA analogue → F.A. (Bacteria) vital

Methotrexate (anti – cancerous) → DHF Reductase → THF (Human) vital

Decumarol (anti –coagulant) → VIt K analogue → (Coagulation)

Ethanol → Methanol Poisoning (Alcohol DH)

Statins → HMG CoA Reductase (RLE) Cholestrol Synthesis

Non – competitive inhibitors

Iodoacetate → Glyceraldehyde – 3 – PDH

NaF → Enolase

Fluoroacetate → Aconitase

Arsenate → Alpha – Keto – Glutarate DH

Cyanide → ETC (Comp IV)

Heavy Metals → SH group (Present at the active site of many enzymes in the body)

Dimercaprol → SH

Disulfuran (Antabuse) → Aldehyde DH

Di–iso–Propylfluorophosphate → Serine Proteases

UN-COMPETITIVE INHIBITION (ANTI-COMPETITIVE)

Both Km and Vmax decreases.

Inhibitor can only bind ES complex

e.g. Uncompetitive Inhibitor: Acetylcholine inhibits Placental ALP (Alkaline Phosphatase)

	K_M	V_{max}
Competitive Inhibitors	Increased	Same
Non-competitive Inhibitors	Same	decreased
Uncompetitive Inhibitors	decreased	decreased

→ Allosteric Inhibition occurs naturally in the body

Allosteric site = Active site + Regulatory site

- Ex: During fed state, gluconeogenesis is allosterically inhibited
  During fasting, gluconeogenesis is allosterically activated

Allosteric Inhibition is normally observed in regulation of enzymes and pathways

→ Non-competitive Inhibition occurs unnaturally

- Ex: Drugs

FEED BACK INHIBITION / END PRODUCT INHIBITION

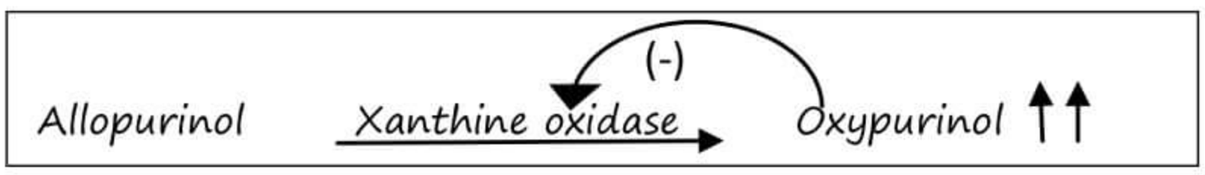
→ End product itself inhibits the reaction

→ Ex: Cholesterol inhibits HMG CoA Reductase

→ Ex: Haem inhibits ALA Synthase [RLE] & stops haem Synthesis

SUICIDAL INHIBITION / MECHANISM BASED INHIBITION

→ Ex: Allopurinol Inhibits Xanthine oxidase by a proper mechanism



→ Feed back Inhibition is a natural phenomenon occurring in body. It is normally observed in regulation of enzymes and pathways.

→ Suicidal Inhibition is unnaturally occurring phenomenon e.g Drugs

ENZYME USES

DIAGNOSTIC USES→ Ex: SGOT , SGPT For Liver diseases

THERAPEUTICS USES

1. Lactase → Lactose Intolerance
2. Lactamase → Penicillin Allergy
3. Urokinase / Streptokinase → Converts Plasminogen → Plasmin
   → Used for lysis of Intravascular clots

4. Trypsin / chymotrypsin → for pain + inflammation in chronic back pain and sprain.

→These protein breaking enzymes are helpful in breakdown of proteins collected at site of inflammation.

5. Collagenase → Skin ulcers (reduces the formatⁿ of scar tissue)
6. Pepsin → Pancreatic insufficiency & chronic indigestion
7. Asparaginase/ Glutaminase → All (Acute Lymphoblastic Leukemia)
8. Uricase → Gout
9. Alpha – 1 – Anti trypsin → Emphysema

→ IN ALL (Acute Lymphoblastic Leukemia), the cancer cell has high demand for Asparagine & Glutamine

→ These enzymes break down these AA & ALL cell will die

ENZYME REGULATION

Various ways of Enzyme Regulation

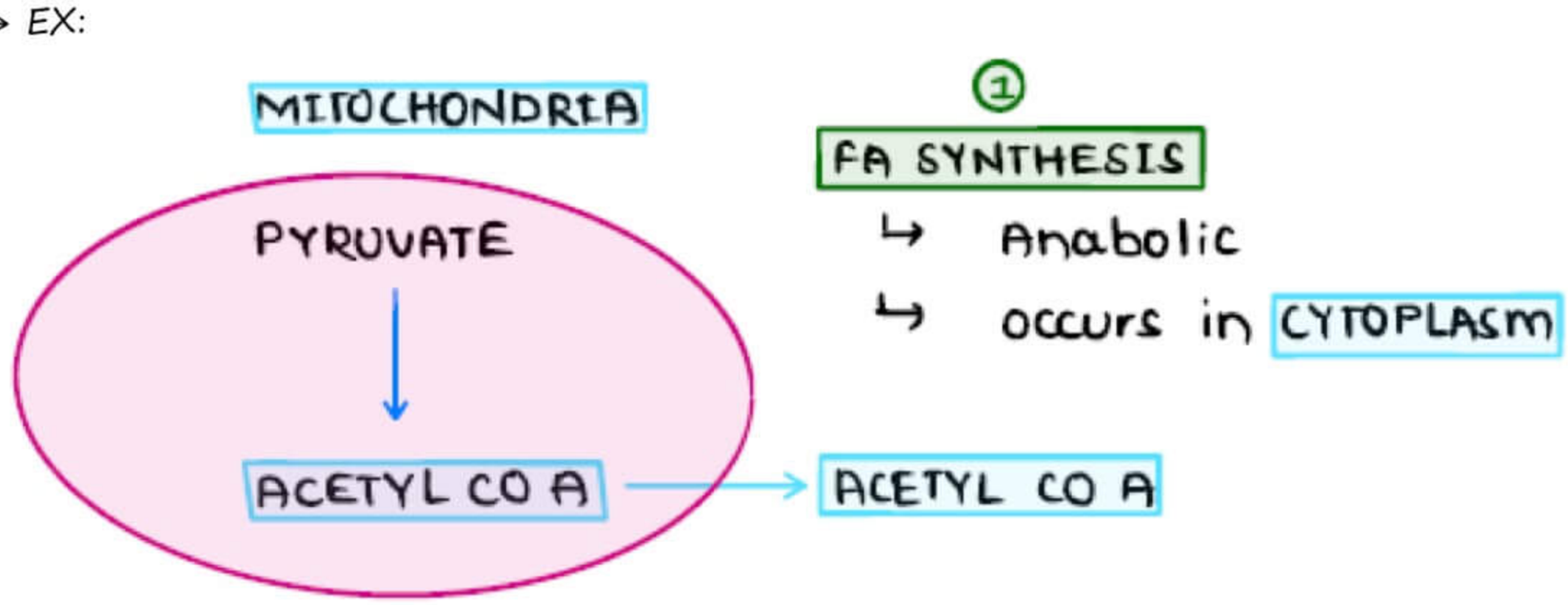
1. Allosteric
2. Covalent
3. Compartmentalization of Cells
4. Rate of synthesis
5. Rate of Degradation of Enzymes
6. Synthesis of Inactive Zymogens

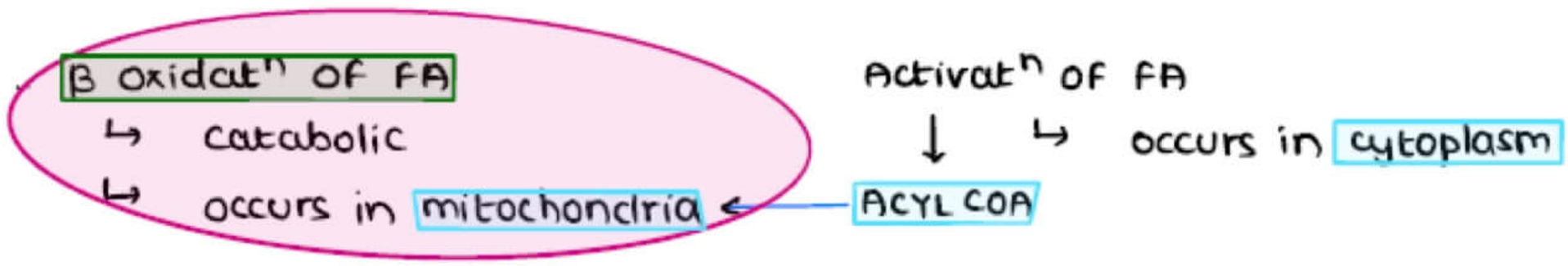
COVALENT → mc covalent modification is phosphorylation & dephosphorylation.

COMPARTMENTALIZATION OF CELLS

→ Different enzymes are kept in different compartments of cell

→ EX:





→ According to body needs, compartmentalization of cells changes

RATE OF SYNTHESIS→ Synthesis of enzymes occurs at the level of genes

1. HOUSEKEEPING / CONSTITUTIVE GENES
   - always active (Ex: TCA cycle genes)
2. INDUCIBLE GENES
   - induced whenever required [Ex: Gluconeogenesis enzyme genes are induced during fasting]

RATE OF DEGRADATION OF ENZYMES

1. **UBIQUITIN PROTEASOME PATHWAY**

→ specific pathway
→ requires ATP
→ Regulated pathway
→ occurs in cytoplasm and nucleus
→ tagged with UBIQUITIN (protein which is highly conserved during evolution)

2 **LYSOSOMAL PATHWAY**

→ ATP Independent
→ non specific
→ enzyme involved is Acid hydrolase
   - Can breakdown protein & other macro molecules

SYNTHESIS OF INACTIVE ZYMOGENS

→ Enzymes are synthesized as inactive enzymes at the site of production
→ Those inactive enzymes are activated at the site of action
Ex: Chymotrypsinogen → Chymotrypsin
Trypsinogen → Trypsin

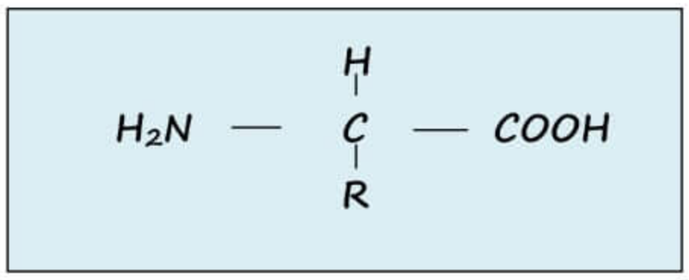
AMINO ACID & PROTEINS

BASICS OF AMINO ACIDS

AMINO ACID

→ Amino group is always on left side
  Acid group is always on right side
} **Functional groups of Amino Acids**

→ Central carbon atom is Asymmetric
- Can show both Optical & Structural Isomerism



→ ALL AA HAVE 1 ASYMMETRIC CARBON

EXCEPTIONS

0 → Glycine

2 → Isoleucine & Threonine [Both are essential AA]

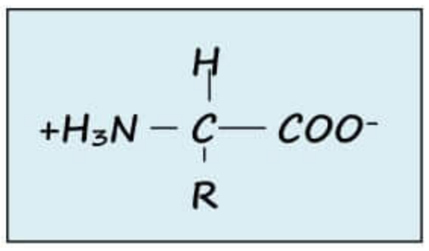
SEMI ESSENTIAL AA → Arginine > Histidine

AA that is essential in children but not in Adults → Histidine

SOLUBILITY

→ Property of charges

→ Anything in body is ionised
- Net charge is zero
- Zwitter Ion / AMPHOLYTE
- Insoluble → Precipitate
- pI → Isoelectric pH, where zwitter ion exits
- pH = pI → Precipitation occurs, no charge
- pH < pI → Acidic pH, protein has positive charge
- pH > pI → Alkaline pH, protein has negative charge

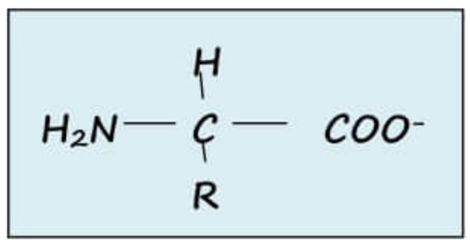


DEPROTONATED FORM of AA

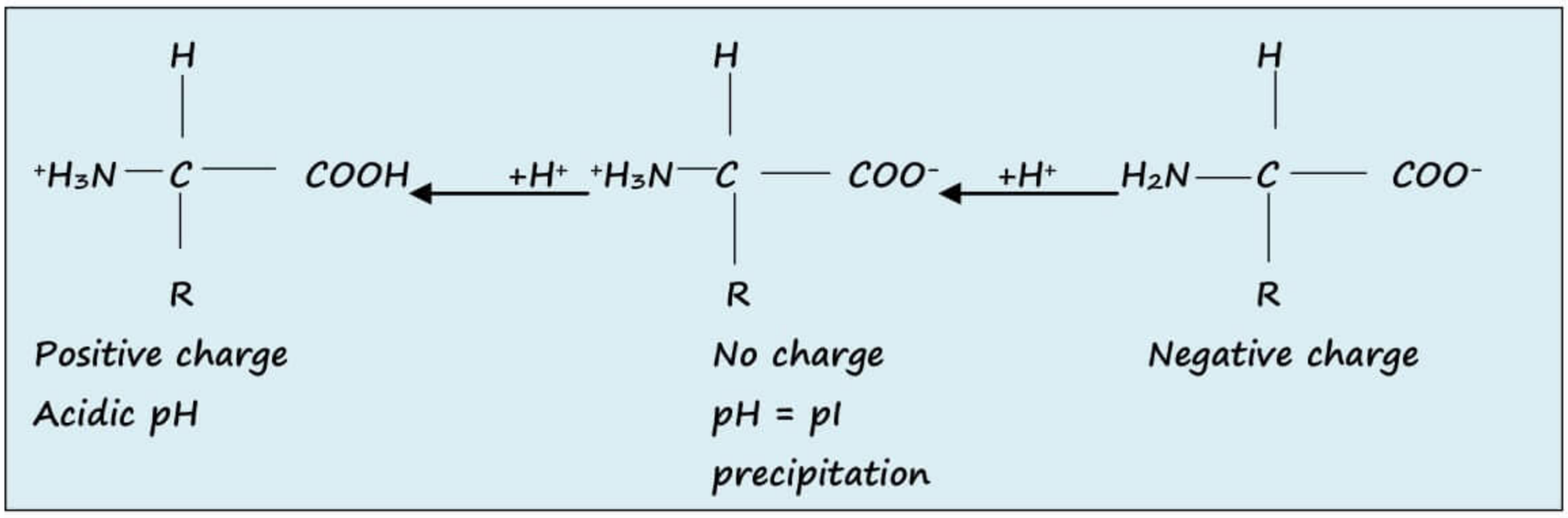
→ Protein is not there on both the functional groups

→ Present in alkaline pH

→ Has negative charge on it



ADDITION OF PROTONS TO DEPROTONATED FORM



→ Acidic AA → Negatively charged

Basic AA → Positively charged

ALL AA are L – α AMINO ACIDS

L→NH_2 group is on left side

α carbon has amino group

→

$C^4 - C^3 - C^2 - COOH$

γ β α

$H_2N -$ $^2C - COOH$ (α) → α carbon has amino group

→ AA in protein is → Always α

Free AA → α

β [Ex: β Alanine – catabolic end product of pyrimidines]

γ

→ AA in protein is → always L

Free AA → L

D [Ex: D – Serine, D – Aspartate – Found in Brain]

CLASSIFICATION AND METABOLISM OF AMINO ACIDS

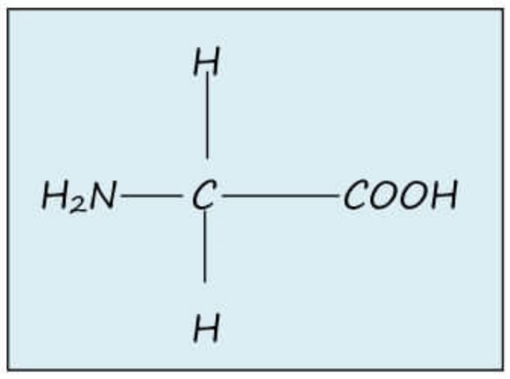
ALIPHATIC AMINO ACIDS

1. Glycine
2. Alanine
3. Valine
4. Leucine
5. Isoleucine

GLYCINE

→ Side chain → Simple hydrogen atom

- Non essential AA
- Smallest & Simplest AA
- No isomers



→ USES

- Haem Synthesis
- Glutathione Synthesis
- Creatine Synthesis
- Serine Synthesis → Non-essential AA

Q **Which AA is responsible for the flexibility of proteins** → **GLYCINE**

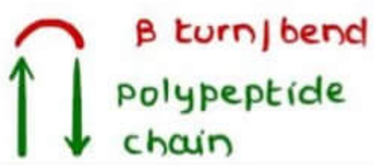
→ Has smallest side chain → Can fit in a small space → Create 'BENDS' in proteins

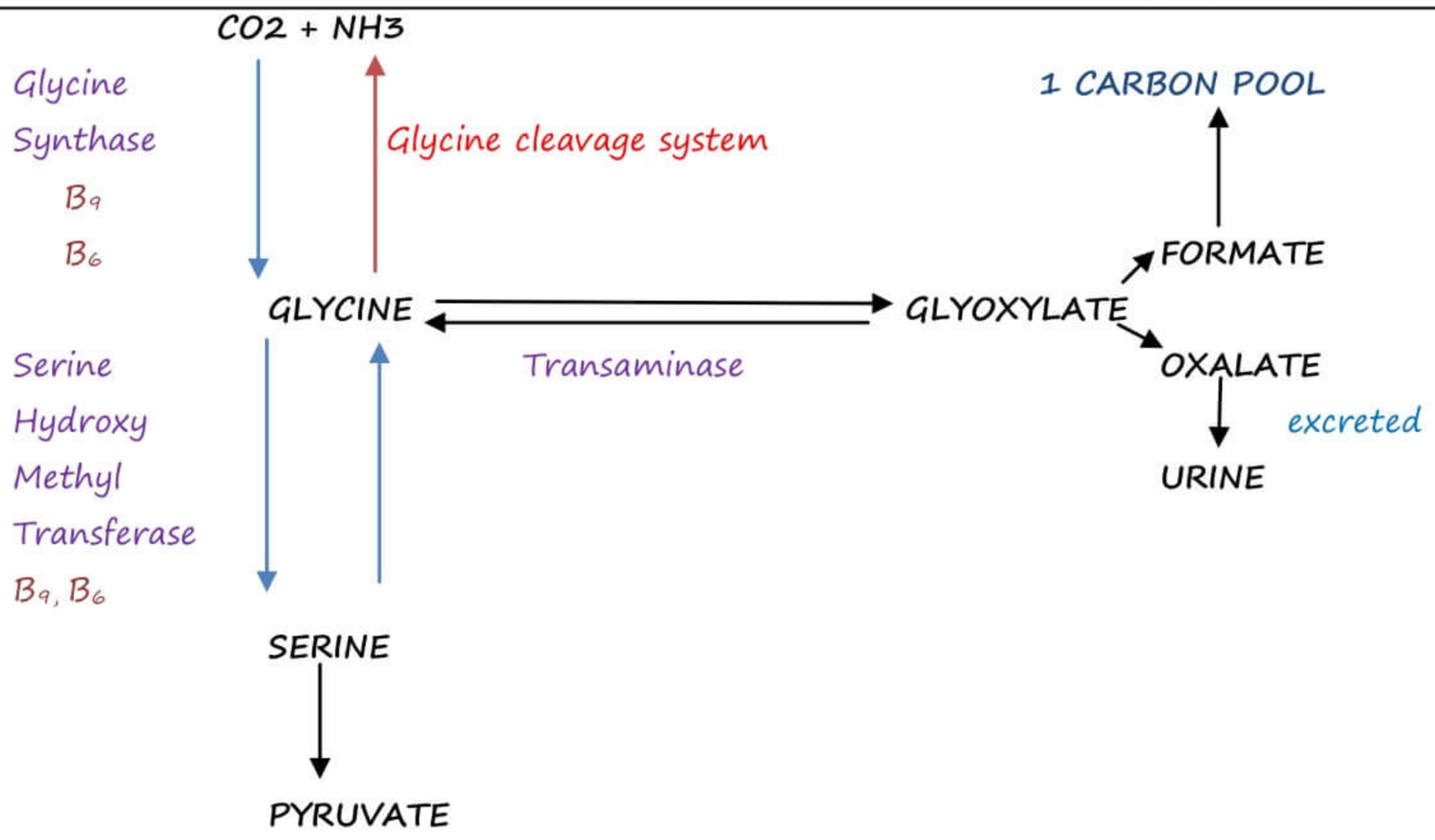
- Ability to bend is known as Flexibility

→ Found in β turn or β bend

Glycine & Proline can be found

→ Never found in α – helix





PRIMARY HYPEROXALURIA

→ Transaminase not working
  Glyoxalate not converted to formate
} ↑↑ Glyoxalate → ↑↑ Oxalate

→ Oxalate stones present in urine

→ Restriction of Oxalate rich foods is advised

- Green leafy vegetables, beetroot & tea

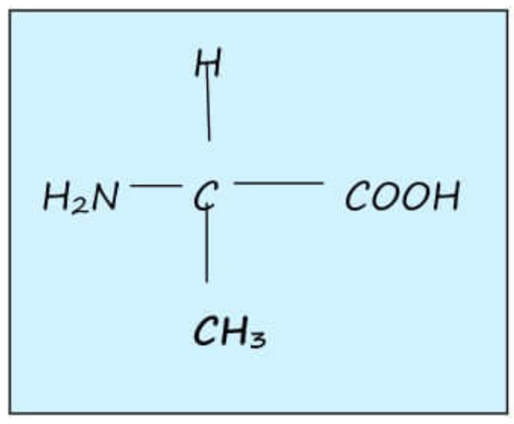
SECONDARY HYPEROXALURIA

→ CAUSES

1. Excess Vit C
2. B_6 Deficiency
3. Ethylene Glycol poisoning
4. Methoxy Flurane

2. ALANINE

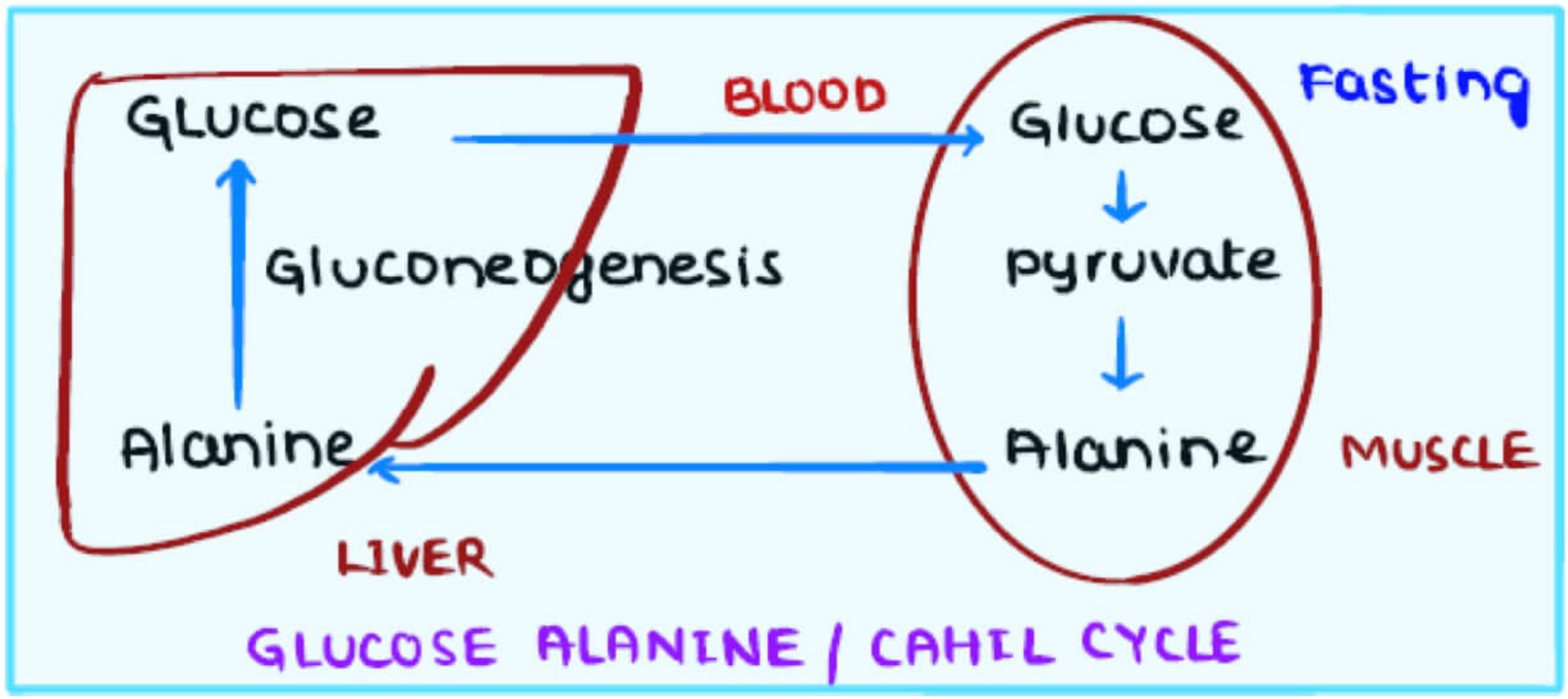
STRUCTURE



- Side chain → Simple methyl group
- Non essential AA

- 3 Carbon compound → Good substrate for Gluconeogenesis
  - Most Glucogenic AA

CAHILL CYCLE OR GLUCOSE ALANINE CYCLE



LEUCINE, VALINE, ISOLEUCINE

→ Branch chain amino acids

→ Essential AA

→ MAPLE SYRUP URINE DISEASE

- Defect in Catabolism of branch chain AA
  - Oxidative Decarboxylation do not occurs
  - Enzyme involved →α – Keto Acid Dehydrogenase / α – Keto acid Decarboxylase
  - → C/F
  - Burnt Sugar like odour from urine QQ
  - Ketosis
  - _ MR (Mental Retardation)
  - Abnormal muscle tone
  - Coma, Death (High mortality Rate)

→ ISOVALERIC ACIDURIA / ACIDEMIA

- Defect present only in catabolism of Leucine
- Cheesy odour of urine
- Enzyme involved → Isovaleryl CoA Dehydrogenase

POLARITY

→ All aliphatic AA are non polar

- Most non polar → Isoleucine > Valine
  Least non polar → Glycine [controversy]

Q	Which is polar
A	glycine
B	Alanine
C	Valine

Q	Which is polar
a	glycine
b	Alanine
c	Aspartate

II AROMATIC AA

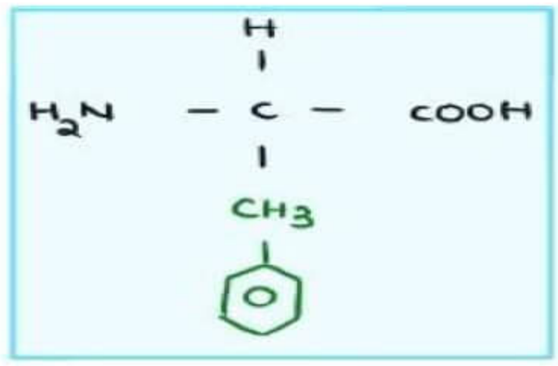
1. Phenyl alanine
2. Tyrosine
3. Tryptophan
4. Histidine

PHENYL ALANINE

STRUCTURE

→ Essential AA

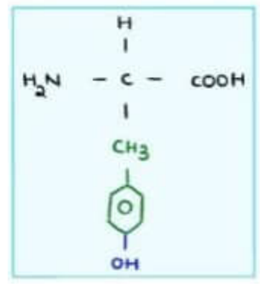
→ Non polar



Phenyl Alanine

TYROSINE

PHENYL ALANINE —Hydroxylase, [O]→ TYROSINE



Tyrosine

→ Non-essential amino acid

→ Polar (controversy)

TRYPTOPHAN

→ Essential AA

→ Non polar AA

→ Never found in α helix

USES

1. Tryptophan → Serotonin → Melatonin
2. Tryptophan —(B_2, B_6)→ Niacin (Vit B_3)

- Vit B_2 & B_6 deficiency also leads to Vit B_3 deficiency
- Vit B_3 (Niacin) → Atypical vitamin (formed in the body)

→ ATYPICAL VITAMINS → VITAMIN D & VITAMIN B_3

TRYPTOPHAN CATABOLISM

TRYPTOPHAN

↓ Catabolism

3 – OH – KYNURENINE

↓ Kynurenine hydroxylase, PLP required (Vit B_6)

NEXT INTERMEDIATE

→ Xanthurenic acid
Formed in B_6 Deficiency

HARTNUP'S DISEASE

→ Autosomal recessive

→ Failure to reabsorb tryptophan from kidneys

→ C/F

- Amino Aciduria [Tryptophan]
- Pellagra like symptoms (due to Niacin deficiency)

CARCINOID SYNDROME

→ All tryptophan in used for the formation of Serotonin

- Leads to deficiency of Niacin → Pellagra

HISTIDINE

→ Side chain contain IMIDAZOLE RING

→ Valency of Nitrogen is → 2

In the Imidazole ring, Valency of Nitrogen is → 3

- 2 Valencies are occupied already in the ring & there is space for 1 Hydrogen

→ $NH_2 \xrightarrow{ionised} + \longrightarrow$ Polar

In ring NH $\xrightarrow{ionised}$ Less + $\longrightarrow$ less Polar

→ Histidine is less polar & semi essential

→ Histidine has max. buffering capacity

III BASIC AA

→ Positively charged

→ Polar

→ Essential AA

→ Arginine [most polar]

Lysine

Histidine [least polar]

IV ACIDIC AA

→ Negatively charged

→ Polar

→ Non-essential AA

BASIC AA

ARGININE	>	LYSINE	>	HISTIDINE
+ + +		+ +		+
Semi essential		essential		Semi essential

ACIDIC AA

OXALOACETATE → ASPARTATE → ASPARAGINE

α KETO GLUTARATE → GLUTAMATE → GLUTAMINE

Q Which of the following moves fastest towards cathode

A	Valine	→	Non polar	Cation	→	(+)
B	Aspartate	→	-	Anion	→	-
C	Histidine	→	+	Cathode	→	-
D	Arginine	→	+ +	Anode	→	+

OH CONTAINING AA

Serine

Non-essential AA

Polar

Threonine

Has 2 Asymmetric carbon

- Essential AA

Polar

Tyrosine

Non-essential AA

Polar

→ AA with max. tendency to bind phosphate → OH containing AA
→ AA which is site for covalent modification → OH containing AA
→ AA responsible for O – Glycosidic bonds → OH containing AA
AA responsible for N – Glycosidic bonds → Asparagine
- has CONH

→ **SERINE** is the best option to select among OH containing AA

SULFUR CONTAINING AMINO ACIDS

CYSTEINE

→ Has Sulfhydryl group

→ Polar

→ Non-essential

METHIONINE

→ Sulfur is attached to 2 carbons with strong bond (C – S – C)

→ Non-polar

→ Essential AA (diet)

IMINO ACID – PROLINE

→ NH_2 is not free

→ Not found in α helix

→ Found in β turns

→ Non polar

→ Non-essential

21st AA ⟶ SELENOCYSTEINE ⟶ Given by UGA
22nd AA ⟶ PYRROLYSINE ⟶ Given by UAG
} Stop Codons

→ STOP CODONS do not give AA

EXCEPTIONS → Selenocysteine & Pyrrolysine
- by CO–TRANSLATIONAL MODIFICATION
(not by Post translational modification)

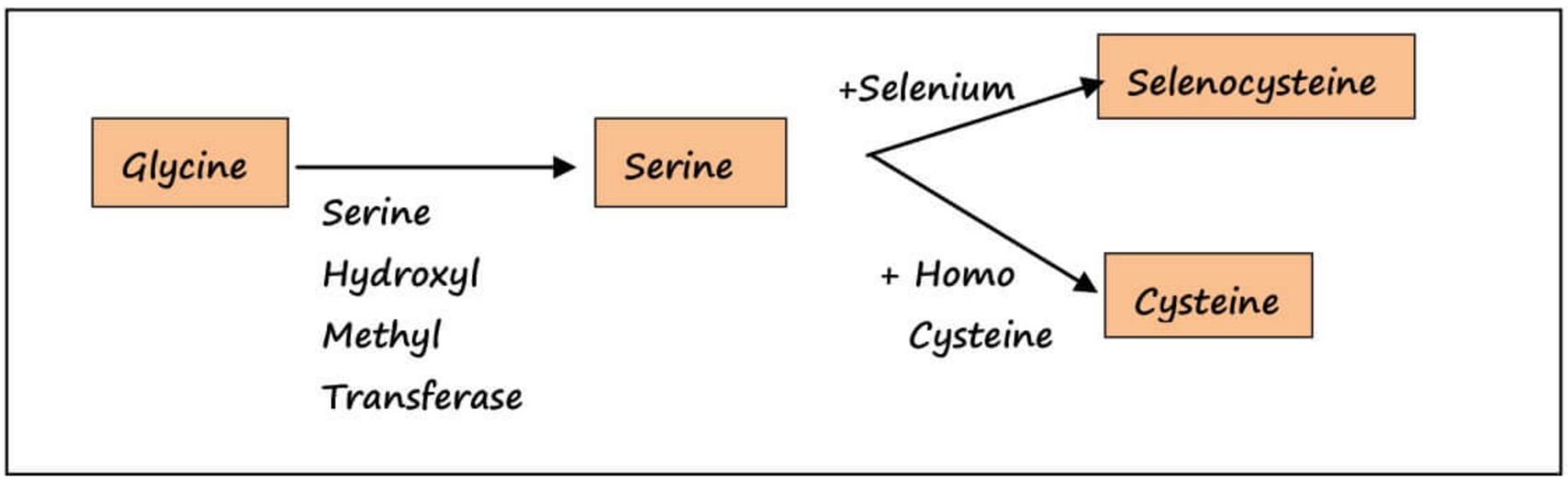
SELENO PROTEINS

→ Enzymes which require Selenocysteine at catalytic site

→ Mainly Reductases & Peroxidases

→ Ex:

1. Glutathione Peroxidase
2. Thioredoxine Reductase
3. Iodothyronine deiodinase



→ Precursor is serine

→ Homocysteine is derived from Methionine

POLAR

CHARGED

1. Basic AA (+)
2. Acidic AA (-)
   Ex: Aspartate
   Glutamate

UNCHARGED

1. OH containing (OH)
2. Cysteine (SH)
3. Asparagine & Glutamine (CONH)

DERIVED AMINO ACIDS → Not coming from codons

- Seen in Proteins
  - Hydroxy proline
  - Hydroxy lysine
- Not present In proteins
  - Ornithine → Involved in Urea cycle
  - Citrulline → Involved in Urea cycle
  - Homocysteine → Involved in Methionine metabolism

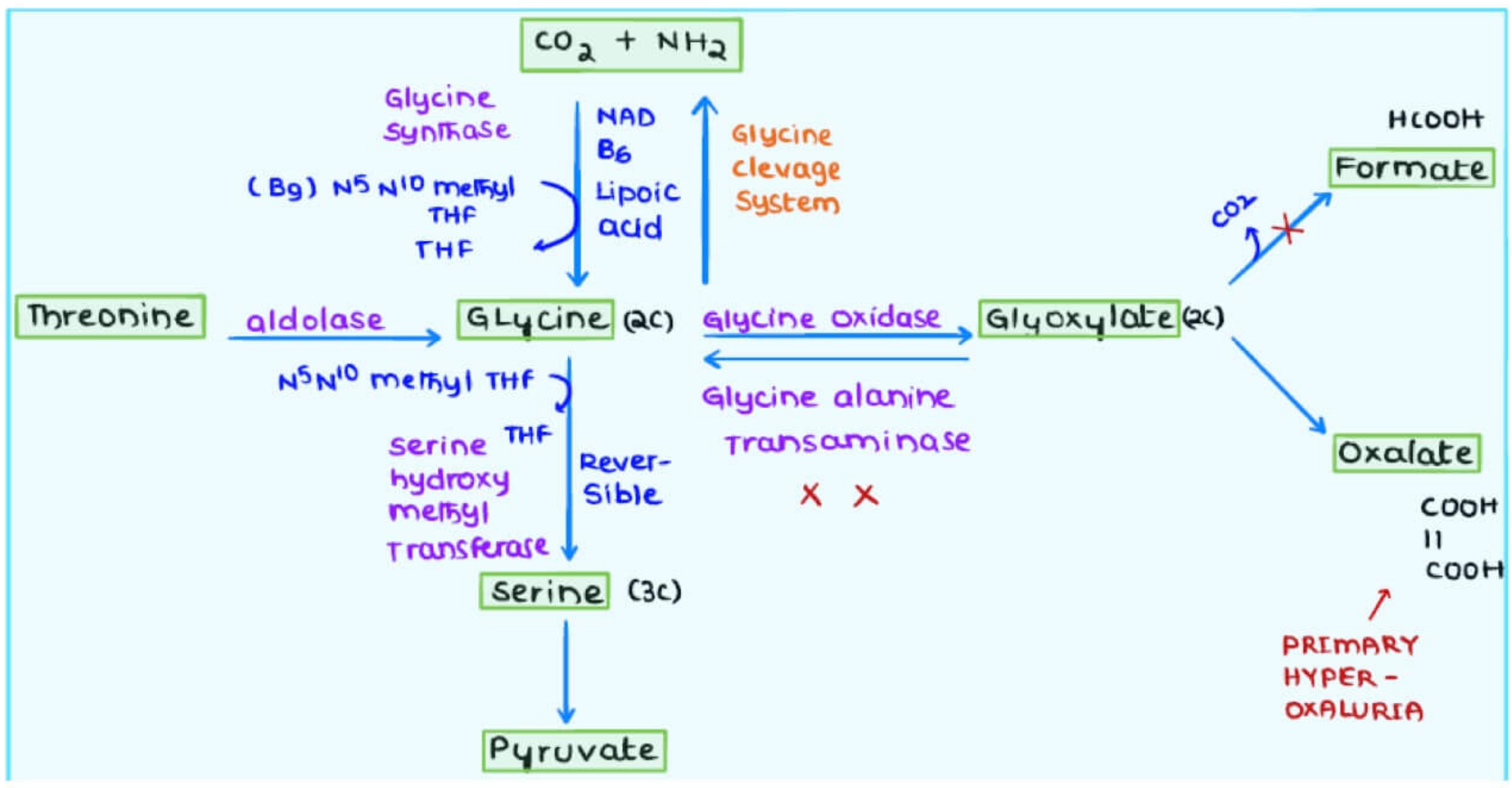
GLYCINE METABOLISM DETAIL

→ Simplest & smallest AA

→ Non polar (controversy)

→ Non-essential AA

→ Glucogenic AA



PRIMARY HYPER OXALURIA

→ Protein targeting disorder

→Oxalate stones in kidneys

→ oxalate depositions in extra renal tissues

→ Rx → hydratⁿ to prevent oxalate stone

→ restriction of oxalate rich foods

SECONDARY HYPER OXALURIA

CAUSES

1. Excess Vit C [Dehydroascorbic Acid → oxalic Acid]
2. B_6 Deficiency
3. Ethylene Glycol poisoning (glycolic acid → glyoxylate → oxalate]
4. Methoxy flurane
5. Bariatric surgery

NON KETOTIC HYPERGLYCINEMIA

→defect in Glycine clevage system

→ ↑glycine

- inhibitory NT in CNS
- excitatory NT in spinal cord

→ **C/F**

- Mental retardation
- Seizures
- Lethargy
- Apnea
- ↑ glycine in blood, CSF & urine
- aka glycine encephalopathy
- Ketone bodies not increased

→ No effective Rx

Ketotic hyperglycinemia occurs in propionic acidemia

GLYCINURIA

→defective reabsorptⁿ of 2 AA

1. Glycine
2. Proline

} Transporter for both is same

→ occurs d/t defect in the transporter

→serum glycine levels are normal

→ has ↑ risk of oxalate stone but urine oxalate is normal

USES

→ Haem

→ Glutathione

→purine rings

→serine

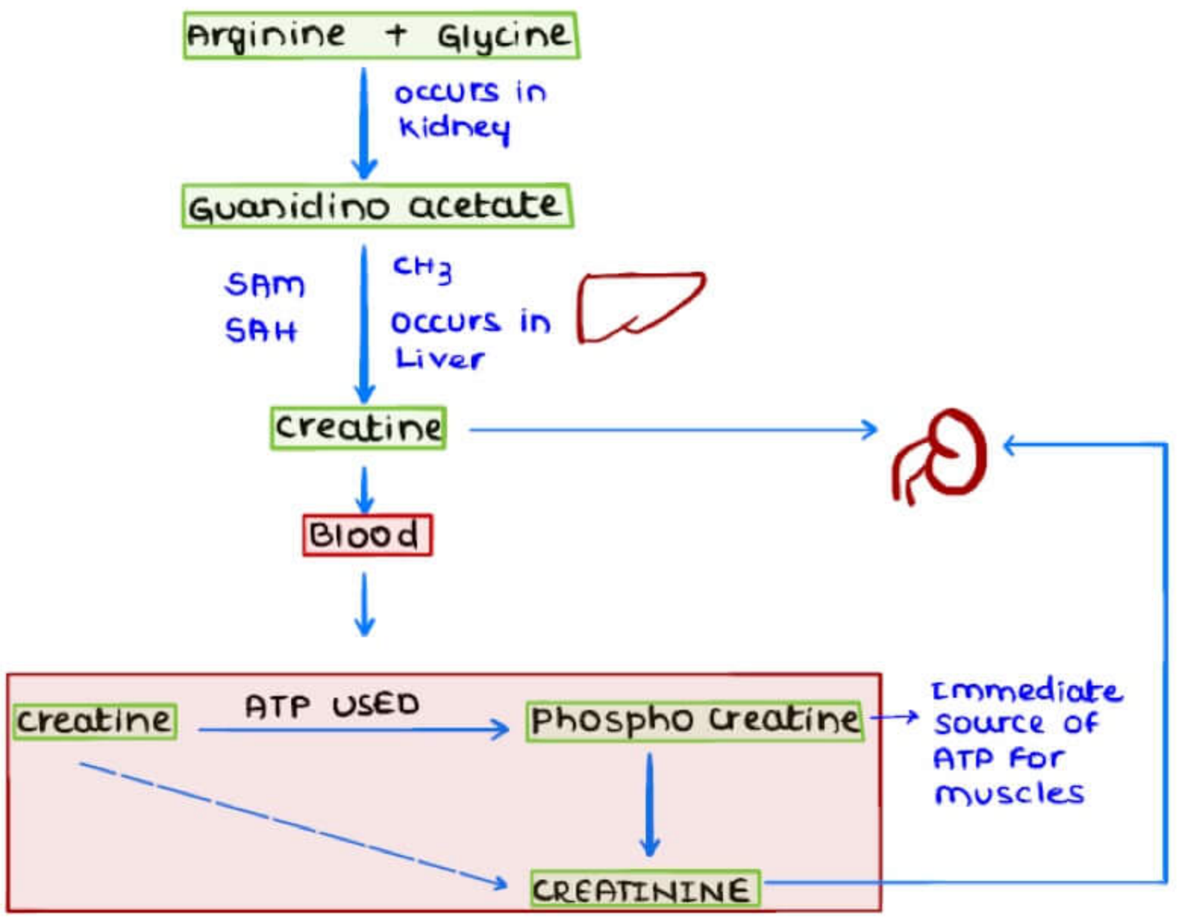
→ conjugating agent

→ NT

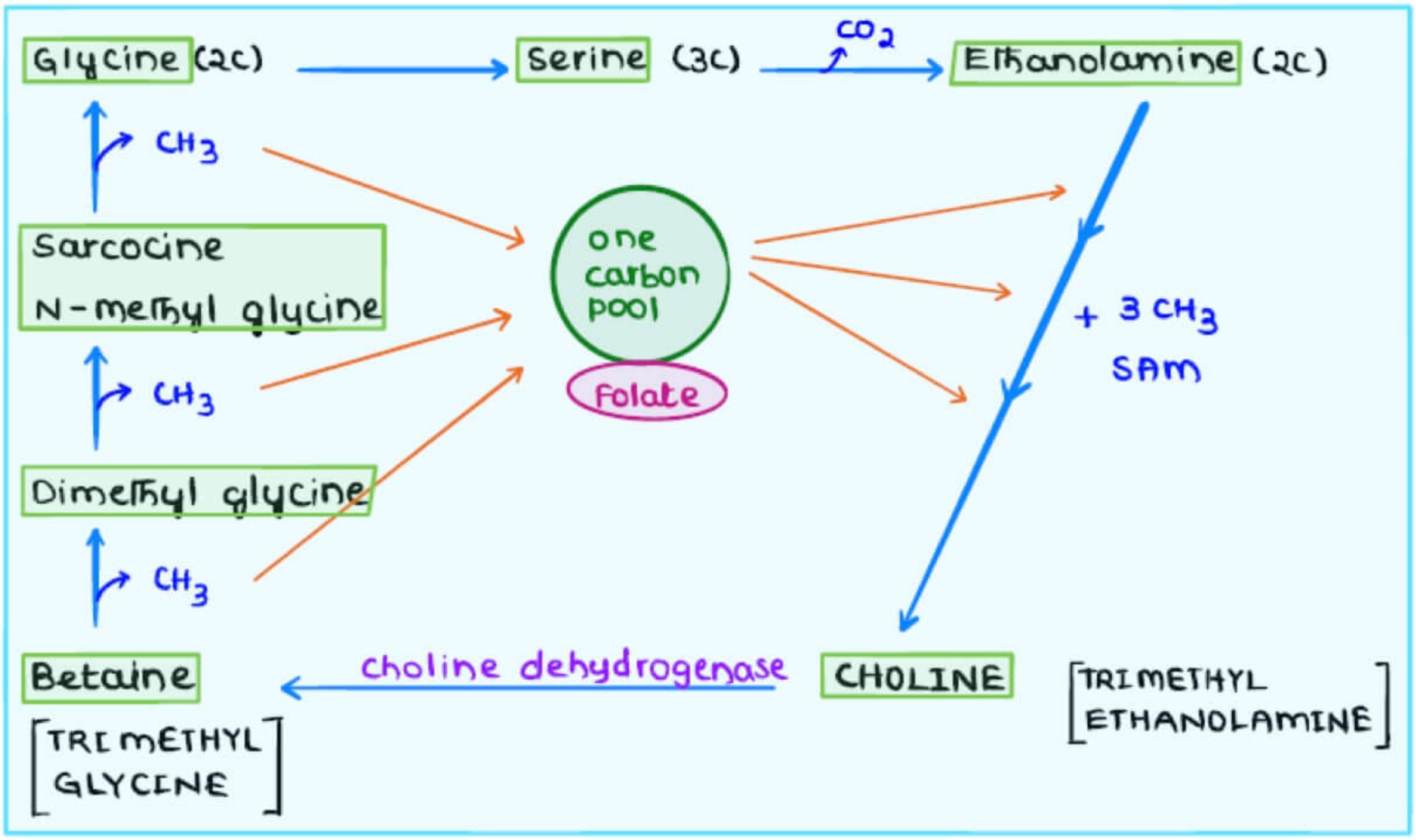
→ forms creatinine

→ forms choline

FORMATION OF CREATININE



FORMATION OF CHOLINE



Glycine metabolism is interlinked with folate metabolism

Choline & Betaine metabolism is also linked to tetrahydrofolate metabolism

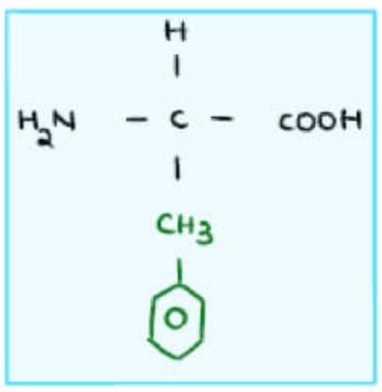
PHENYLALANINE & TYROSINE METABOLISM DETAIL

PHENYL ALANINE

Structure

→ Essential AA

→ Non polar

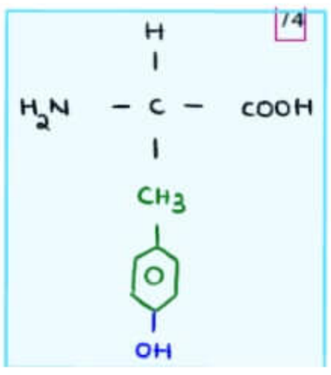


Phenylalaine

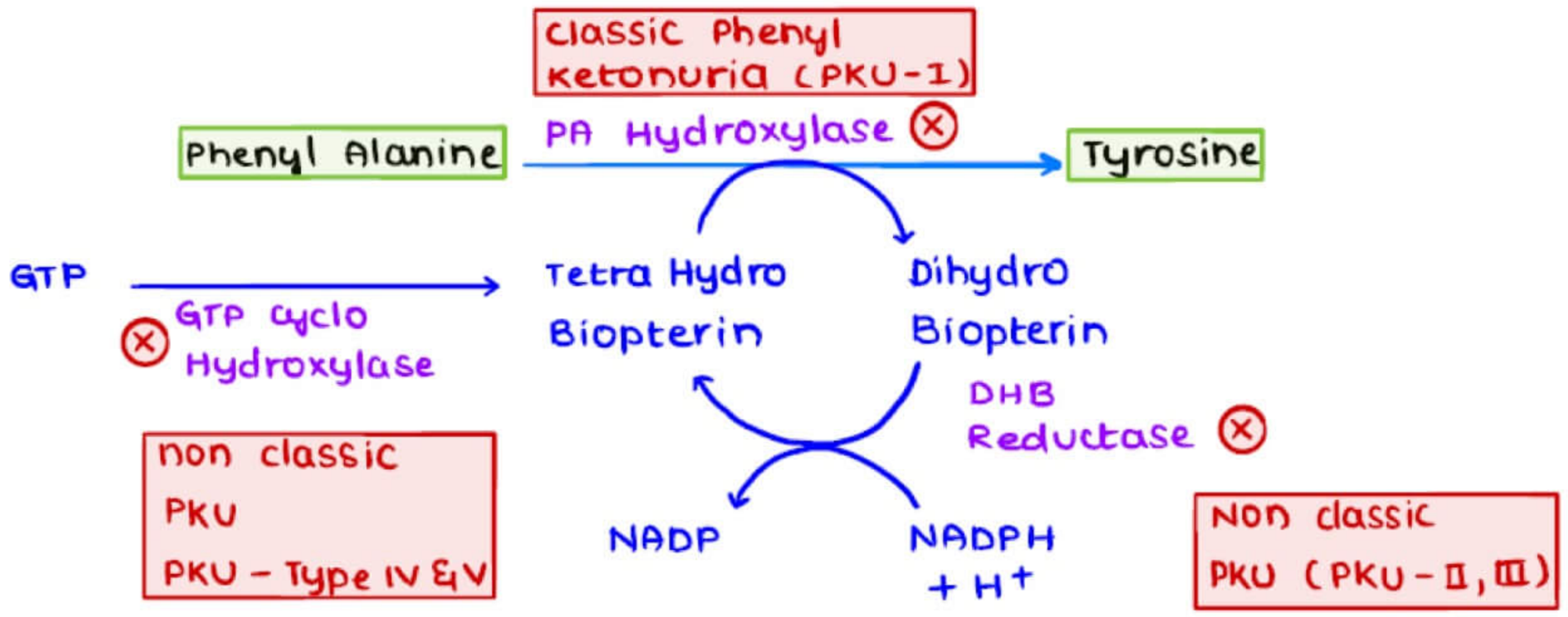
PHENYL ALANINE $\xrightarrow[[O]]{\text{Hydroxylase}}$ TYROSINE

→ non-essential amino acid

→ Polar (controversy)



Tyrosine



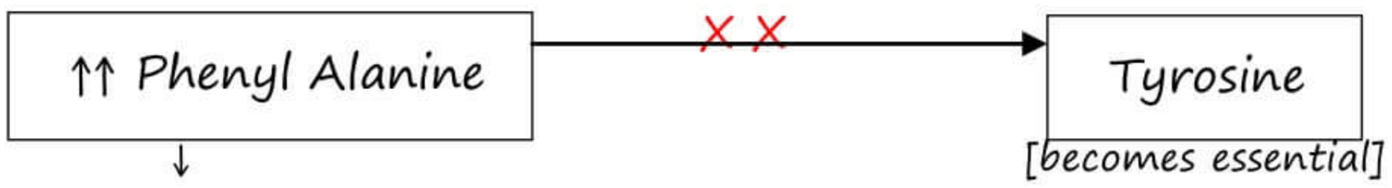
1. PAH
2. Tyrosine Hydroxylase
3. Tryptophan Hydroxylase

PHENYLKETONURIA

→ mc metabolic disorder of AA

→autosomal recessive

→ PAH deficient



Phenyl Pyruvate → Hypopigmentatⁿ
Phenyl lactate - Fair skin
Phenyl Acetate - Blue eyes

C/F

→ mousy/musty body odour d/t phenylacetate

→severe MR d/t Phenylalanine

Dx

1. $FeCl_3$ urine test → Green colour → positive

→ d/t Phenyl pyruvate

- 3 carbon keto acid comes in urine

2 DNPH Test → Positive

3. Bacterial Guthrie's test → positive

→ Bacillus subtilis is used

Screening

→ should be done after 2-3 days of birth

at birth → levels are N d/t maternal enzymes

MATERNAL PKU

→ d/t lack of proper diet in pregnancy

→child have

- Microcephaly
- MR
- Growth retardation
- congenital Heart defects

BRAIN INVOLVED DUE TO

1. ↓ Neurotransmitter

→ ↑↑ Phenyl alanine in blood → into brain cells

→ Tyrosine & Tryptophan unable to reach brain

- ↓ catecholamines (↓ Tyrosine)
- ↓ serotonin (↓ tryptophan)

2. ↓ Thyroxine

TREATMENT

1. Restrict phenyl alanine in diet

→ Lifelong restrictⁿ required

→ ASPARTAME

- artificial sweetener

- C/I
- Dipeptide
  → Aspartate + Phenylalanine

2. Give tyrosine & tryptophan
   - because Tyrosine becomes essential in this condition
   - these amino acids will enter the brain cells

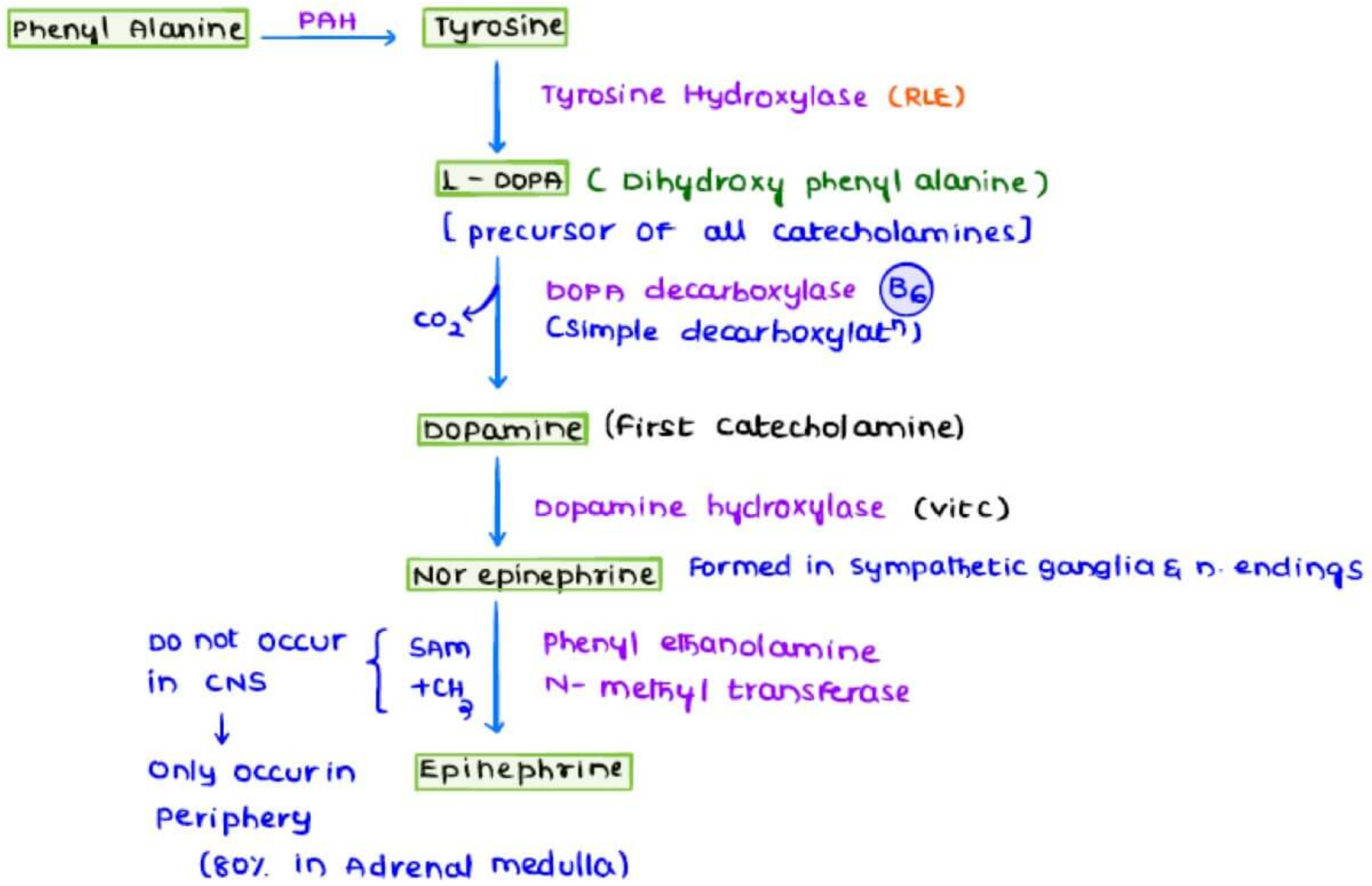
3. Tetra hydro Biopterin Supplementatⁿ specially for non-classic PKU

→ THB LOAD TEST → distinguish classic & non-classic PKU

USES OF TYROSINE

1. Catecholamine
2. Thyroid hormones
3. Melanin pigments

CATECHOLAMINE BIOSYNTHESIS PATHWAY

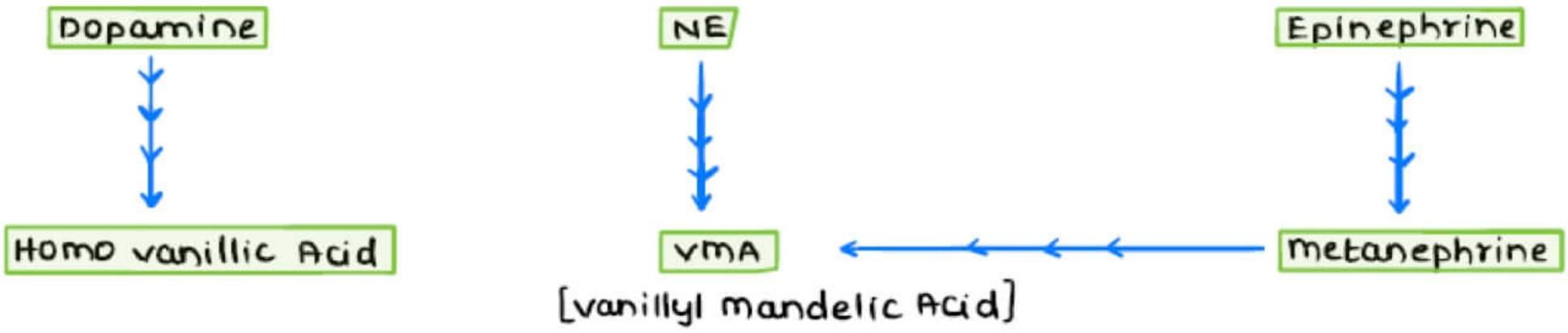


→ Catecholamine pathway has organ specific termination

→ CATABOLISM

COMT → catechol – o – methyl Transferase

MAO → mono amine oxidase



VMA [Vanillyl Mandelic Acid]

→ ↑in pheochromocytoma

- Tumor of adrenal gland
- Headache
- palpitatⁿ
- profuse sweating

→ ↑in Neuroblastoma of Adrenal gland

Dx

→24 hr urine samples collected & VMA levels measured

- (N) VMA → 2 - 6 mg / day

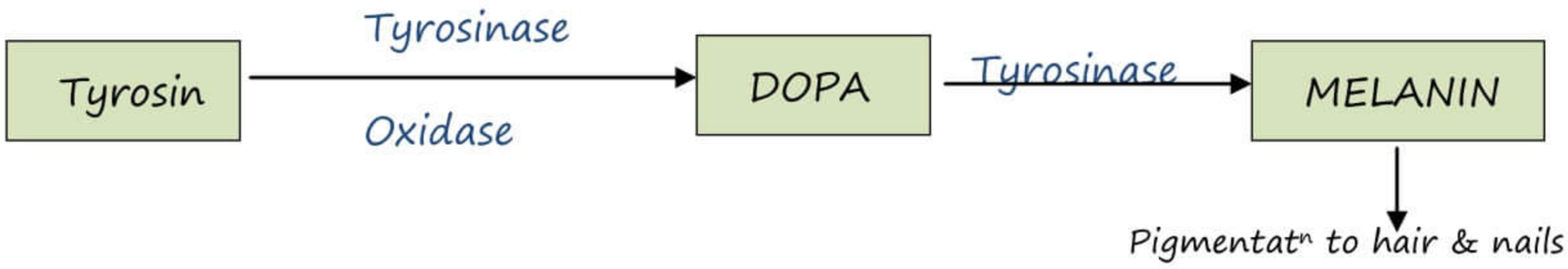
THYROID HORMONE SYNTHESIS

Tyrosine + I ⟶ Mono Ido tyrosine (MIT)

MIT + I ⟶ DIT

MIT, DIT } T_3 & T_4 (Thyroxine)

MELANIN PIGMENT SYNTHESIS



Tyrosinase

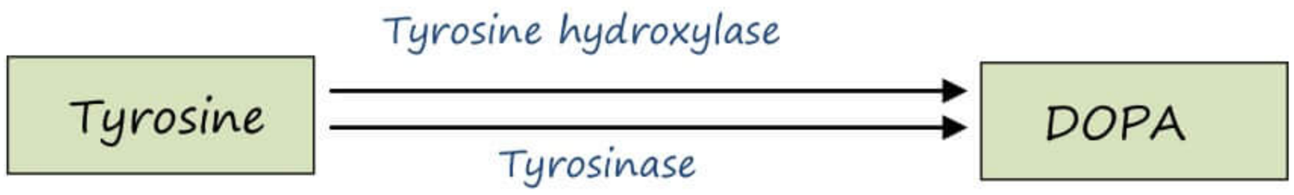
→ synthesized in melanosomes present in melanocytes of skin & hair

→deficiency causes **ALBINISM**

- milky white skin
- white hair
- red eye colour

→ **VITILIGO**

- Tyrosinase is normal
- Lack of melanoblast in regional areas



Tyrosine Hydroxylase

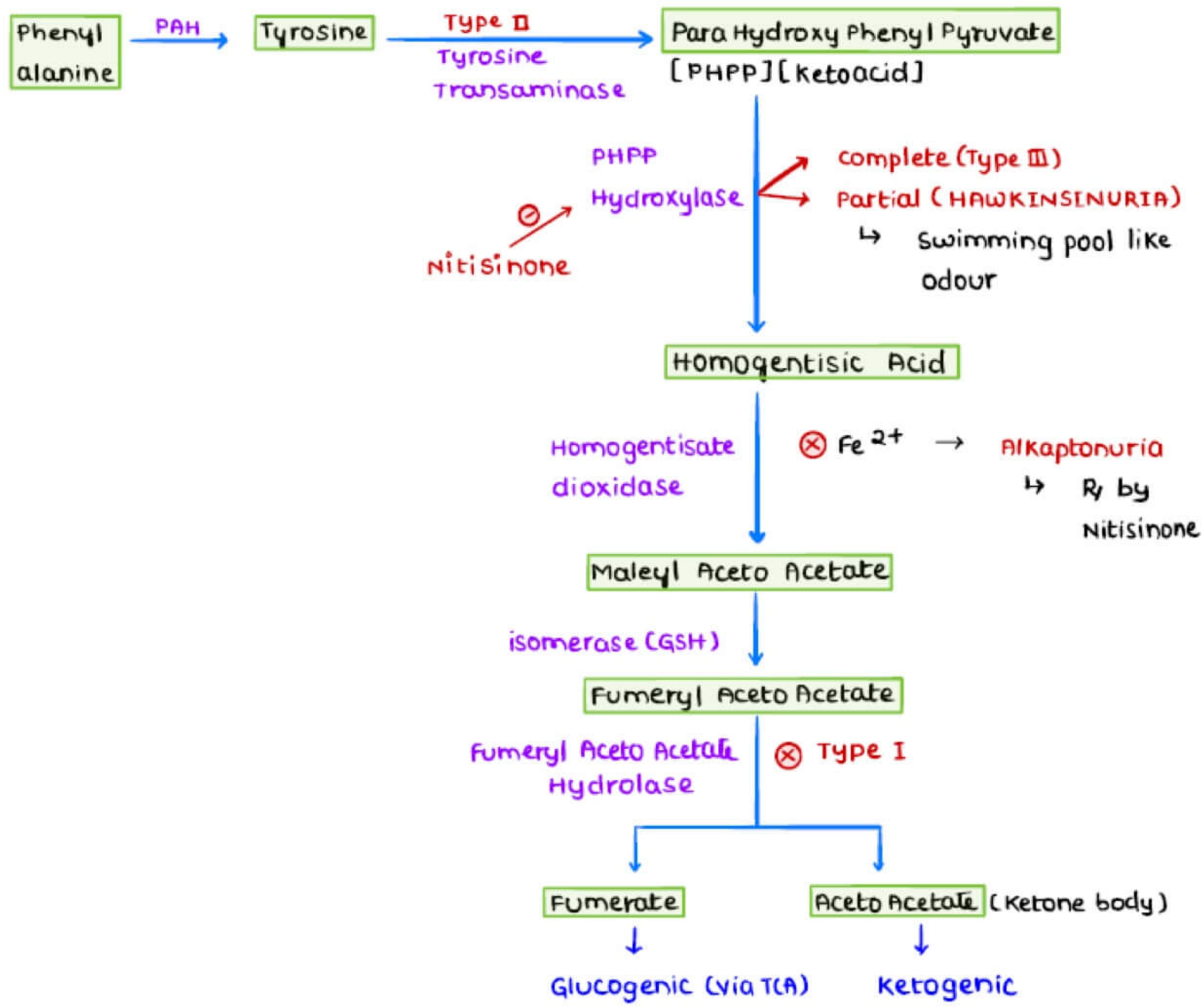
→ present in adrenal medulla, sympathetic ganglia & nerve endings

Tyrosinase

→ synthesized in melanosomes present in melanocytes of skin and hair

Diet → Phenyl alanine→ tyrosine →catabolized

CATABOLISM OF PHENYL ALANINE & TYROSINE



ALKAPTONURIA

→ d/t deficiency of Homogentisate dioxygenase (requires iron)

→Rx → NITISINONE

→ Homogenetic acid accumulated →oxidised→ Black urine

- fresh urine is normal in colour
- on standing or exposed to air → turns Black

→ GARROD 'S TETRAD

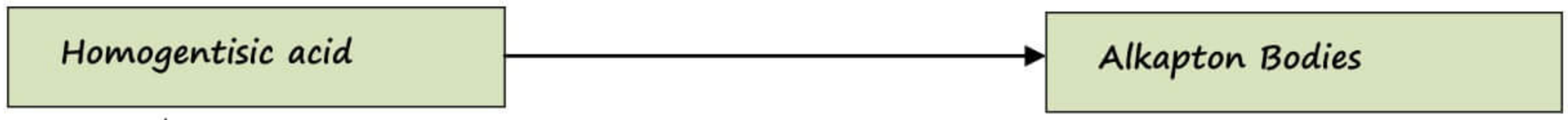
1. cystinuria
2. Alkaptonuria
3. Albinism
4. Pentosuria

Age of onset → 30 - 40 yrs

→ presents with lower back pain

→no MR

→ ALK



↓

Accumulated in

→ Cartilages → Nose, ear pinna, IV disc → Arthritis

→ Connective tissue → bluish black colour

→ Ochronosis → accumulation of Homogentisic acid in body

→ Arthritis is also known as ochronotic arthritis

→ Benedict's test is positive

→ d/t Homogensitic Acid (Reducing substance)

→ Dx by $FeCl_3$ urine test → positive

TYROSINEMIAS

TYPE I → TYROSINOSIS/ Hepatorenal Tyrosiniemia

→ mc

→ Fumaryl acetoacetate hydrolase is deficient

TYPE II → OCULO CUTANEOUS TYROSINEMIA

→ Tyrosine transaminase deficient

→ Eyes affected → corneal ulcers

Skin affected → Hyperkeratotic plaques

TYPES III → NEONATAL TYROSINEMIA

→ d/t deficiency of PHPP hydroxylase

TRYPTOPHAN METABOLISM DETAIL

side chain has INDOLE RING / NUCLEUS

→ essential AA

→ Bulky AA

- Not found in a helix

→ Non polar

USES

1. serotonin, Melatonin synthesis

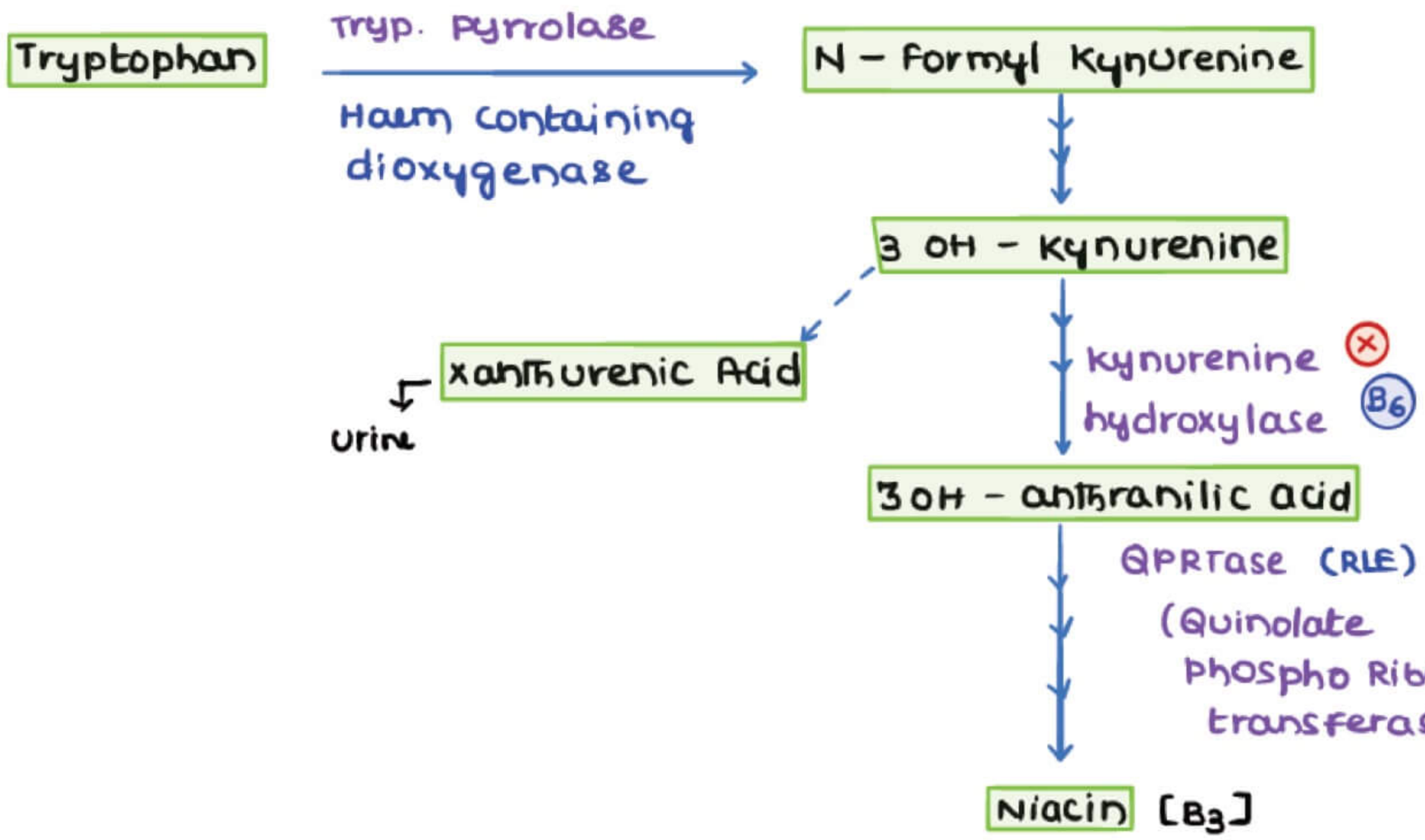
2. Niacin [Vit B_3] →Atypical vitamin

3 donor of formyl group to 1 carbon pool of Body

- C_2 of purines
- formyl methionine formation [prokaryotes]

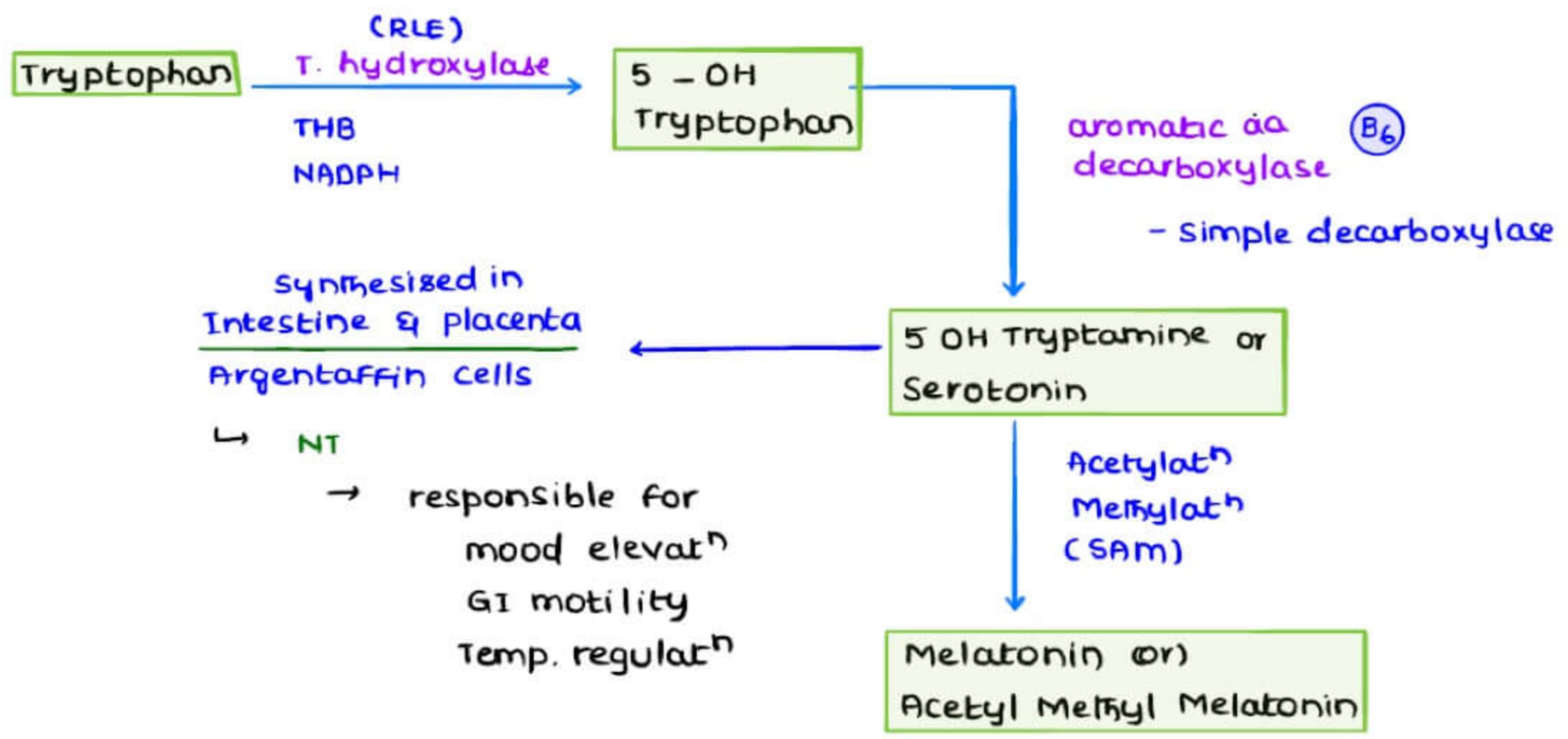
NIACIN SYNTHESIS

KYNURENINE – ANTHRANILATE PATHWAY



→ 60 mg of Tryptophan → 1 mg of Niacin

SYNTHESIS OF SEROTONIN & MELATONIN



→ Excretory end product

- 5 hydroxy Indole acetic Acid

MELATONIN

→ Neurotransmitter synthesized in penial gland

→Responsible for biological rhythm of the body

HARTNUP DISEASE

→autosomal recessive

→Failure to reabsorb & absorb neutral AA & tryptophan

- Neutral AA & tryptophan has same transporter in intestine and kidney
- These transporters are defective

CIF

→ Amino Aciduria (Tryptophan)

→pellagra like symptoms

→Tryptophan (that is not absorbed from intestine)

↓

Bacterial action

↓

Indoxyl compounds formed

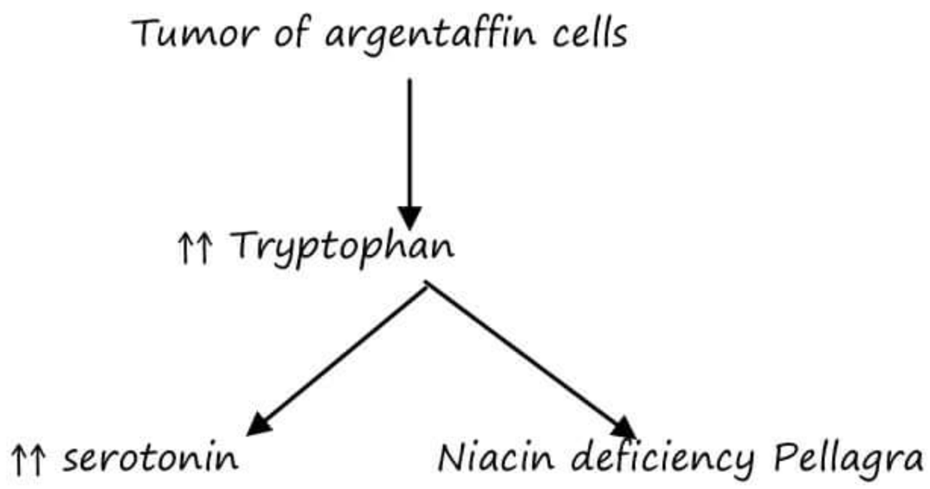
↓

Blue colour diaper

→In intestine, Indican compounds are formed

→ detected by OBERMEYER TEST [positive]

CARCINOID SYNDROME



C/F

→ profuse sweating

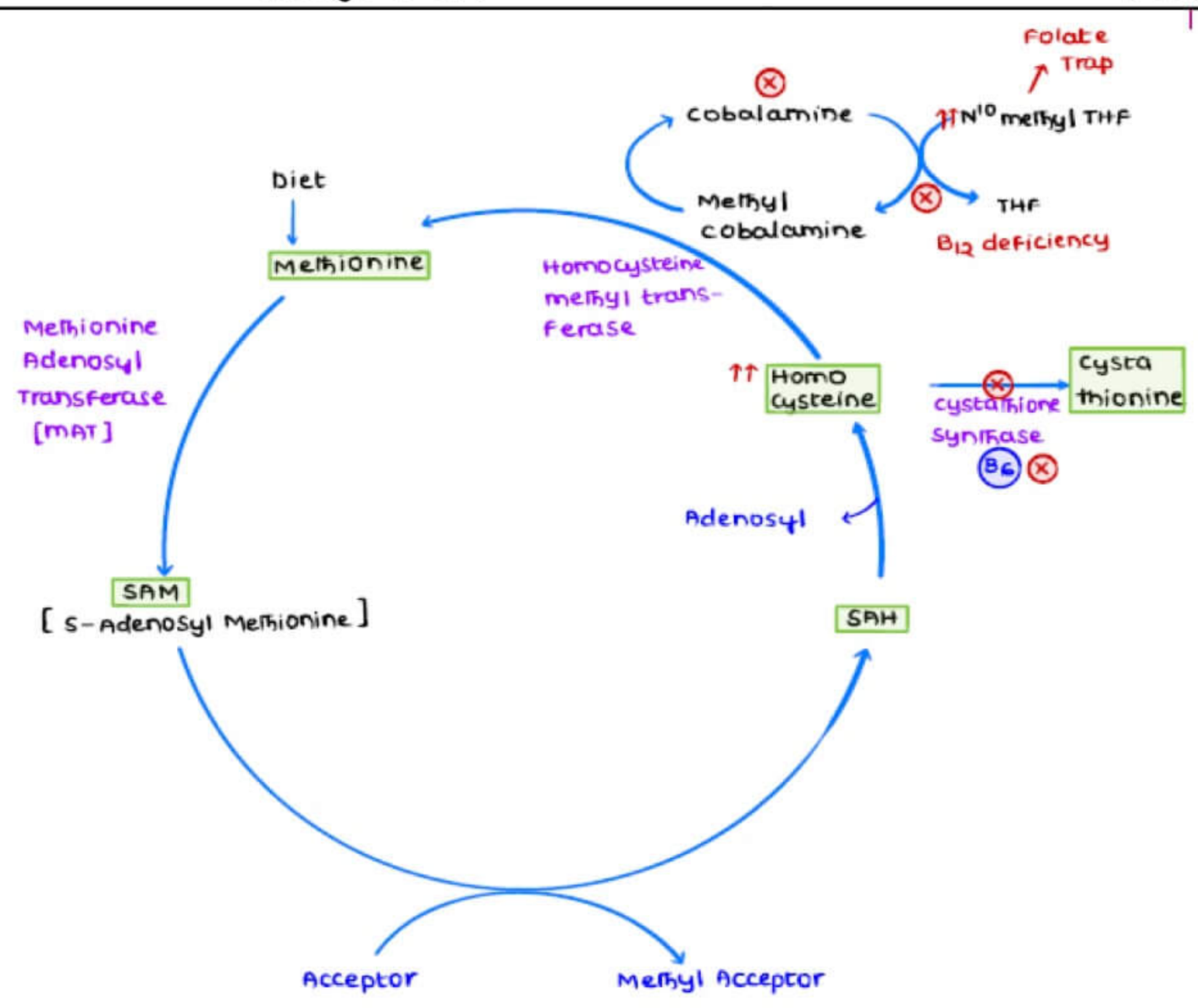
→ flushing

→ GI motility

→ 5 Hydroxy Indole Acetic Acid (HIAA) comes in urine

METHIONINE & CYSTEINE DETAIL

CYSTEINE	METHIONINE
H - S - C	C - S - C
Polar	Non-polar
Non-essential AA	Essential AA
Glucogenic AA	Glucogenic AA



Reaction requiring Methyl groups

1. 7-methyl guanosine cap of mRNA

2. NE → Epinephrine

3. cephalin [ethanolamine]→ Lecithin [Choline]

4. Guanidoacetate → creatine

5. Acetyl serotonin → melatonin

→ MAT Type I & 3 → present in Liver

→ MAT type 2 → present in extra hepatic tissues

→Homocysteine Metabolism

1. If enough of methionine available in diet

Homocysteine ⟶ cystathionine

2. If enough of methionine is not available in diet

Homocysteine ⟶ Methionine

FOLATE TRAP

→ occurs due deficiency of Vit B_{12}

→ ↑↑ methyl THF ⟶✕ THF

→ Functional deficiency of folate

- folate accumulated in the form of methyl THF

Homocysteine accumulation occurs in case of B_6, B_9 , B_{12} deficiency

→ Homocysteine

- irritates endothelium of blood vessels
- causes stroke, atherosclerosis, pulmonary embolism & MI

OTHER C/F

→ MR

→ Ectopia lentis

→ seizures

→ Osteoporosis

→ Marfanoid habitus

- Similar to Marfan syndrome

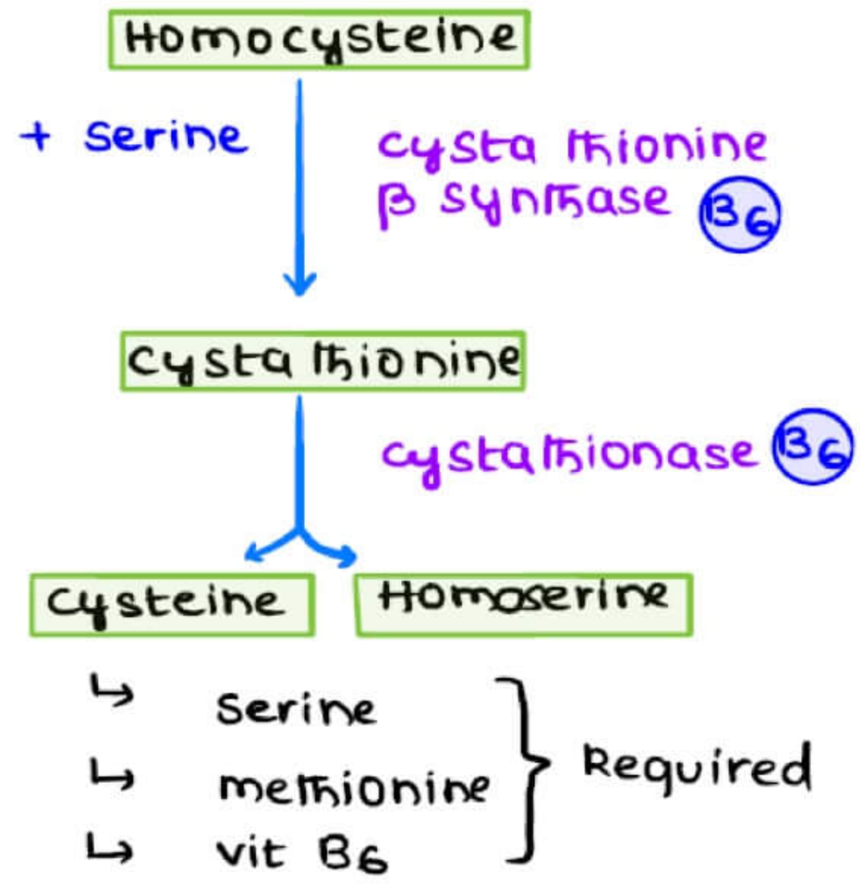
Except

→ Lens dislocated outwards & upwards in Marfan syndrome

→ Lens dislocated downwards & inwards in homocystinuria

TYPES

1. GENETIC → d/t enzyme deficiency
2. ACQUIRED → d/t vitamin deficiency (B_6, B_9, B_{12})



→ Serine
→ Methionine
→ Vit. B6
} Required

Cysteine is non-essential as it is derived from Methionine

It becomes essential, once methionine is deficient in diet

GENETIC HCU

Type I/ Typical/ Classical type	Type II/ Non- Classical type
Cystathionine β synthase deficiency (more common)	Main defect is in conversⁿ of homocysteine to methionine (Less common)
↑ Methionine	↓ Methionine
Cysteine becomes essential	Cysteine is non-essential
Rx: - ↓ Methionine in diet - Give cysteine	Rx - Betaine (Trimethyl glycine) ↓ Homo cysteine ⟶ Methionine

Dx

1. Cyanide Nitroprusside Test

→positive for

- cysteine
- cystine
- Homocystine
- Homocysteine

→ CN breaks the S ~ S bond → SH liberated

Na Nitroprusside react with & gives Magenta/ Red purple colour

Homocystinuria	Cystinuria	Cystinosis
→ AR	→ AR	→ LSD
→ CNT ⊕	→ CNT ⊕	→ CNT ⊕
	→ Defect in dibasic AA transporter	

CYSTINURIA

→d/t dibasic Amino Acid Transporter

- present in intestine & kidney
- defective absorption & reabsorption of

C - Cystine

O - Ornithine

L - Lysine

A - Arginine

Rx Of CYSTINURIA

→ aim is to ↑ solubility of cystine stones

1. Good hydration
2. Alkalisation of urine
3. Chelating agents like penicillamine

Penicillamine + cystine → soluble compound

CYSTINOSIS

→ CNT ⊕

→ Generalised Lysosomal storage disease

→ Defect in cystine transporter (cystinosin) in lysosomes

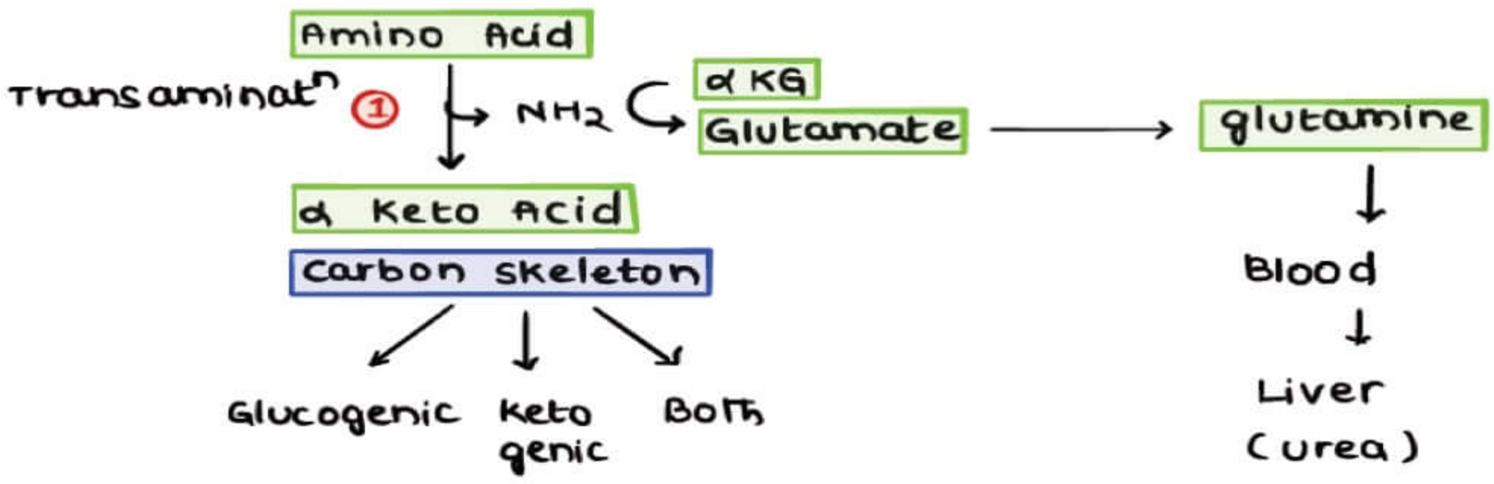
→ Cystine deposits occur in

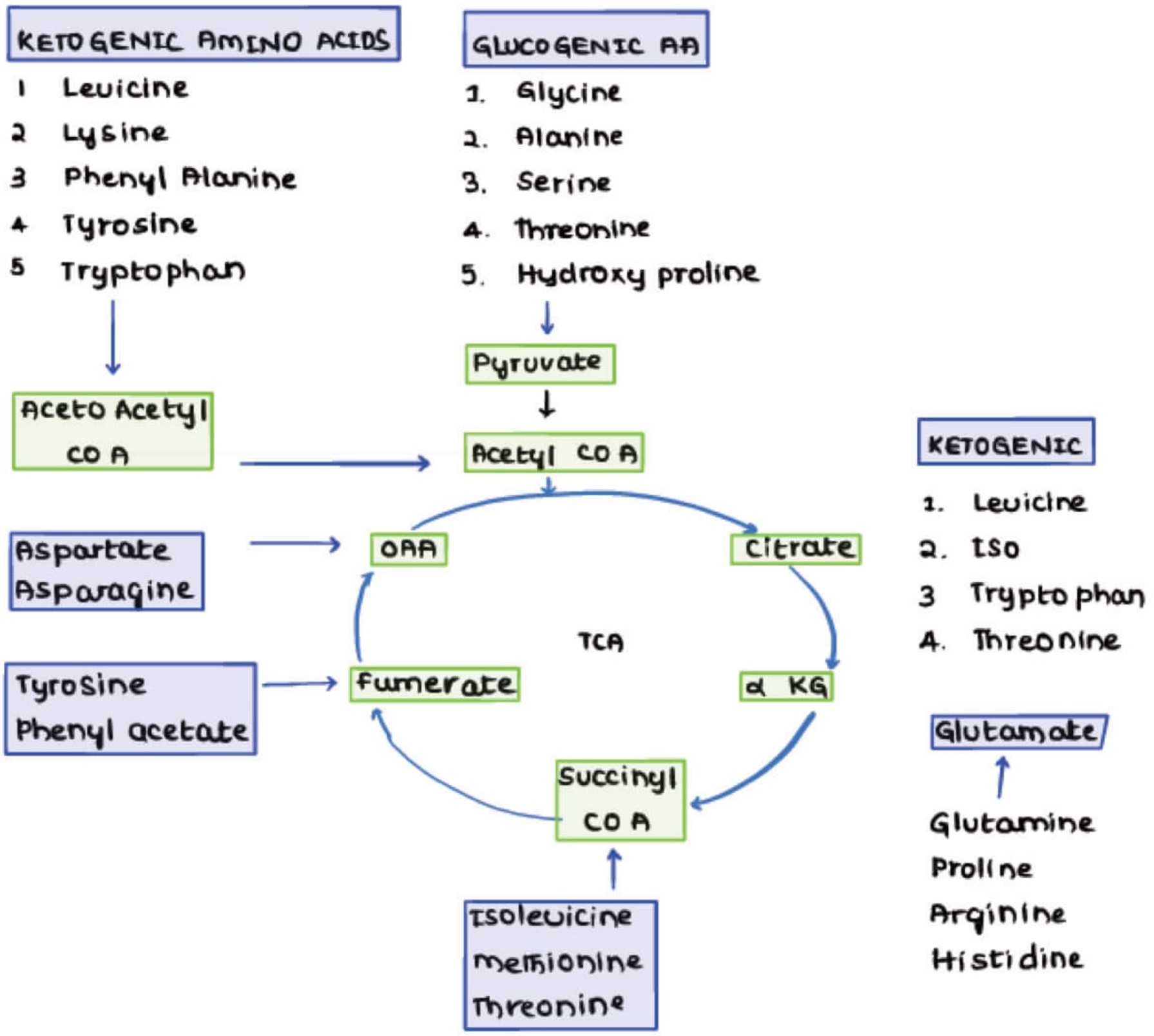
Bone marrow, cornea, Liver & kidney etc.

→ Rx→ CYSTEAMINE → forms complex with cystine

UREA CYCLE

CATABOLISM OF AMINO ACIDS





Any AA forming intermediate of TCA CYCLE is glucogenic

→PROLINE

- Forms Glutamate as a end product of carbon skeleton of proline
- As an end product of nitrogen of proline , proline can never form glutamate via transamination

NITROGEN EXCRETION

TRANSAMINATION REACTION

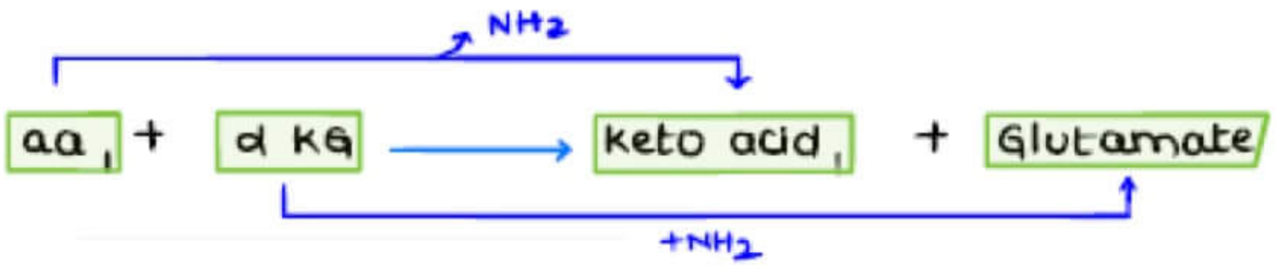
→reversible

→ require (B_6) → PLP (pyridoxal phosphate)

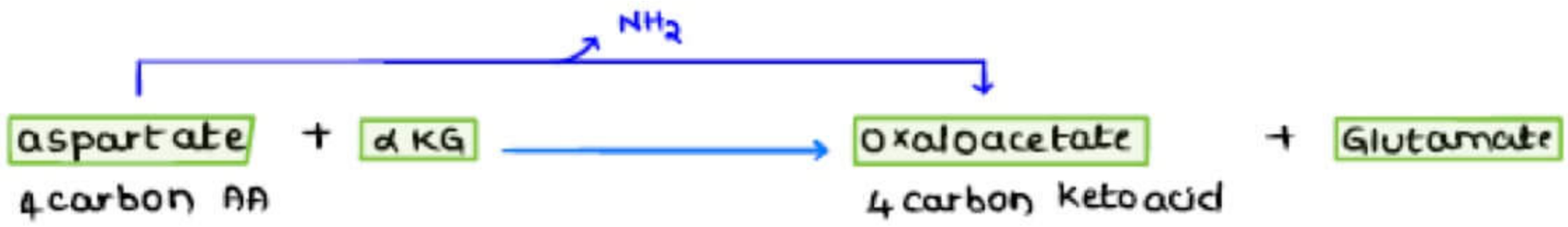
→have covalent catalysis → strong covalent bond formed

→ 1st react^n in the catabolism of AA

→common AA → Glutamate



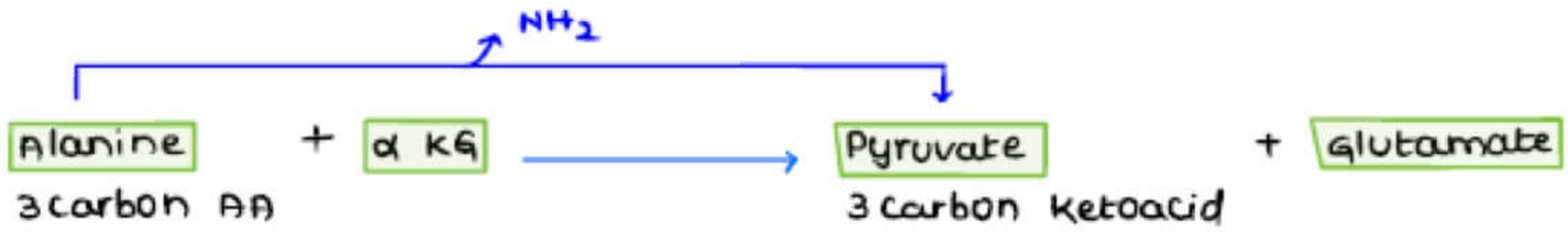
SGOT/ AST



SGOT→ Serum glutamate oxaloacetate transaminase

AST → Aspartate transaminase

SGPT/ALT



SGPT → Serum Glutamate Transaminase

ALT → Alanine Transaminase

→ specific for 1 pair of substrate

Asp —SGOT→ OAA

→Name is given after the AA from which amino group is removed

→ Important for the synthesis of non-essential AA.

EX:

OAA → Aspartate

Pyruvate → Alanine

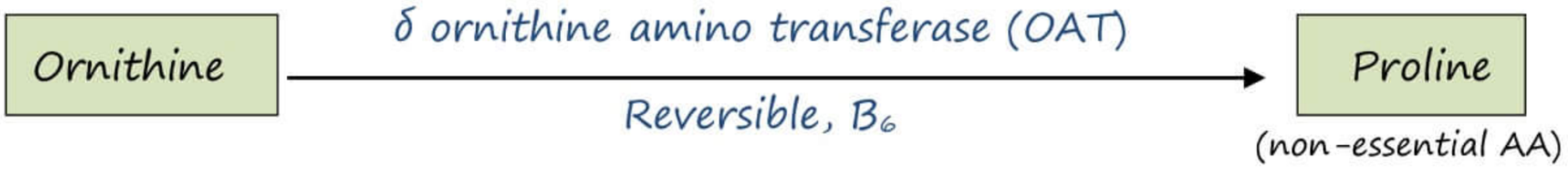
α-KG → glutamate

glyoxalate→ glycine

→ only α amino group can take part in transanimation

Exception

→ δ amino group of ornithine



→ OAT → Present in liver kidney, retina, brains

→ deficiency → Gyrate atrophy of choroid & retina

-rare

-autosomal recessive

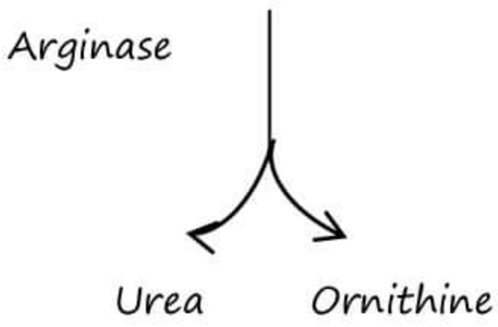
-↑↑ ornithine

-Proline not formed

-Vision loss, cataract

- Rx → Vit B_6

Restrict Arginine



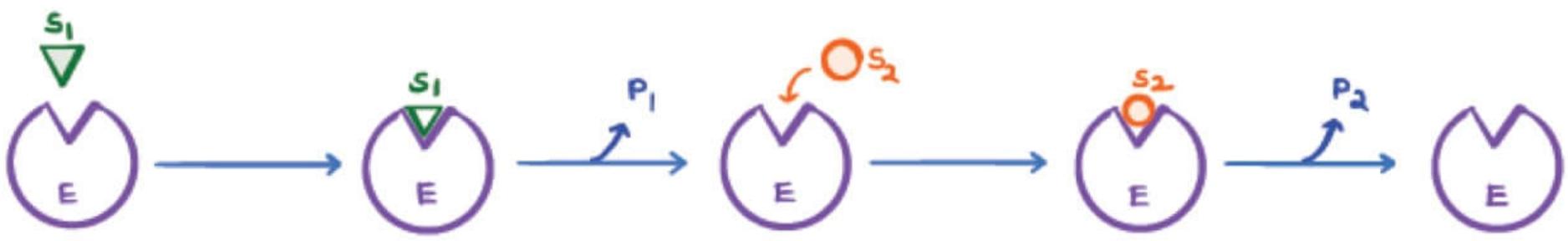
Transaminases

→Covalently bound to PLP with

1. Electrostatic interactions
2. Shiff base formation b/w aldehyde group of PLP & δ-amino group of lysine residue in the enzyme (Apoprotein)

PING PONG MECHANISM OF BI BI REACTIONS

→ Prosthetic group is modified during reaction & 1st product released before 2nd substrate binds

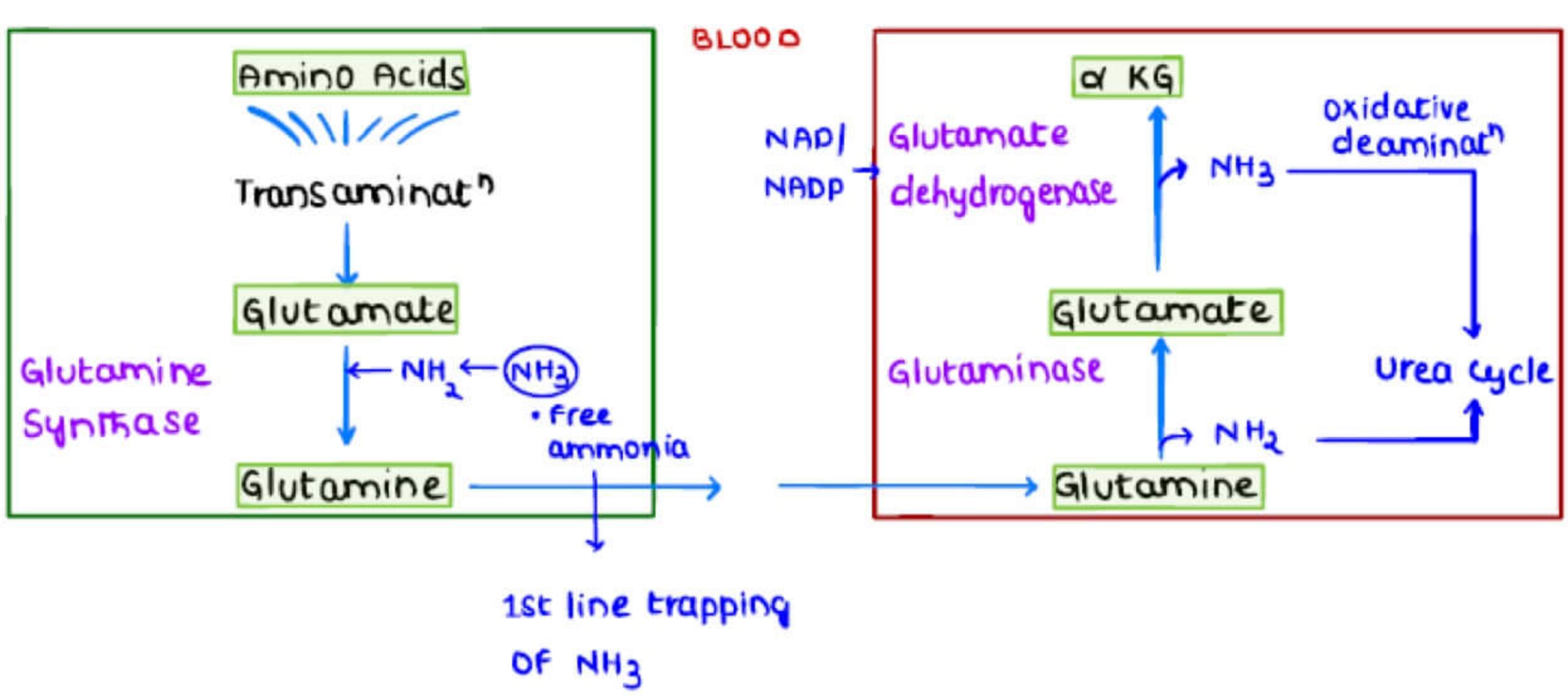


→ 17 amino acids can take part in transamination

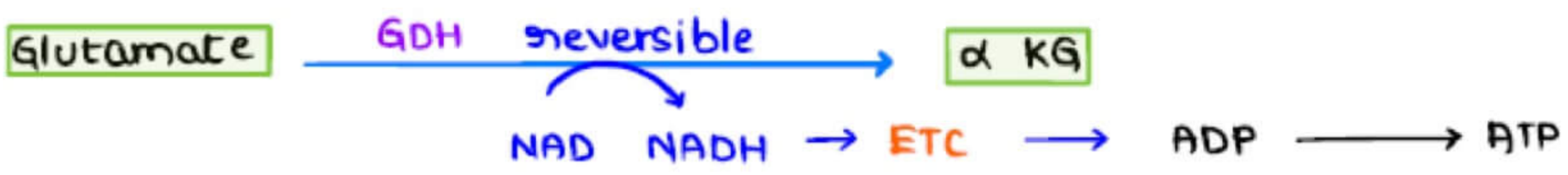
→ 17 amino acids can form glutamate

Rest 3 Amino acids can't take part in transamination

1. PO Proline, OH - proline
2. LY Lysine
3 THENE Threonine



GDH



Activators of GDH	Inhibitors of GDH
1. NAD	1. ATP
2. ADP	2. GTP
3. GDP	3. NADH

→ Occur only in Liver

→ Glutamate is the only AA, that can undergo oxidative damination to release amine group in the liver

GLUTAMINE SYNTHETASE

Asparagine synthetase	Glutamine synthetase
Aspartate → Asparagine	Glutamate → Glutamine
Source of nitrogen → glutamine	Source of N_2 → Free NH_3
No role in nitrogen exretion	Has a role in nitrogen excretion

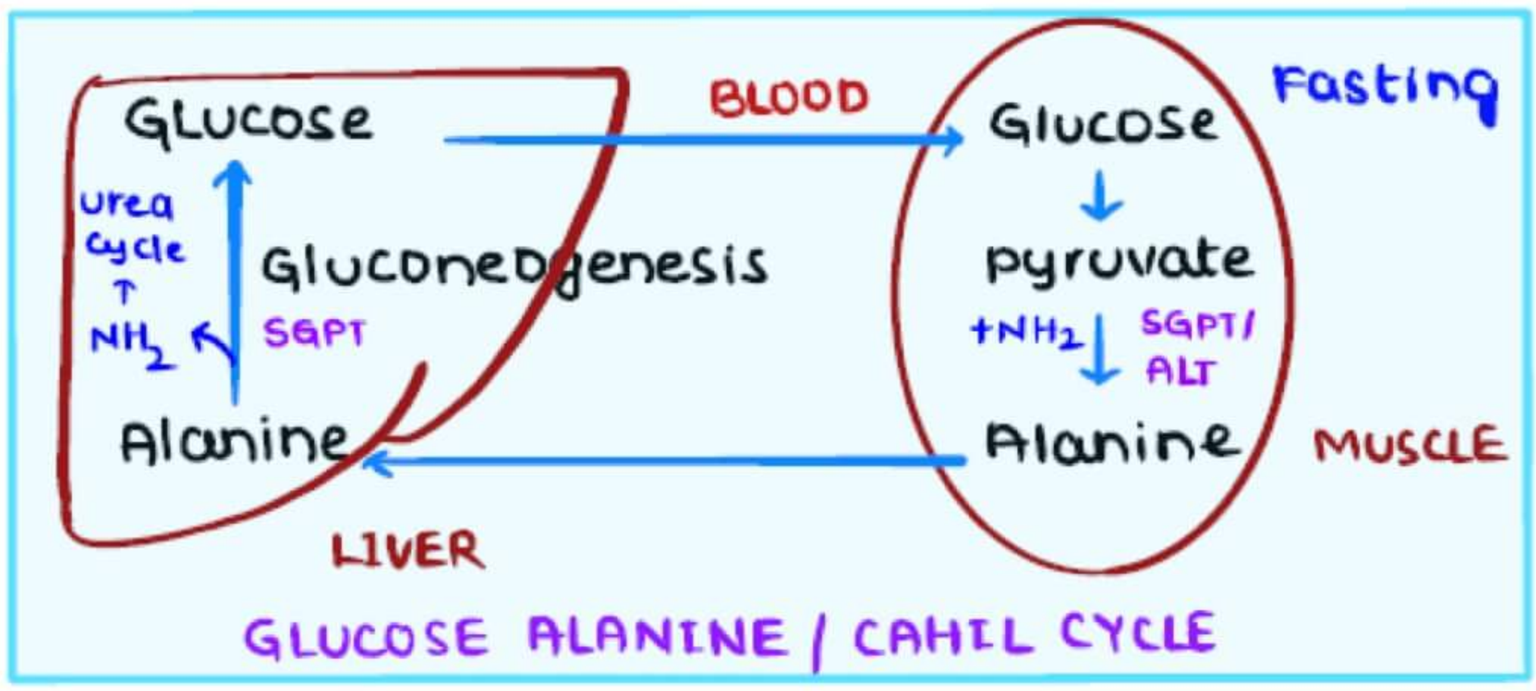
Transport form of NH_3

→ From body / most tissues } Glutamine

Brain

Muscles } Alanine (Cahill cycle)

CAHILL CYCLE OR GLUCOSE-ALANINE CYCLE



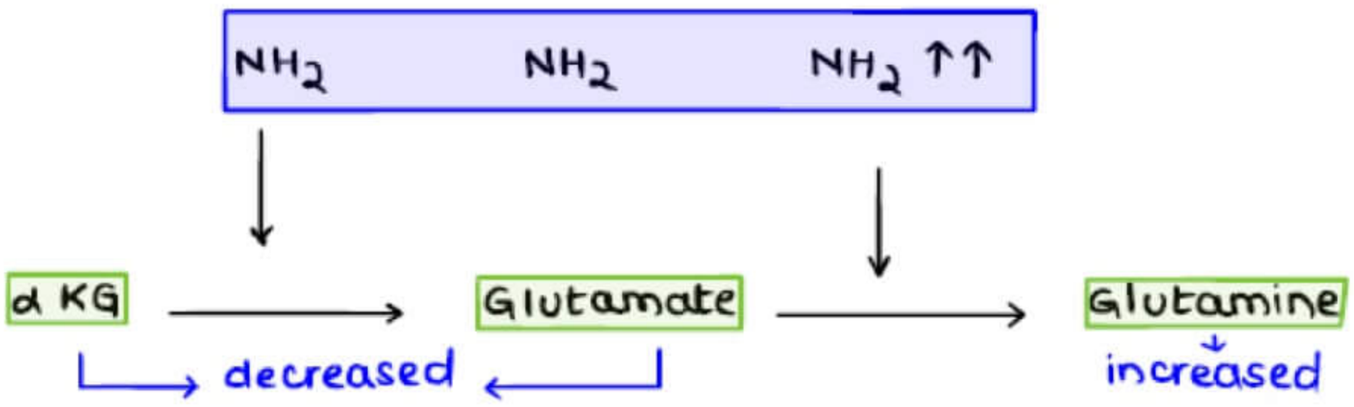
TRANSDEAMINATION: Transamination [All organs] + Oxidative deamination [liver]

SOURCES OF NH_3

1. α- NH_2 group of AA by transamination (mc & most abundant)
2. Porphyrins acted upon by PBG deaminase to release NH_3
3. Purines & pyrimidines

HYPERAMMONEMIA

→ Occurs in any urea cycle enzyme disorder



1. Depletion of α KG

→ TCA cycle affected & ATP not produced

→ Brain affected First

2. Depletion of Glutamate

→ Excitation in Brain → Fine Tremors

3. ↑ Glutamine → Osmotically active

→ ↑ Blood ↑ CNS → Cerebral edema

C/F of Hyperammonemia

1. Blood glutamine ↑
2. Blood alanine ↑
3. Blood urea nitrogen ↓
4. Cerebral edema
   - Vomiting
   - Fine tremors
   - Lethargy
   - Slurred speech
   - Blurred vision
   - Hyperventilation
   - Coma & Death (if not treated d/t Respiratory Failure)

UREA CYCLE / KREB'S HANSELEIT CYCLE / ORNITHINE CYCLE

→Ornithine is regenerated → ornithine cycle

→ CPS-I [Carbamoyl phosphate synthetase - I]

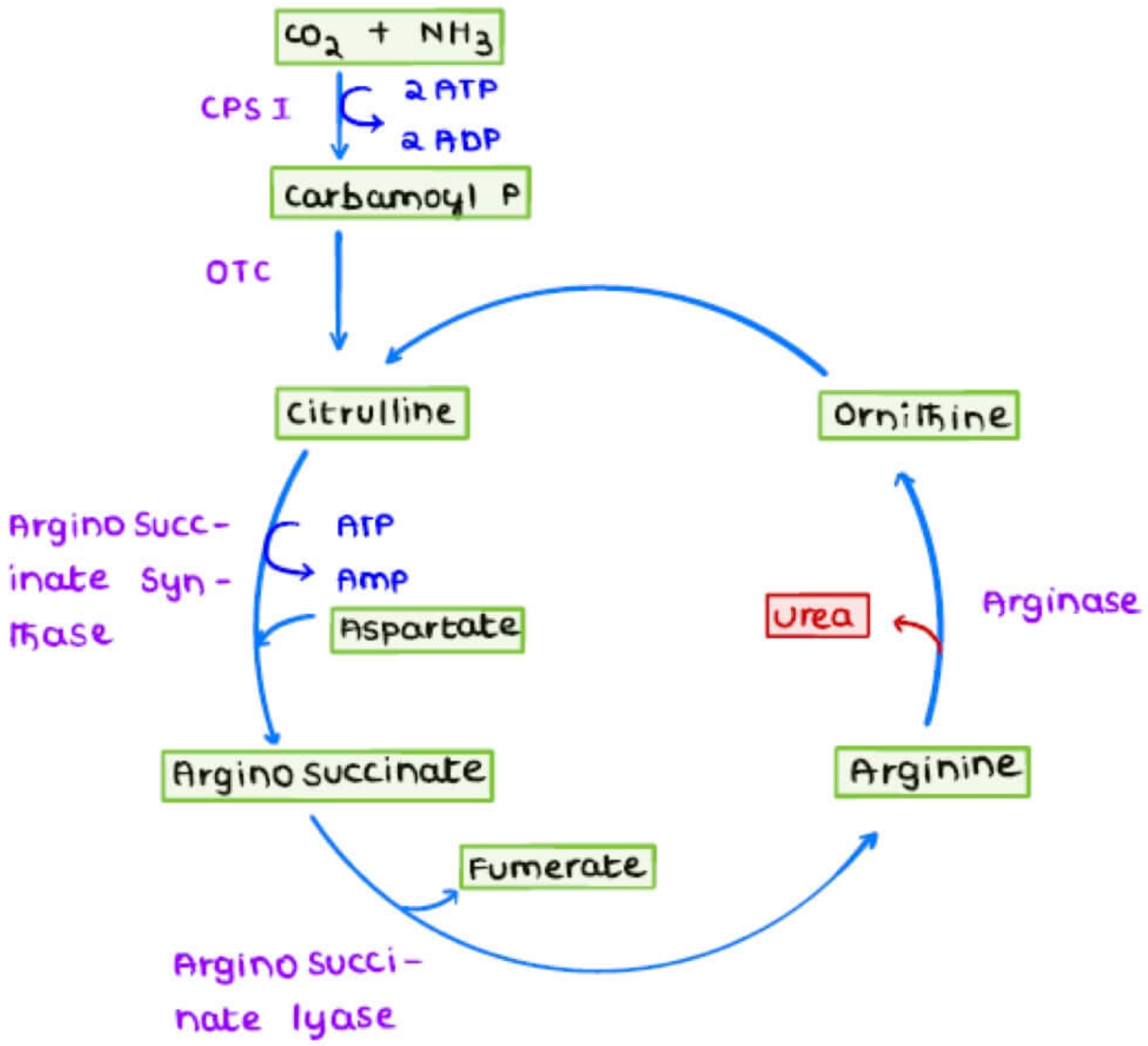
- RLE
- Pacemaker enzyme
- Committed step

→ CPS-II → involved in Pyrimidine synthesis

→ Organ → only liver

→ Compartments → Both in mitochondria & cytoplasm

MITOCHONDRIA



OTC - Ornithine Transcarbamoylase

→Absent in brain

→ OTC Deficiency is mc urea cycle defect

ARGINASE

→ Absent in kidneys

- End product of urea cycle in kidneys → Arginine

→ Arginine

- Semi essential AA
- Major source of Arginine → Kidney

CPS II

Cytoplasm

2 ATP + CO_2 + Glutamine

↓ CPS - II

Carbamoyl P

↓

Orotic Acid

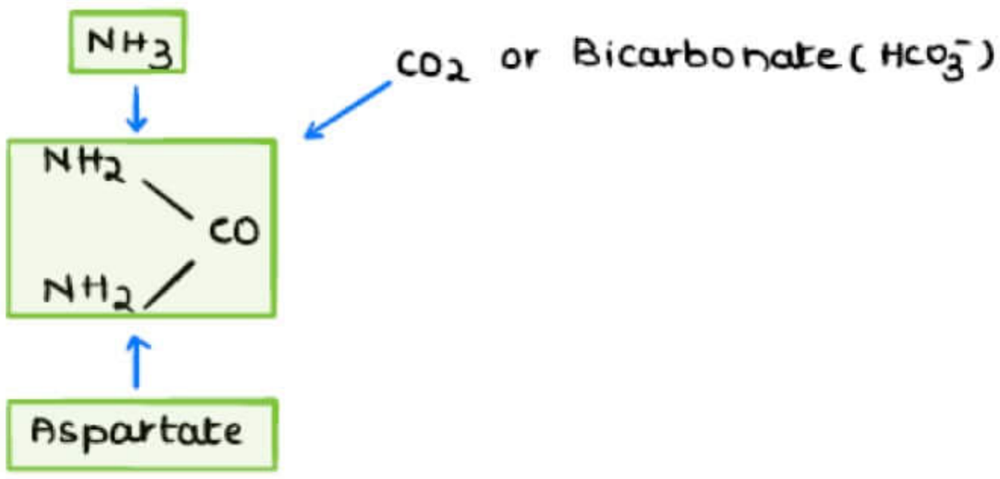
↓

OMP [Orotidine monophosphate]

↓

UMP [Uridine monophosphate]

UREA



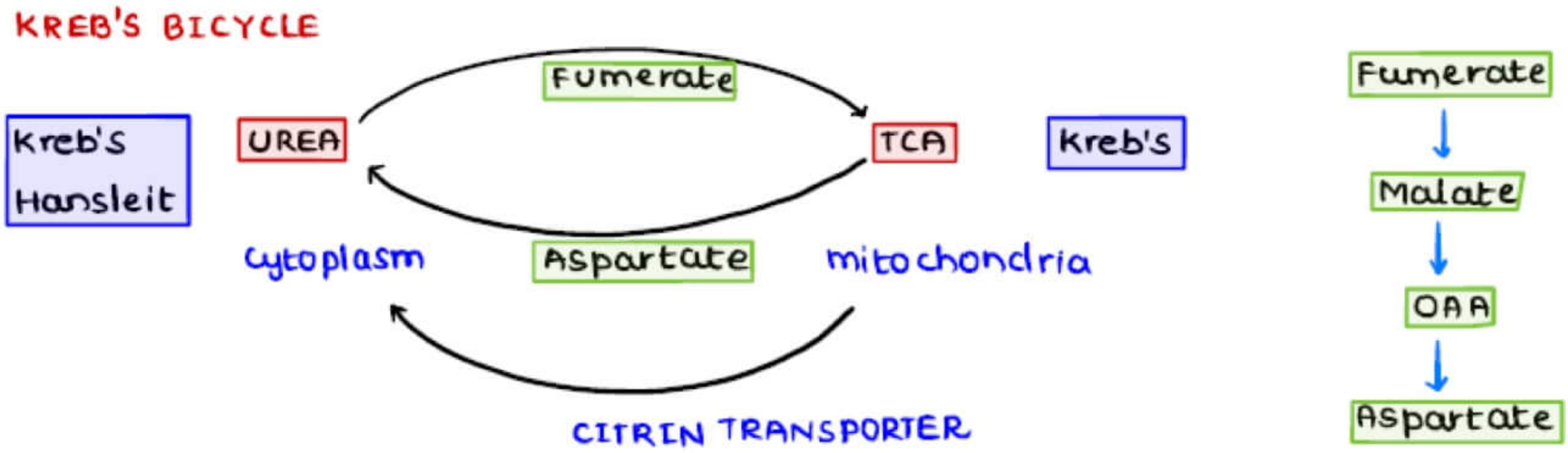
Energetics (controversy)

CPS - I → 2 ATP → 2 ADP

As synthetase → ATP → AMP (1 ATP)

} 4 ATP

(3 ATP + 4 high energy phosphates)



CITRIN TRANSPORTER

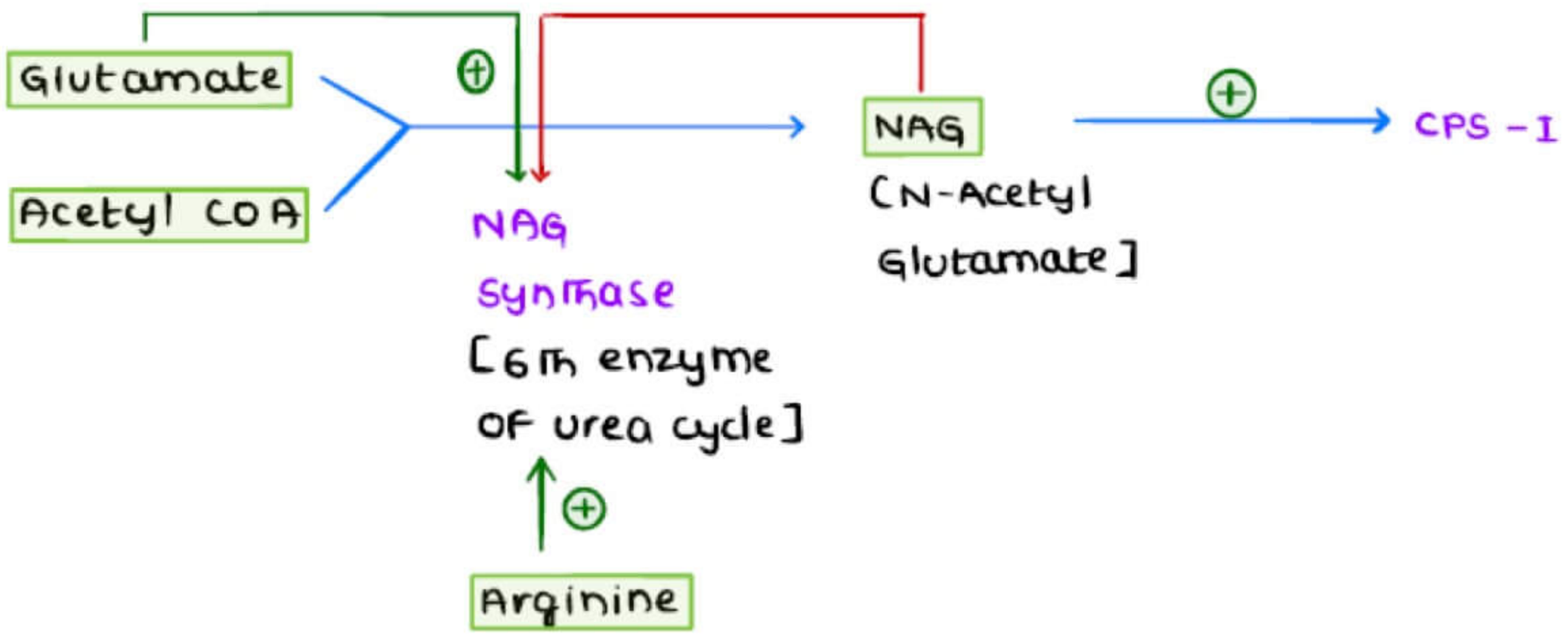
→Also needed in Malate-Aspartate NADH Shuttle

→Defect causes CITRULLINEMIA TYPE II

REGULATION OF UREA CYCLE

→During starvation, cycle activity increases d/t ↑ protein catabolism

→Protein rich diet → ↑ Urea cycle activity



→ without activation by NAG, CPS will not work

- Deficiency OF NAG synthase ≡ Deficiency of CPS-I

UREA CYCLE DISORDERS

ENZYME	SUBSTRATE ↑ ↑	DISORDER
1. CPS – I	NH_3 only	Hyperammonemia type 1
2. Ornithine trans carbamoylase	NH_3, OMP, UMP, orotic acid	Hyperammonemia type 2
3. Argino succinate synthetase	NH_3, citrulline	Citrullinemia type 1
4. AS lyase	NH_3, Arginosuccinic Acid	Arginosuccinic aciduria
5. Arginase	NH_3, Arginine	Hyper argininemia

Hyperammonemia type 1
Hyper ammo hernia type 2
} More severe
- NH_3 present in inorganic form

Citrullinemia type 1
Arginosuccinic aciduria
Hyperargininemia
} Mild
- NH_3 present in organic form

ALL UREA CYCLE DISORDERS ARE AUTOSOMAL RECESSIVE

Except → OTC Deficiency → X linked Recessive → Most Common

AS Lyase deficiency → TRICHOREXIS NODOSA
- brittle hair
- tufted hair
- habitual plucking of hair

Arginase deficiency → has least hyperammonemia
- milder symptoms (misdiagnosed as cerebralpalsy)

→spasticity → progressive spastic diplegia
→ scissoring of the gait

UREA CYCLE TRANSPORTER DEFECT

1. CITRULLINEMIA TYPE II → defect in Citrin transporter
2. Ornithine transporter defect → HHH Syndrome
   - Hyper ammonemia
   - Hyper ornithinemia
   - Homo citrullinemia/urea

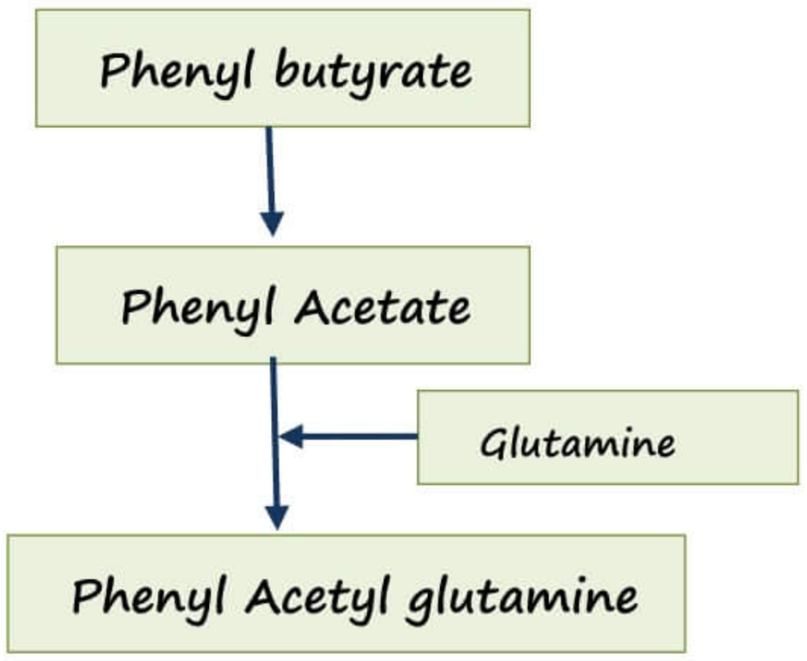
TREATMENT OF OCD

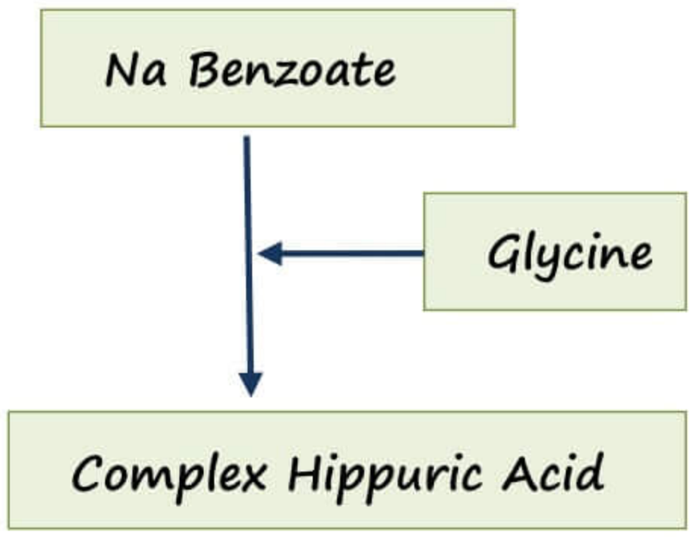
1. ARGININE

→ 1st line Rx

- an essential AA
- Source of ornithine
- Activator of 6th enzyme (NAG synthase)
- C/I only in arginase deficiency

2. ACYLATION THERAPY OR NH_3 SCAVENGING AGENTS

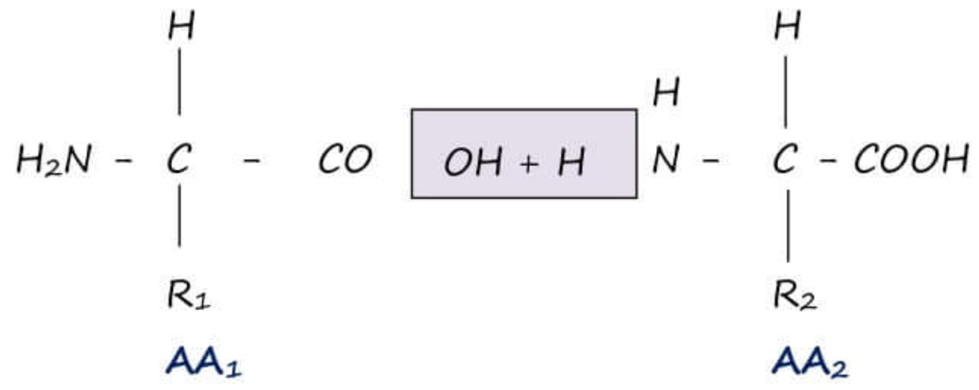




→ As effective as urea

3. Restrict protein intake to 50%

PROTEINS BONDS AND STRUCTURES



- CONH -

Amide

If amide bond is present in protein, it is known as PEPTIDE BOND

→ Covalent bond [on denaturation, this bond do not break (primary structure)]

→ Has double bond character

- Double bond is in 'trans' configuration
- In unsaturated fats, double bond is in 'cis' configuration

PROTEIN STRUCTURE

1° Structure → Sequence of amino acids

2° structure → Obtained from folding of 1° structure
- α Helix
- β Sheets
- β Turns

3° structure → Further folding → fully folded structure

4° structure → > 1 polypeptide chain
- Hb

MONOMERIC PROTEIN → Those proteins which have only one moment, they donot have Quaternary structure

α HELIX

→ Symmetrical helical structure

→ Secondary structure

→ mc helix found in the body

→ Right handed

AA not found in α-helix

1. Proline → Introduce 'kink' in α-helix
2. Glycine → Cause 'bend' in α helix
3. Tryptophan → Has bulky side chain
4. Aspartate or Glutamate
5. Valine

FEATURES	1°	2°	3°	4°
Bond	Covalent	H-bond	S-S, Hydrophobic, Hydrogen, Ionic	Hydrophobic, Hydrogen, Ionic
Functional Activity	Absent	Absent	Present	Present
Denaturation	Retained	lost	lost	lost
Detection	Mass Spectrometry (best) and Edman's Technique			

2° structure →
3° Structure
4° Structure

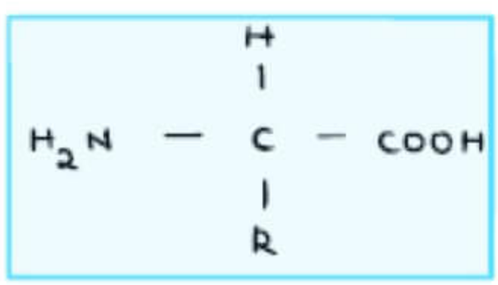
X ray crystallography (Best)
- For Detecting 3 D structure of proteins or any other macromolecules (DNA)
- Non-crystallisable structure can't be detected

→ NMR spectrometry
- For non-crystallisable proteins

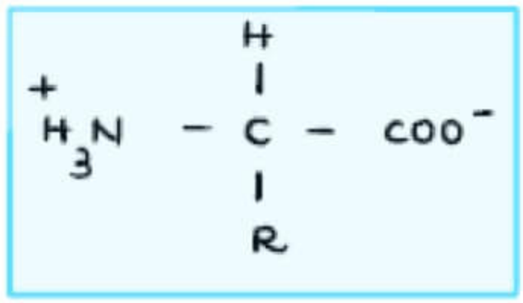
BEST TECHNIQUE TO DETECT PROTEIN STRUCTURE → X-RAY CRYSTALLOGRAPHY

PROTEIN PRECIPITATION

AMINO ACID



→ Anything in body is ionized



- Net charge is zero
- ZWITTER ION/ AMPHOLYTE
- insoluble → precipitate
- PI → Iso electric pH , where zwitter ion exists
- pH = PI → precipitatⁿ occurs, no charge

pH < PI → Acidic pH, protein has positive charge

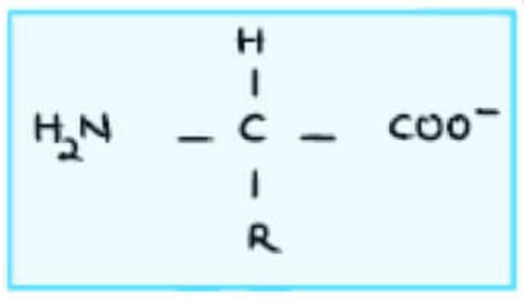
pH > PI → alkaline pH, protein has negative charge

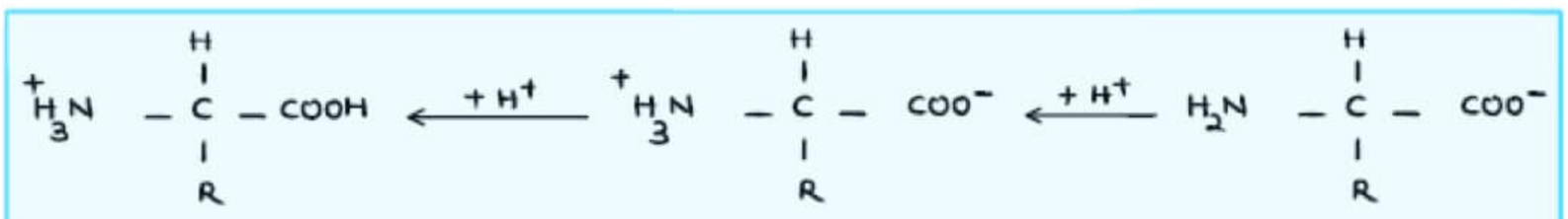
DEPROTONATED FORM of AA

→ Proton is not there on both the functional groups

→ Present in alkaline pH

→ has negative charge on it





Diprotonated Structure	Zwitter ion	Deprotonated AA
Acidic PH	pH = pI	Alkaline pH
pH < pI		pH > pI
positively charged (dlt charge on amino group)		Negative charged (dlt charge on carboxy group)

USES OF PROTEIN PRECIPITATION

→ purification of enzymes / Proteins

→ Preparation of protein free filtrate (PFF) for various biochemical tests

PRECIPITATION REACTIONS OF PROTEINS

→ Any factor which

1. causes denaturation
2. Neutralizes charge
3. causes dehydration

VARIOUS METHODS

1. Heat
2. strong mineral acids

} causes precipitation by denaturation

3 Heavy metal salts in alkaline medium

4. Alkaloidal reagents (Trichloro acetic acid, phospho tungstic acid, sulfosalicylic acid]

→ cause precipitation by neutralization of charges

HEAVY METAL SALTS IN ALKALINE MEDIUM

→ In alkaline medium, proteins are negatively charged

→ Heavy metals are positively charged

→ Neutralization of charges occur which leads to precipitation known **as POSITIVE ION**

PRECIPITATION

ALKALOIDAL REAGENTS [Trichloroacetic acid, Phospho tungstic acid, Sulfo salicylic acid]

→ Alkaloidal reagents are negatively charged

→ In acidic medium, proteins are positively charged

→ Neutralizatⁿ of charges occur & leads to **NEGATIVE ION PRECIPITATION**

SALTING OUT

→ When salt is used for protein precipitation → Salting OUT

→ EX: Heavy metal salts

Neutral salts [EX: Ammonium sulfate]

PRECIPITATION OF ALBUMIN & GLOBULIN BY AMMONIUM SULFATE SALT

→ Albumin are precipitated by → Full saturation

→ Globulins are precipitated by→ half saturatⁿ

GLOBULINS	ALBUMINS
↓	↓
More molecular weight, large size	Less molecular weight, smaller size
↓	↓
Less surface area	More surface area
↓	↓
Less hydration	More hydration
↓	↓
Less salt required for precipitation	More salt required for precipitation

Q Which is not a method of protein precipitation

a salting out with heavy metals

b Acetone & Alcohol

c changing pH other than isoelectric pH

d Trichloro acetic Acid

SUMMARY

1. ZWITTER ION → Net charge is zero, so insoluble, so precipitatⁿ occurs

2. PI (Isoelectric pH) → that pH at which Zwitter ion exists

3. Heat or strong mineral acids cause denaturation

4. Heavy metal salts & Alkaloidal reagents causes neutralization of charges

5. Organic solvents and Neutral salts causes Dehydration.

COLOUR REACTIONS OF PROTEINS & AMINO ACIDS

→ **Colour reactions** - Reagents reacts with Proteins and Amino acid to give colour.

→ They are used for qualitative and quantitative detection of amino acids and proteins.

NINHYDRIN TEST

→ The α – amino acids react with Ninhydrin to form a purple, blue or pink colour complex (Ruhemann's spray).

→ Finger prints are taken by Ninhydrin Spray.

→ Ninhydrin reaction is effectively used for the quantitative determination of amino acids & proteins.

→ Proline & Hydroxyproline give yellow colour with Ninhydrin.

→ Ninhydrin is also used in chromatography.

BIURET TEST

→ Positive → Purple colour

→ Minimum 2 peptide bonds are required

→ Dipeptides do not give this test positive

→ Tripeptides & protein will give this test positive.

TESTS FOR AROMATIC AMINO ACIDS

1. XANTHOPROTEIC TEST	→	For all aromatic amino acids Except Phenyl Alanine
2. HOPKIN COLE'S TEST	}	For Tryptophan
3. OBERMEYER TEST	}	
4. MILLON'S TEST	→	For Tyrosine
5. PAULY'S TEST	→	For Histidine & Tyrosine

1. XANTHOPROTEIC TEST

→ Positive → Yellow colour

→ Nitric acid is used

2. HOPKIN COLE'S TEST

→ Positive → Purple colour

→ Ring test

→ For Indole ring of tryptophan

3. OBERMEYER TEST / URINE INDICAN TEST

→ For tryptophan

4. MILLON'S TEST / COLE'S TEST

→ Positive → Brownish Red colour

→ aka Cole's Test

→ For Phenol ring of Tyrosine

5. PAULY'S TEST

→ Positive → Red colour

→ For Imidazole ring of Histidine & Phenol ring of Tyrosine

SAKAGUCHI TEST

→ Positive → Red colour

→ For guanidine group of Arginine.

(Basic Amino acid)

TEST FOR SULFUR CONTAINING AA

1. SULFUR TEST / LEAD SULFIDE TEST
2. CYANIDE NITROPRUSSIDE TEST

1. SULFUR TEST / LEAD SULFIDE TEST

→ Positive → Black or Brownish colour

→ For cysteine (SH)

→ Cystine (S~S)

→ Not positive for methionine (C – S – C)

2. CYANIDE NITRO PRUSSIDE TEST

→ Positive in Cystinuria, homocystinuria, cystinosis,

→ Reddish purple colour

FERRIC CHLORIDE TEST

→ For Keto Acids

→ Positive for MAPLE SYRUP URINE diseases

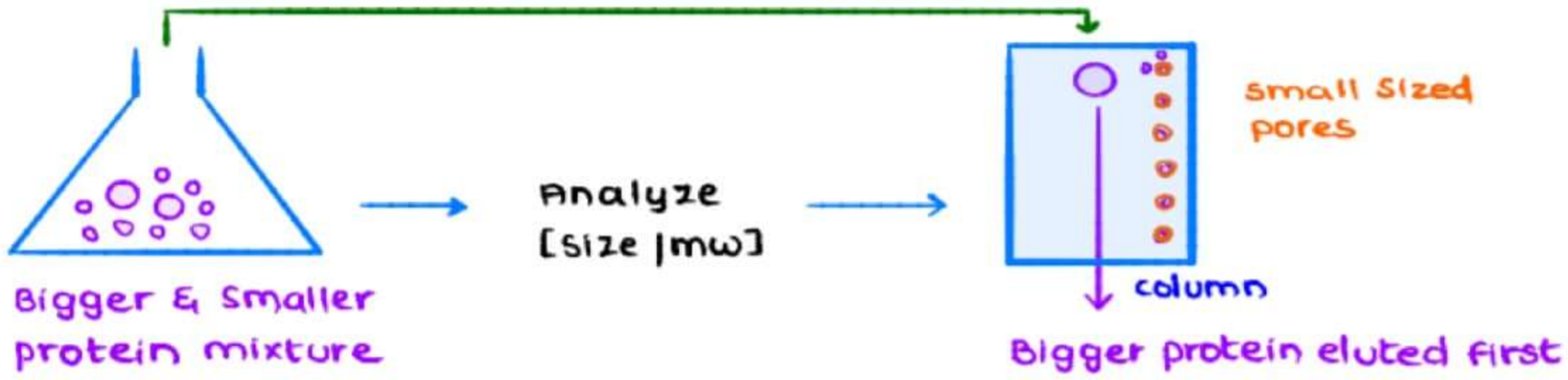
Phenylketonuria (P K U)

DINITRO PHENYL HYDRAZINE TEST (DNPH Test)

→ Used for Screening of PKU & MSUD patients

CHROMATOGRAPHY & ELECTROPHORESIS

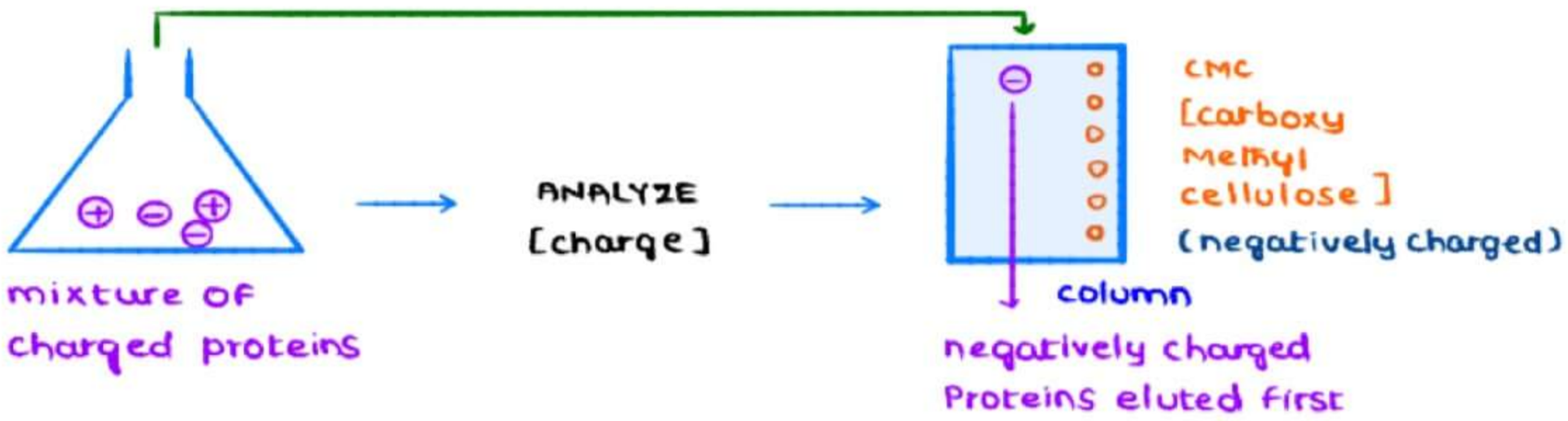
1. GEL FILTRATION CHROMATOGRAPHY / SIZE EXCLUSION CHROMATOGRAPHY



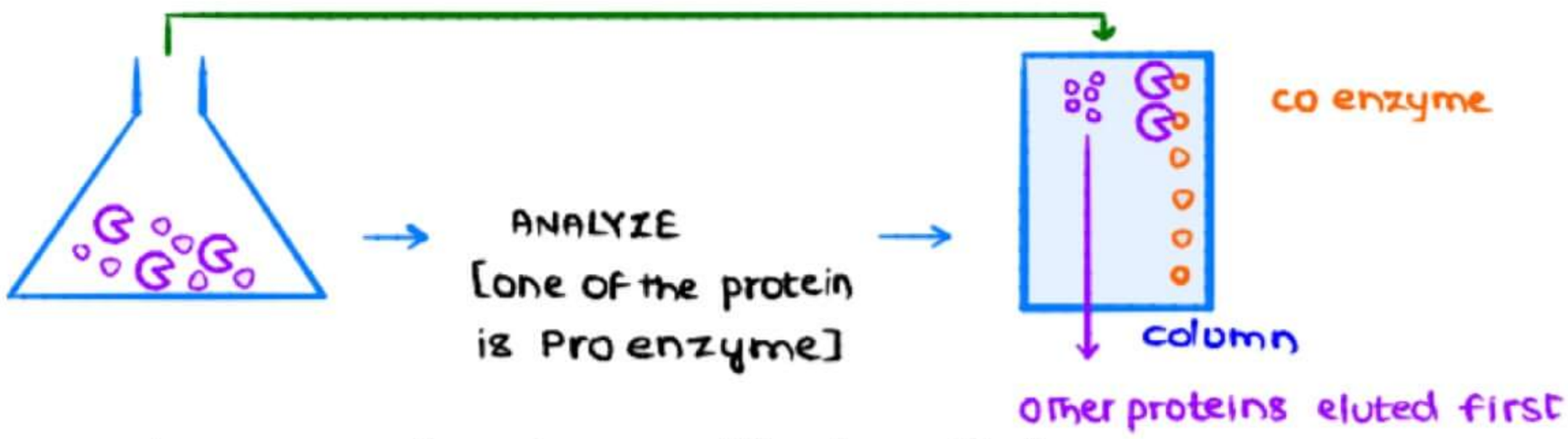
GEL → SEPHADEX

- Biochemically Dextran
  - Dextran used as plasma expander
- Gel in the column do not allow bigger proteins
- Bigger proteins can not enter the gel → ELUTED FIRST
- Smaller proteins enters the gel, & later eluted out

2. ION EXCHANGE CHROMATOGRAPHY



3. AFFINITY CHROMATOGRAPHY



→ Used to separate enzymes, antigens, hormones, Vitamins, antibodies

TYPES OF COLUMN CHROMATOGRAPHY

1. Based on Size / mw → GEL FILTRATION CHROMATOGRAPHY
2. Based on Charge → ION EXCHANGE CHROMATOGRAPHY
3. Based on Affinity → AFFINITY CHROMATOGRAPHY

Stationary Phase → Column
Mobile Phase → Mixture to be separated

OTHER TYPES OF CHROMATOGRAPHY

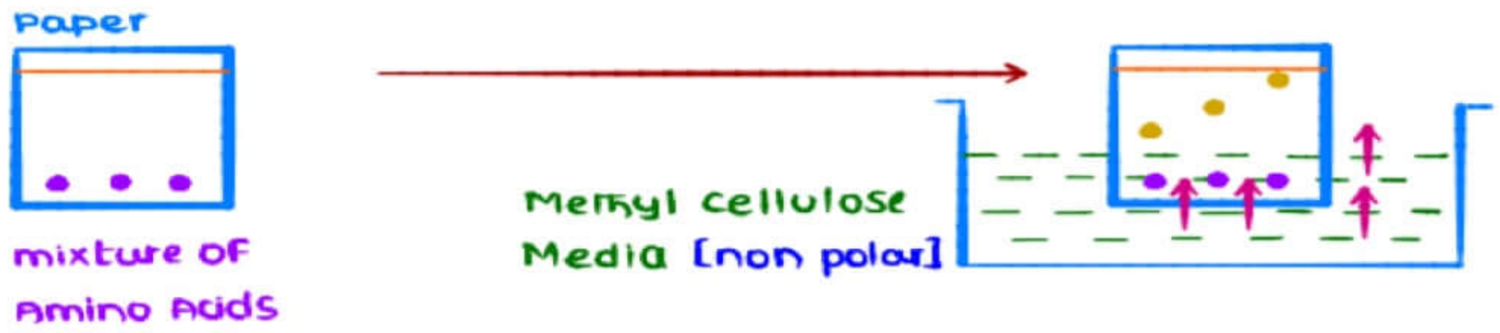
4. Paper chromatography

- Older & cheaper
- Used for teaching purpose
- Stationary phase → Paper

5. Thin layer chromatography

- Newer & Costlier
- Used for research & diagnosis
- Stationary phase → Thin layer of silica

COMMON PRINCIPLE -

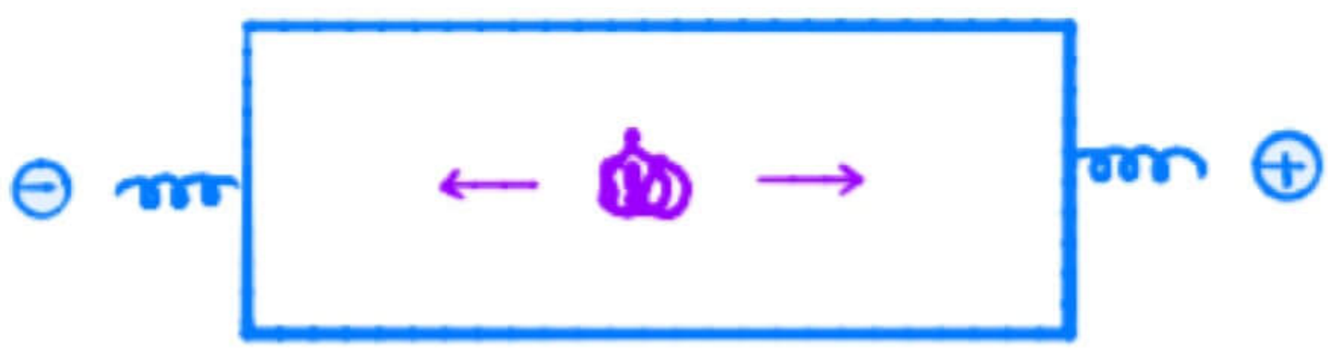


→ On paper, mixture of amino acids taken at one end & marking present at other end
→ It is placed in methyl cellulose media (non-polar)
→ Solvent moves up on the paper, once it touches marked line, then it is removed & staining done
→ Staining indicates the polarity of AA with respect to the Solvent

Early Staining → Polarities of AA & Solvent are different (polar)
Late Staining → Polarities of AA & Solvent are same (non-polar)
Mobile phase → Mixture of amino acid + solvent

ELECTROPHORESIS

→ Movement in electric field
→ Depends on
- Charge (main factor)
- Size
- Shape



SDS – PAGE → Depends only on size

PAGE → Poly Acryl amide Gel Electrophoresis

SDS → Sodium Dodecyl Sulfate

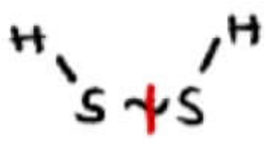
→ Salt derivative of Lauric Acid (12C)

Properties

1. Denature proteins → 2^{o} 3^{o} 4^{o} Structures lost → no shape
2. Anionic detergent → Coat all the proteins with negative Charge
3. 1.4 gm of SDS binds to 1 gm protein
4. SDS cannot break disulfide bond (disulfide bond present in 3° Structure)
   - Disulphide bond can be broken by
     - → Oxidation → done by **PERFORMIC ACID**
     - → Reduction → done by **MERCAPTO ETHANOL**

oxidation

Performic acid

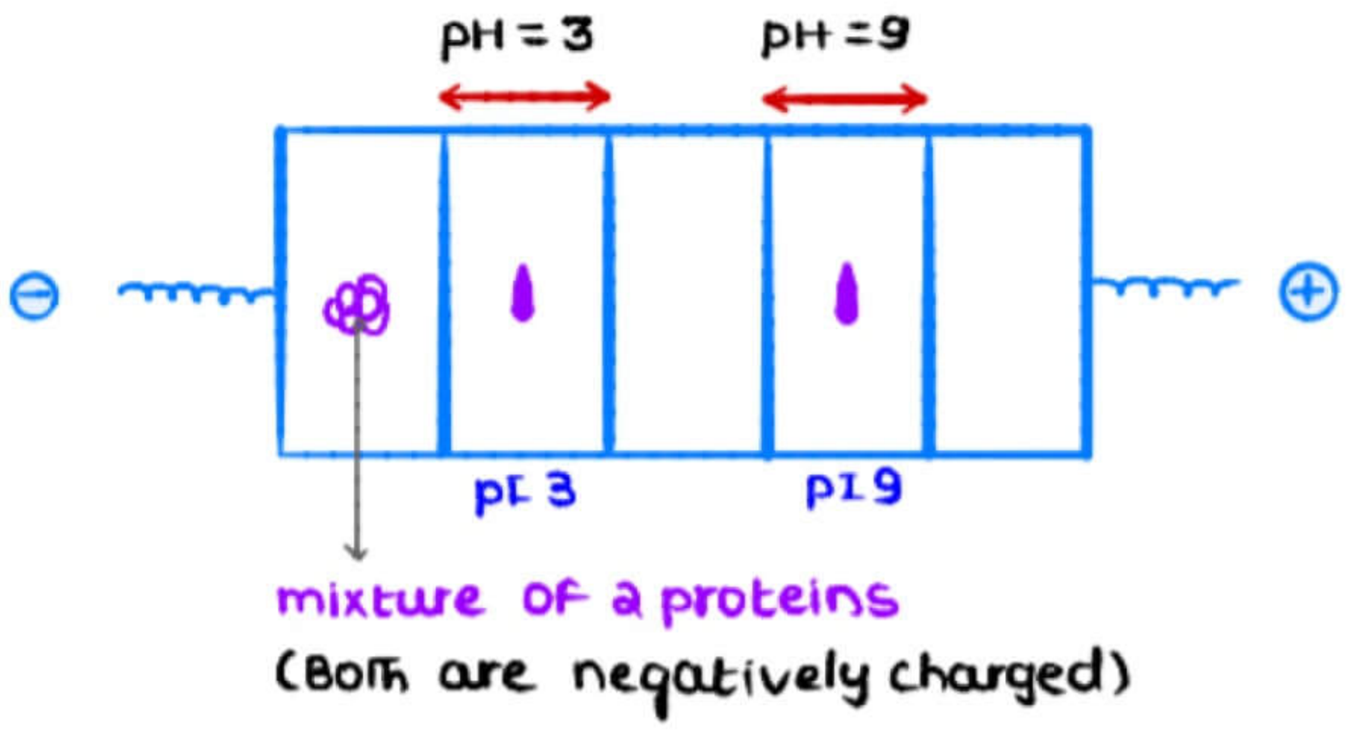


Reduction

mercapto Ethanol

ISO ELECTRIC FOCUSSING

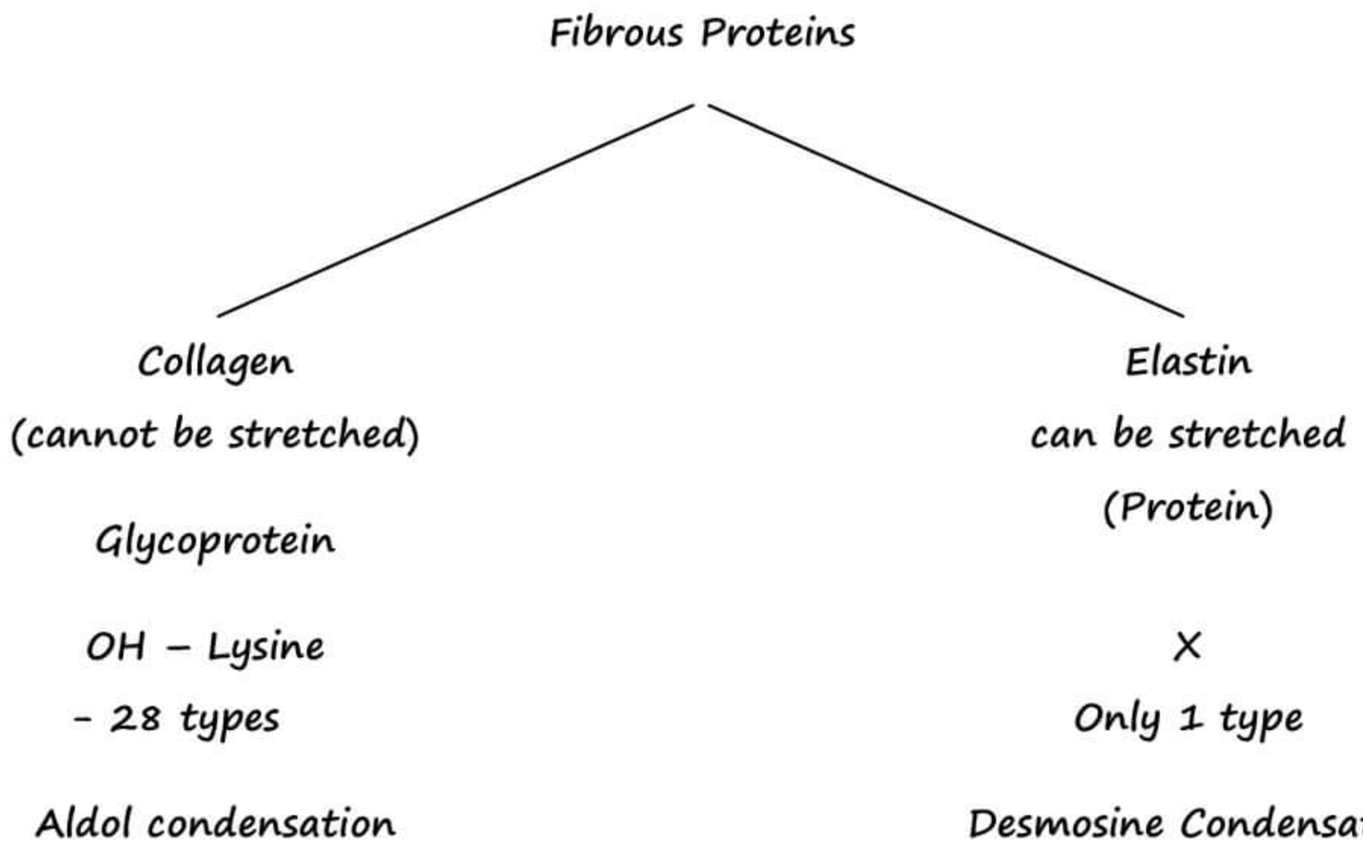
→ Using Iso electric pH, Separation by electrophoresis can be done



mixture of 2 proteins

(Both are negatively charged)

FIBROUS PROTEIN



Collagen → Most abundant of all human proteins.

1° Structure → (Gly – X- Y)n

Every 3 aa. is glycine

X, Y → Pro, OH-Pro, Lys, OH-Lys

Q. Collagen has

(a) Proline

(b) Phenylalanine

(c) OH – Proline

Ans. (c)

Types of Collagen

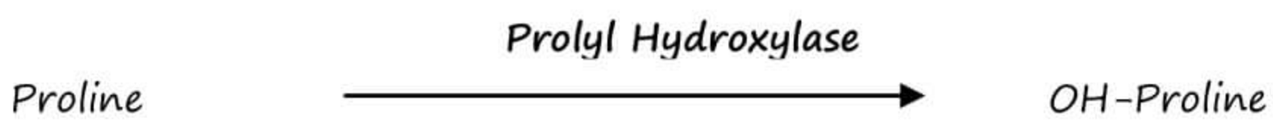
I	Skin (most abundant)
II	Connective tissue
III	Arteries and CVS
IV	Basement membrane of Glomerulus*
VII	Junction of dermis & epidermis*

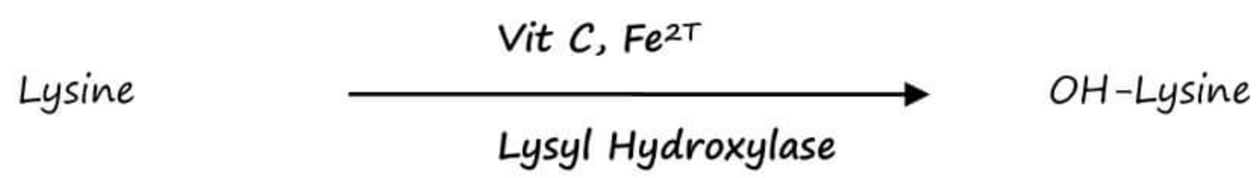
Type IV Defective in disease → Alport Syndrome (Hematuria), (ESRD).

Type VII defective in Epidermolysis Bullosa, Skin Blisters.

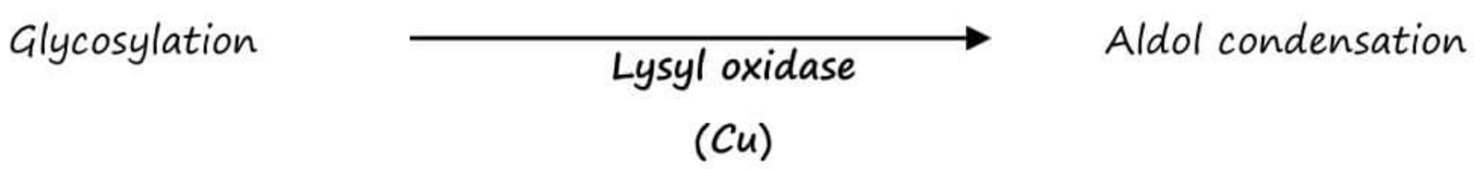
Post Translational Modifications of Collagen.

1. **Hydroxylation → H-bonds**





2.



Dietary deficiency of Cu → Menke's disease

Menke's disease

1. Kinky hair
2. Greying of hair
3. Growth Retardation

Ehler -Danlos Syndrome → A heterogeneous group of disorders characterized by stretchy skin + loose joints

Collagen affected: Collagen Type I, III, V, Lysyl Hydroxylase (Deficient), Lysyl Oxidase (Deficient)

Type I Collagen → Skin, Bone.
→ Defective in EDS VII
→ Osteogenesis Imperfecta
→ Osteoporosis

Type II→ Present in connective tissue, Cartilages
→ Condrodysplasia, Osteoarthritis

Type III → EDS (IV) → Most Severe (Vascular)
Type IV → Alport Syndrome
Type VII → Epidermolysis Bullosa
Elastin → Skin, Lungs, Elastic Ligaments, Vascular tissue, Large arterial blood vessel.

Intramolecular cross links called Desmosine (4 Lysine residues)

α- 1AT
↓ (-)
Elastase

Elastin ⟶ Breakdown

α-1 AT deficient → Elastase active → Elastin broken down → Emphysema

Marfan Syndrome – AD

Mutation in Fibrillin-1

Glycoprotein-Structural component of microfibrils.

Helps is deposition of elastin

C/F → Tall stature, long limbs, lens dislocation, arachnodactyly, media of large arteries is weak.

Death → Rupture of dilated aorta

Keratin → nails, hair outer layer of skin

Collagen → α-chains , Rich in cysteine (SH)

There are many S~S disulfide bonds in Keratin. These disulfides give strength to the molecules.

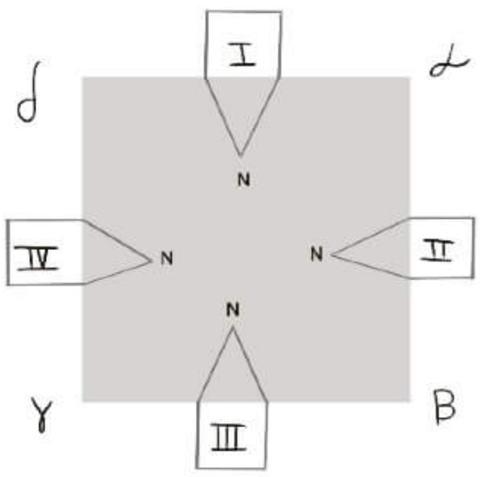
More the (S~S) bonds, harder is the keratin.

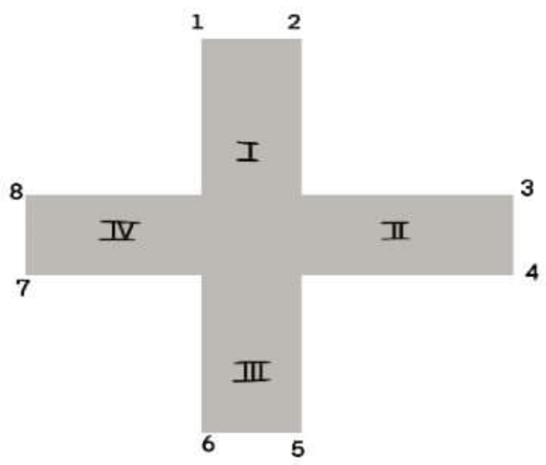
HEAM SYNTHESIS & PORPHYRIA

Hb = Haem + globin (Protein)

Haem = Fe^{+2} + Porphyrin

Porphyrin = 4 Pyrrole





Depending upon different side chains, there are various isomers of porphyrins.

I → Negligible amounts.

III → Belongs to IX series – Most common

Various type of Porphyrins

Uro P → Copro P → Proto P

Various side Chains

1. Methyl
2. Acetyl

} Decarboxylase → Copro P. formed

3. Propionyl
4. Vinyl

} Oxidase → Proto P. formed

Uro P —(Decarboxylase: Acetyl to Methyl)→ Copro P. —(Oxidase: Propionyl to Vinyl)→ Proto P.

Conjugated double bonds → absorb visible light
→ 400 nm

Absorption band – Sorett band

Haem Synthesis

1. Monopyrrole – Porphobilinogen
↓
Tetrapyrrole/ Porphyrins [U, C, P]
↓ +Fe
Haem
↓
Haem Hb/ Mb/ Cytochromes

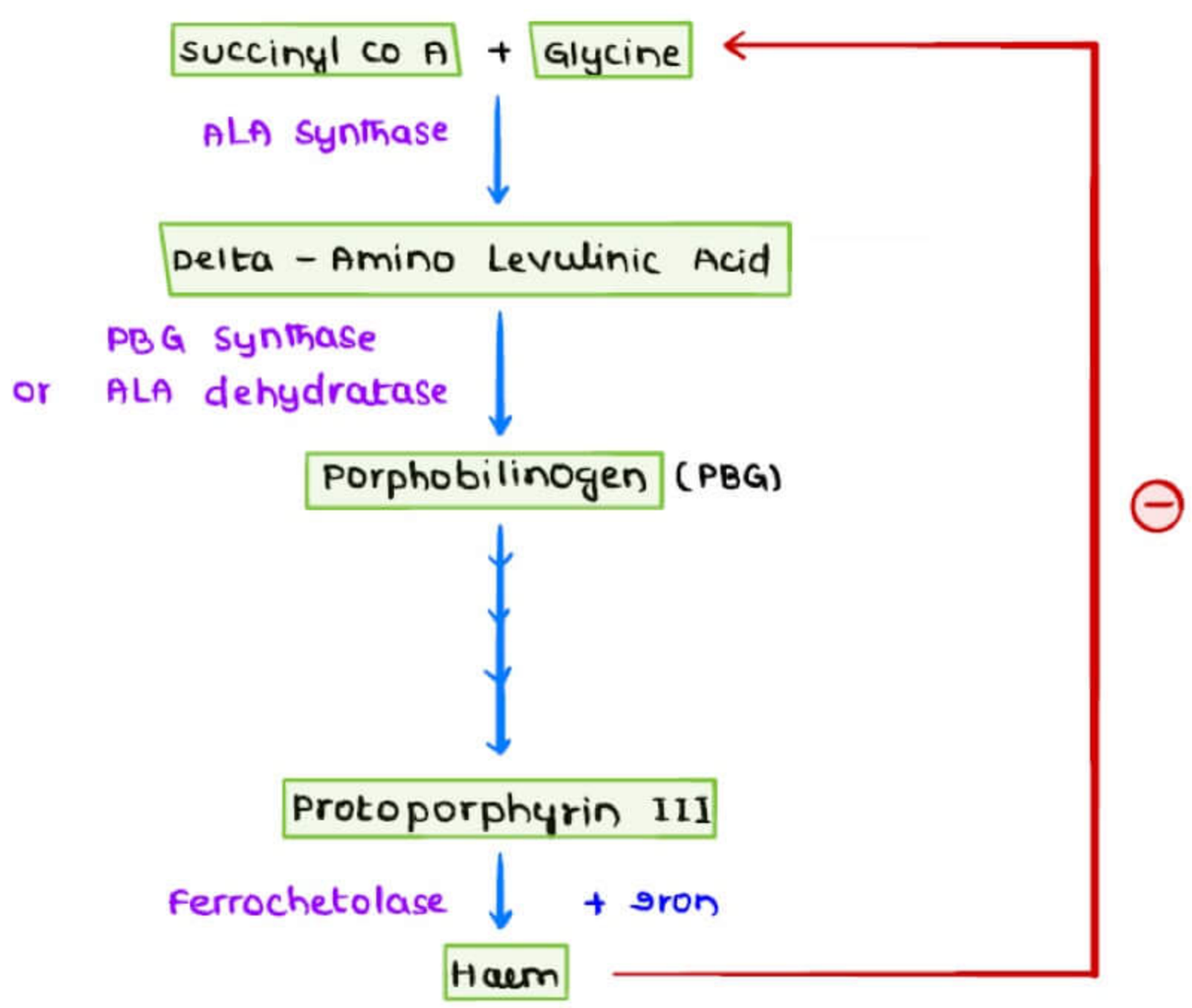
Enzymes→ Tryptophan Pyrrolase
NOS (Nitric Oxide Synthase)
Catalase

Haem Synthesis occurs in all cells of body. It will not occur in Mature RBCs because there is no mitochondria

Compartment → Mitochondria + Cytoplasm

↑

Start



There are two types of ALAS

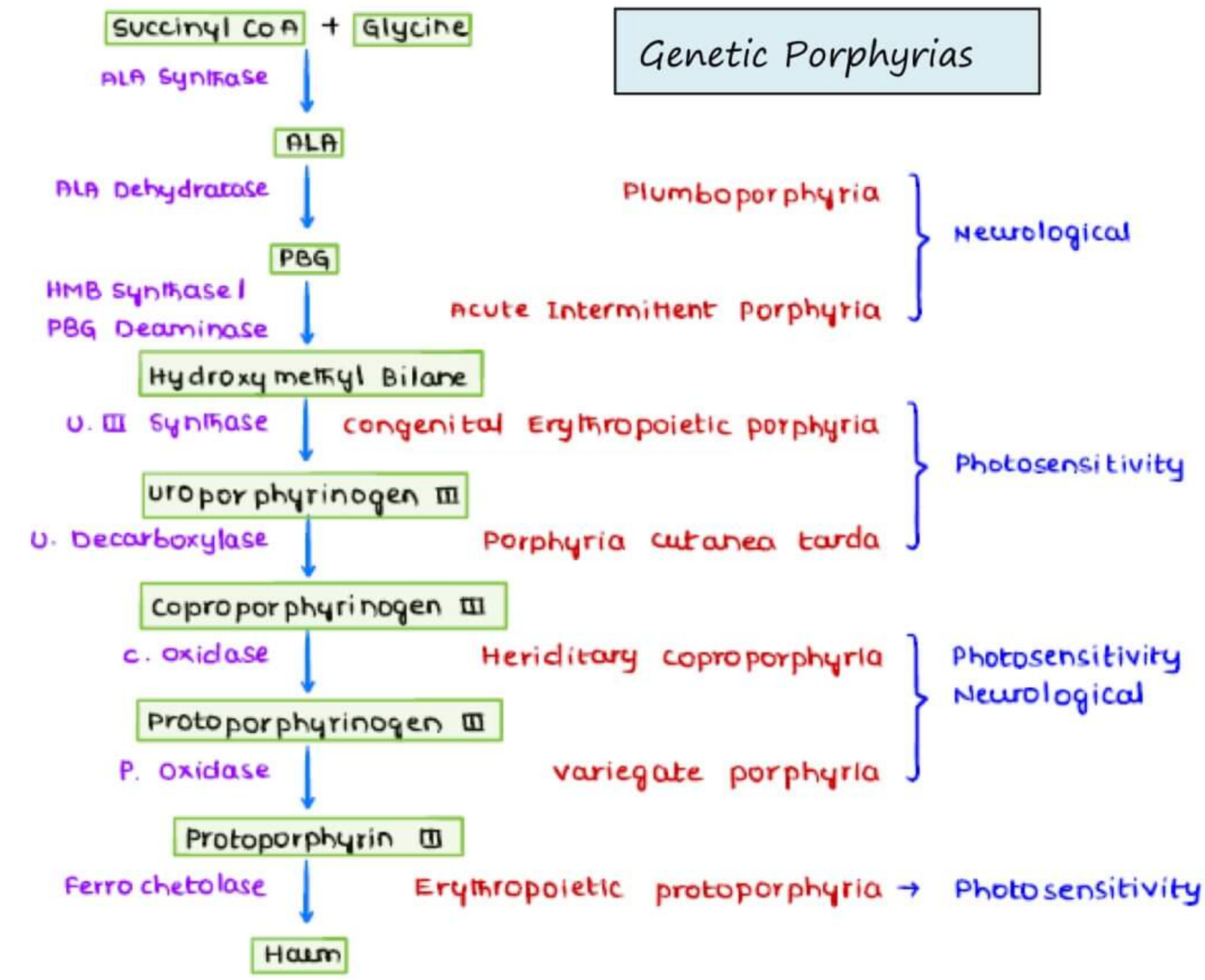
1. All Tissues (Mainly LIVER)
→ Inducible enzyme
→ Inhibited by free Haem

2. In Erythroid tissues
→ Not regulated
→ Onetime event.

Lead Poisoning

Enzyme decreased → PBG synthase/ ALA Dehydratase
Enzyme increased → ALA Synthase
→ ALA will be coming in excess in urine.

PORPHYRIAS



PORPHYRIAS

Haem not getting formed

→ **Genetic** → Enzyme deficiency other than ALAS

→ **Acquired** → Lead Poisoning (M.C. of acquired porphyria), Fe deficiency

→ **ALAS** – Deficiency

- X-linked Sideroblastic Anemia

→ **ALAS** – Gain of function mutation

Fe is limited

Neurological → Due to block early

ALA & PBG ↑↑

↓

Block the action of GABA excitation in visceral pain fiber/ Abdomen pain, Vomiting, Hypertension, Tachycardia.

Photosensitivity → Due to block late in the pathway.

Porphyrinogen ↑

Porphyrin → excited when exposed to light

↓

React with O_2 [excited when exposed to light]

↓

Damage Lysosomal membrane

↓

Lysosomal Enzymes damage

↓

Skin damage

↓

Scarring

Inheritance pattern of Porphyrias

All are AD except:

→ X – linked Proto Porphyria.

→ Congenital Erythropoietic Porphyria

→ Erythropoietic Proto P → M.C in children

→ Genetic ALA dehydratase deficient Porphyria

Drugs like Barbiturates, Phenytoin, Griseofulum

↓

Metabolized using cytochromes

↓

Increase the synthesis of Cyto P450 enzymes

↓ [Haem used]

Free Haem decreased

↓

ALA synthase induced

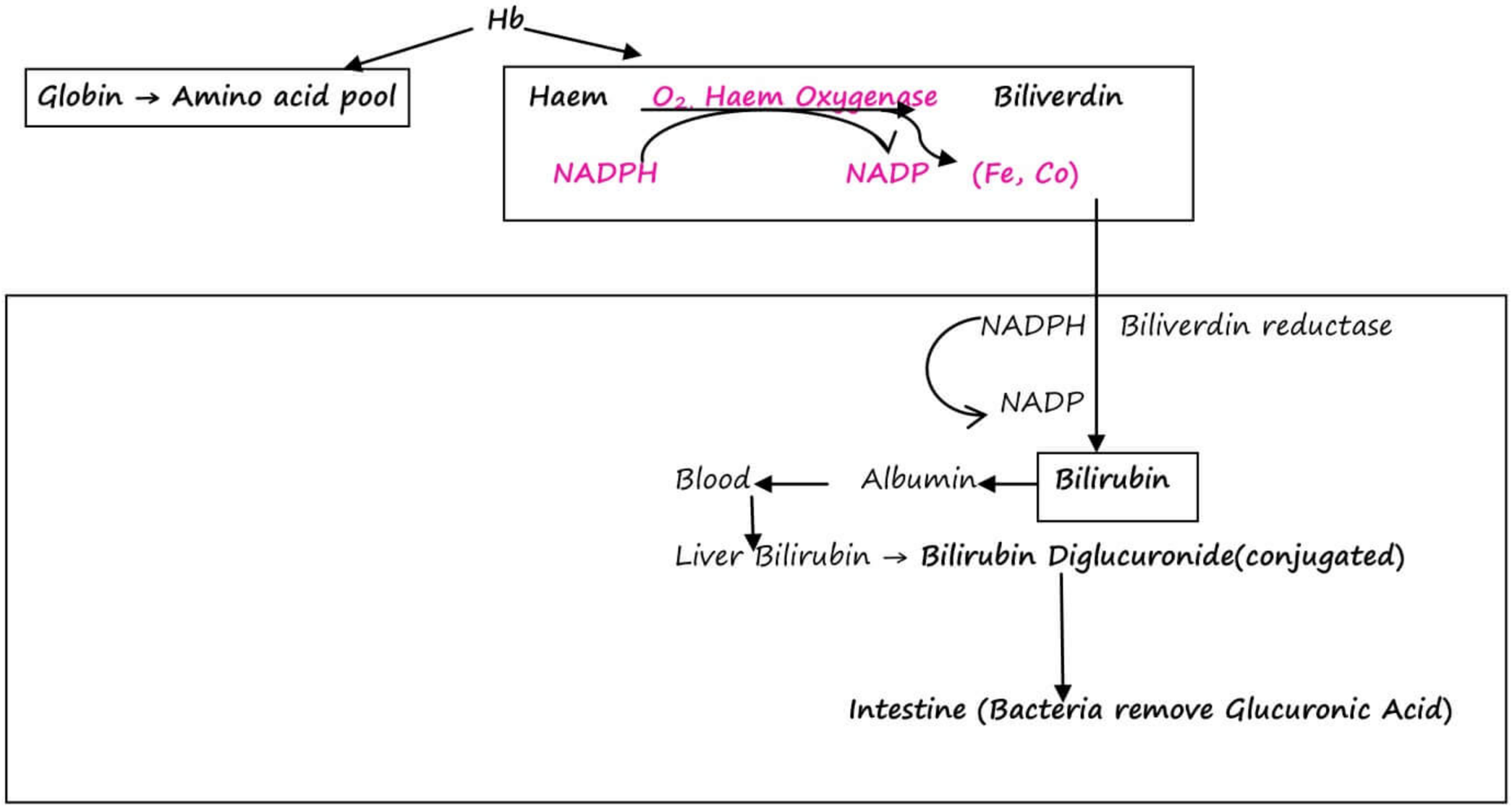
↓

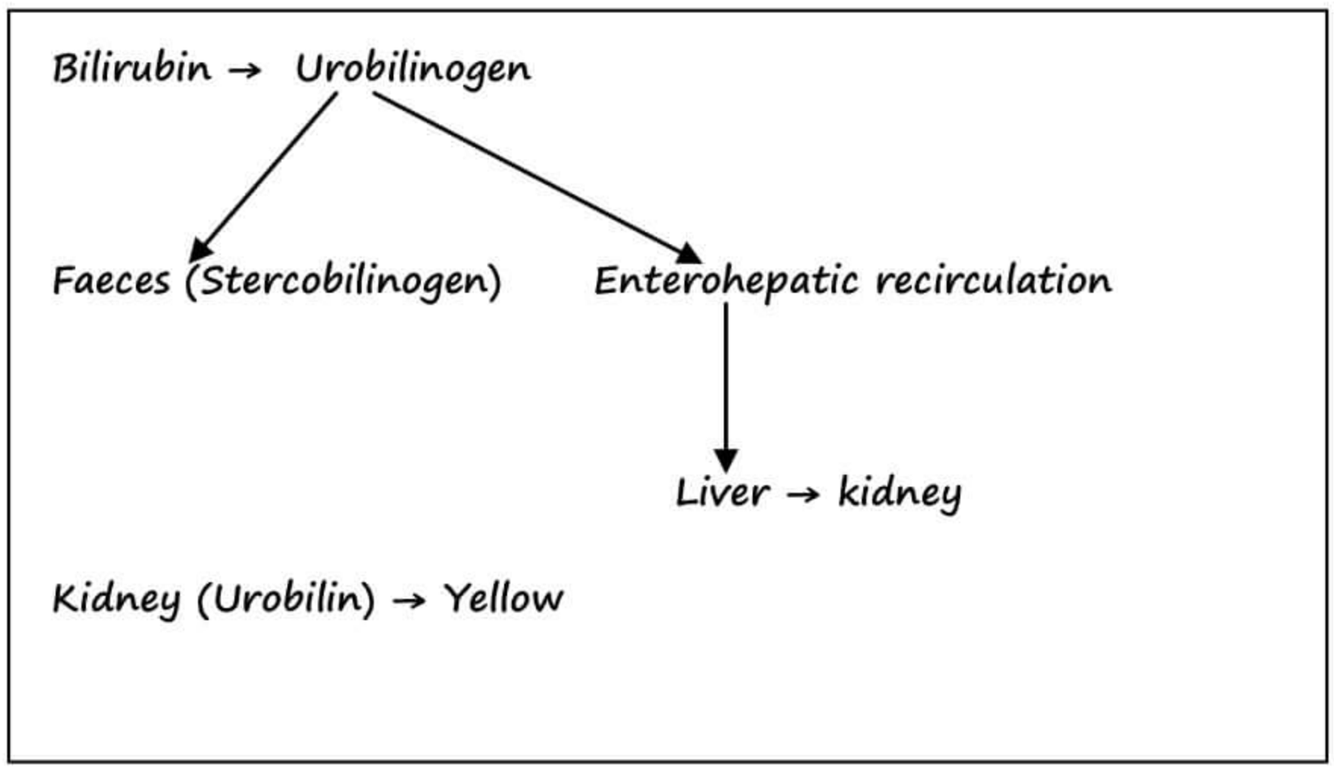
Increase the synthesis of Haem Pathway intermediate

↓

Aggravate Porphyria attack

HAEM CATABOLISM





LIPIDS

LIPID CHEMISTRY

LIPID → Any compound which is insoluble in water & soluble in non-polar organic solvent

FATTY ACID

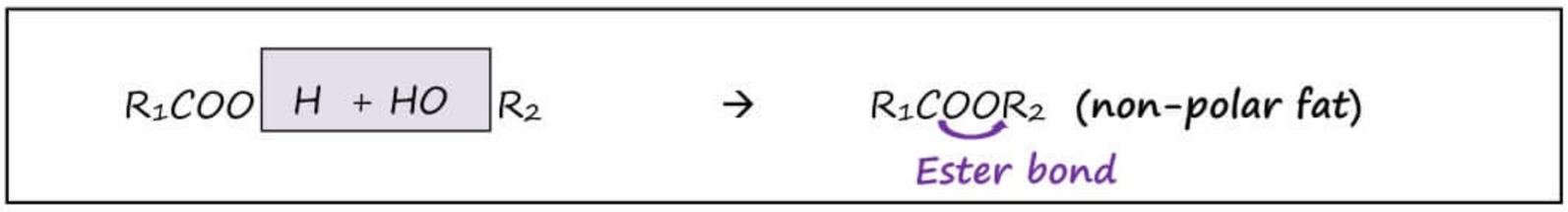
→ Polar

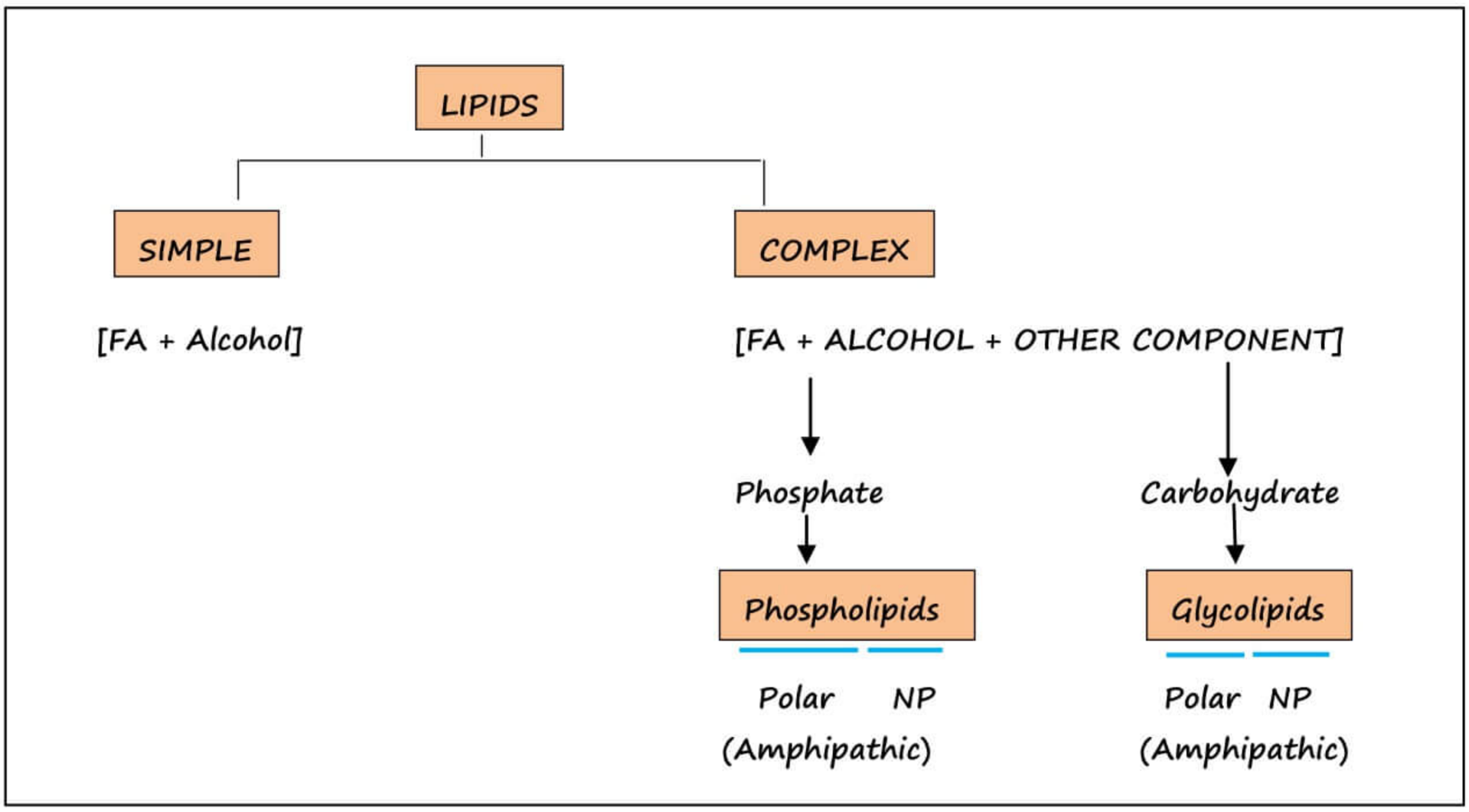
→ Nick name → 'Acyl'

→ FA + alcohol → Non polar fat

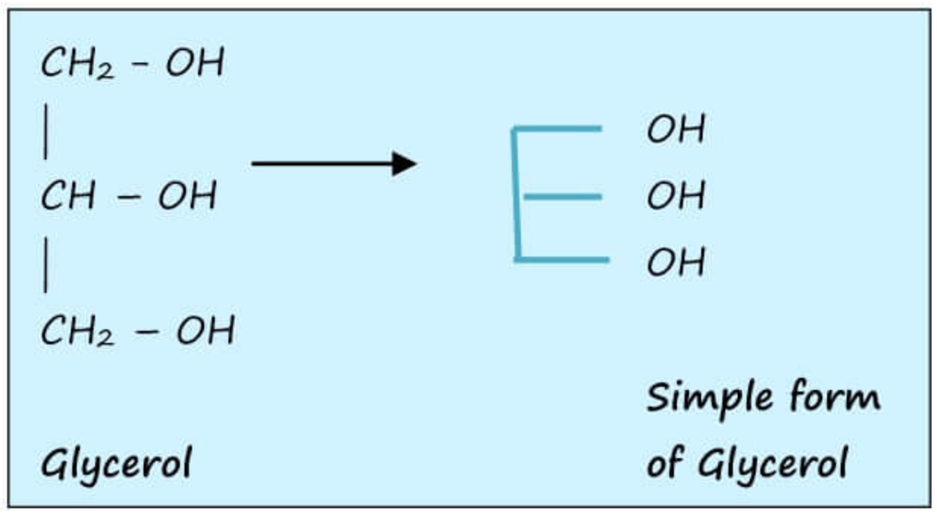
Carboxy + Alcohol → Ester bond

Carboxy + Amino → Amide bond





SIMPLE LIPIDS



Mono Acyl Glycerol

OH – FA_1 ← Ester bond
OH
OH

→ One FA + Glycerol
→ Amphipthic

DiAcyl Glycerol

OH – FA_1
OH – FA_2
OH

→ 2 FA + Glycerol
→ Amphipathic

Triacyl glycerol

OH – FA_1
OH – FA_2
OH – FA_3

→ 3 FA + Glycerol
→ Triglycerides
→ Neutral Fats
(Non polar)

PHOSPHOLIPIDS (Alcohol + FA + Phosphate)

OH – FA_1
OH – FA_2
OH — (P) — Base

Base → CHOLINE → LECITHIN (Surfactant)
Base → Ethanolamine → CEPHALIN (CNS)

Phosphatidic Acid

PRODUCTS OF HYDROLYSIS

Lecithin → 1 Choline, 1 Phosphate, 2FAs & 1 glycerol

PHOSPHOLIPASES

→ Breakdown Phospholipids

A_1
OH – FA_1
OH – FA_2
OH — A2 — P — Base
C D

Action of
D → Phosphatidic Acid + Base
C → Diacyl glycerol + base with (P)
A_2 → FA_2
A_1 → FA_1

CARDIOLIPIN

- Complex phospholipid
- Present in inner mitochondrial membrane
- Can be antigenic

→ Structure of Cardiolipin

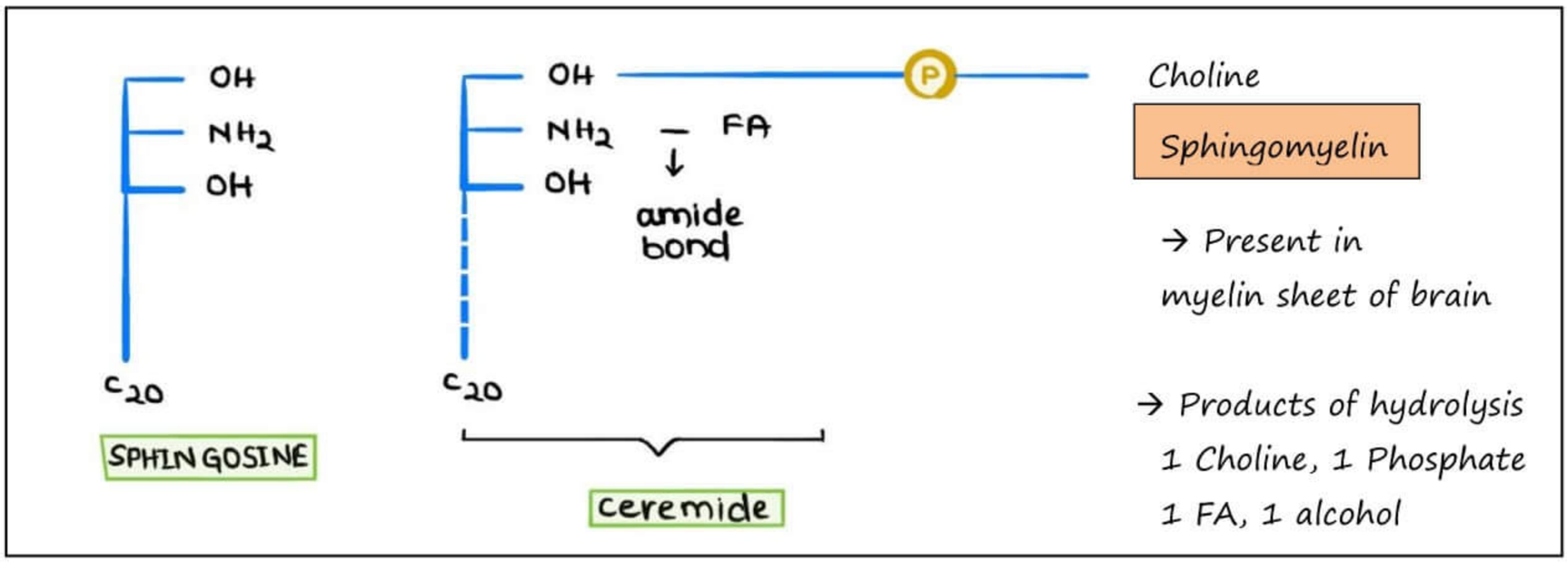
Glycerol → Phosphatidic Acid
Glycerol → Phosphatidic Acid

PHOSPHO LIPIDS – TYPES

1. Glycero Phospholipids (Parent alcohol → Glycerol)
2. Sphingo Phospholipids (Parent alcohol → Sphingosine)

Sphingosine → Unsaturated 20 carbon amino alcohol

SPHINGO – PHOSPHOLIPIDS → Alcohol + FA + Phosphate



GLYCOLIPIDS

→ Alcohol + FA + Carbohydrate

→ Alcohol glycerol is never present

→ Phosphate & base is never present

→ Glucosyl ceramide / Glucocerebroside

→ Sphingosine + FA + glucose

→ Always found in extra neural tissues

→ Never found in CNS

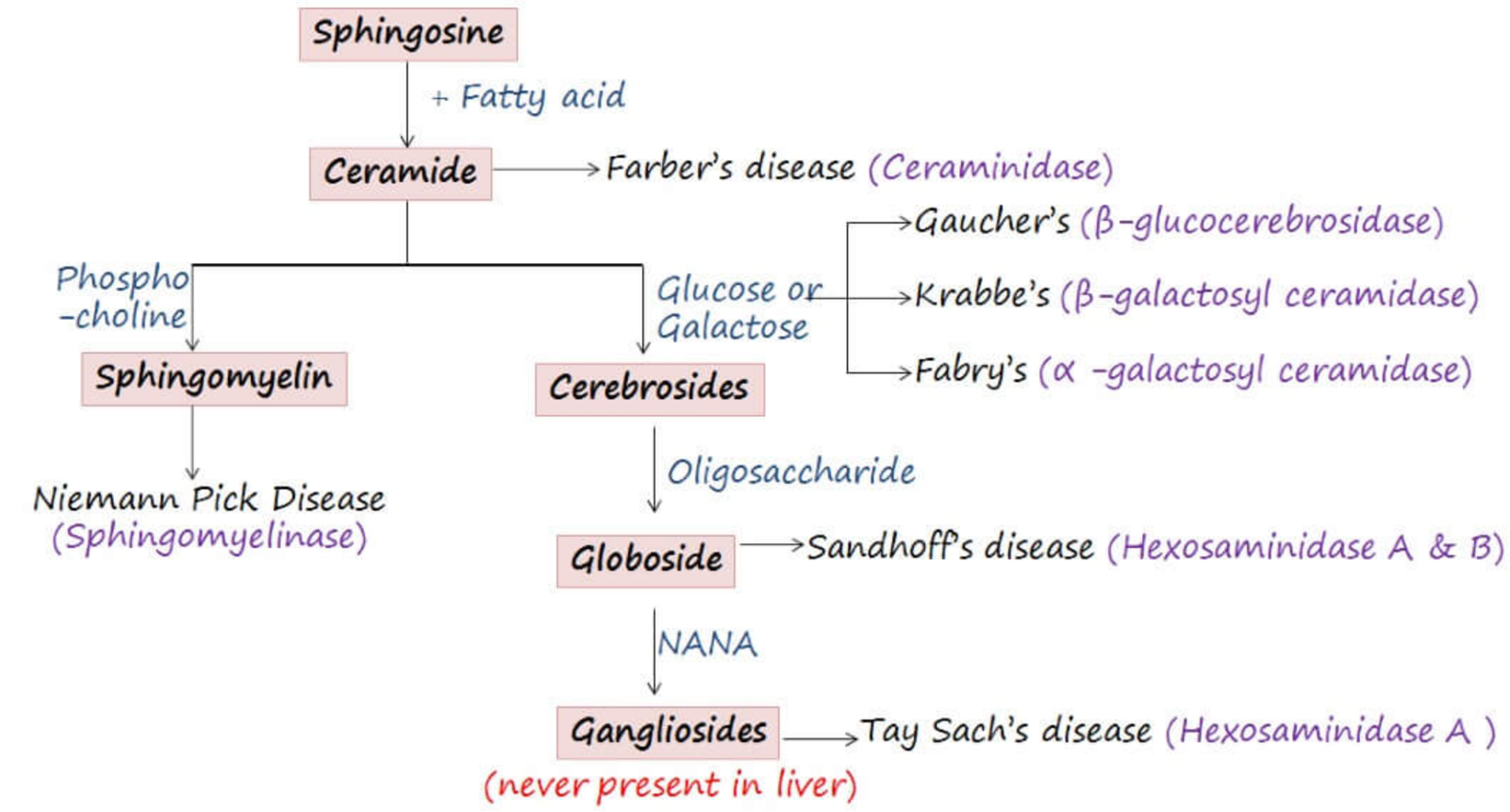
→ Galactosyl | Ceramide | Galacto cerebroside

→ Sphingosine + FA + Galactose

→ Always found in CNS

SPHINGOLIPIDOSES

- Sphingolipids accumulates in lysosomes due to enzyme deficiency



* NANA – N- acetyl Neuraminic Acid

Sphingolipids and their Diseases

Other Diseases are:

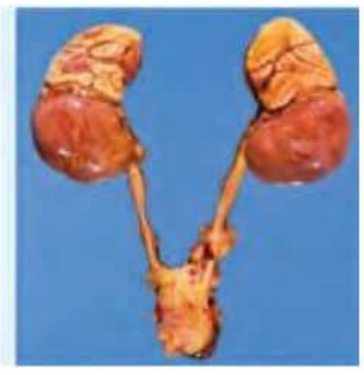
- GMI gangliosidosis
- Metachromatic leukodystrophy

- All are lysosomal storage disorders (LSD)
- Wolman's Disease (A LSD but not a sphingolipidoses)
- All Gangliosidosis are sphingolipidoses (SLP)
- All Sphingolipidoses are autosomal recessive except Fabry's which is X-linked recessive
- All Sphingolipidoses have mental retardation except Gaucher's & Fabry's disease
- All Sphingolipidoses gave cherry red spot except Fabry's & Gaucher's disease
- All Sphingolipidoses have hepatosplenomegaly except Tay Sach's & Krabbe's disease.
- Sphingolipidoses with angiokeratoma – GM1 gangliosidosis & Fabry's disease
- Krabbe's disease (Globoid cell Leukodystrophies)
- SLP resembling Rheumatoid arthritis (Farber's Disease)
- SLP resembling sickle cell crisis (Fabry's Disease)

Note- A patient have organomegaly, who bruise easily and have bony pain- It is due to **Gaucher's Disease**.

Wolman's disease or Cholesterol Ester Storage disease

- Not a sphingolipidosis
- A lysosomal storage disease
- Enzyme deficient is Acid Lipase
- ↑↑ Ch esters and TG
- Watery green diarrhoea
- Relentless Vomiting and failure to thrive
- Hepatosplenomegaly
- Calcification of adrenals is pathognomonic feature



Cholesterol + Fatty acid ⟶ Cholesterol ester
(Amphipathic) (Non-polar)

PUFAs | EFA

→ PUFA [Poly unsaturated FAs] → have ≥ 2 double bonds
→ EFA [Essential FAs]

CATEGORIES

OMEGA 3 CATEGORY

1. CERVONIC ACID / DHA
   → DHA – Docosahexaenoic acid
   → 22 carbons & 6 double bonds present
   → Health drinks are fortified with DHA
   → Requires for brain development of first 2-3 yrs of Life
   → Breast milk contains DHA

2. ALPHA – LINOLENIC ACID
   → 18 carbons & 3 double bonds
   → Essential FA
   → Precursor of omega 3 category

3. TIMNODONIC ACID → 20 carbons & 5 double bonds

OMEGA 6 CATEGORY

1. GAMMA – LINOLENIC → 18 carbons & 3 double bonds
2. LINOLEIC ACID
   - → 18 carbons & 2 double bonds
   - → Most essential FA
   - → Precursor of Omega – 6 category
3. ARACHIDONIC ACID
   - → 20 carbons & 4 double bonds
   - → Important for the Synthesis of PGs & Leukotrienes

CLASSIFICATION BASED ON THEIR CHAIN LENGTH

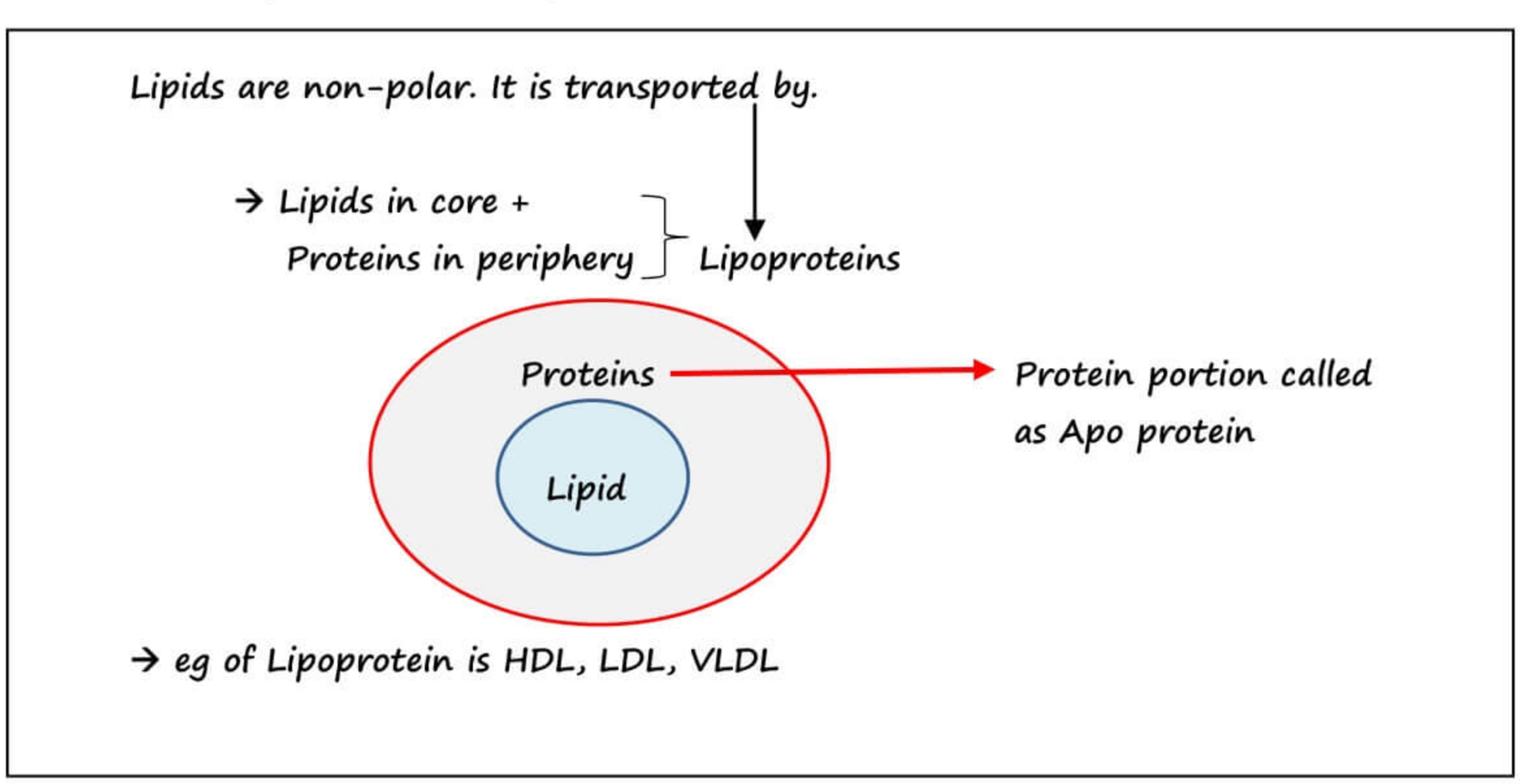
→ Short chain FA	→	2-4C
→ Medium chain FA	→	6-12C
→ Long chain FA	→	14-20C
→ Very long chain (VLCFA)	→	>20C (usually required in brain)

LIPOPROTEINS

LIPID TRANSPORT

NOTE

- → Transport medium in our body is blood
- → Blood is water based, hence it is polar
- → Polar Substance is Soluble in polar medium
- → Non-polar Substance is Soluble in Non-polar medium
- → Carbohydrate & Proteins are polar hence they are easily Soluble in blood and thus can be transported from one place of body to another



→ **Lipids present in Lipoproteins**

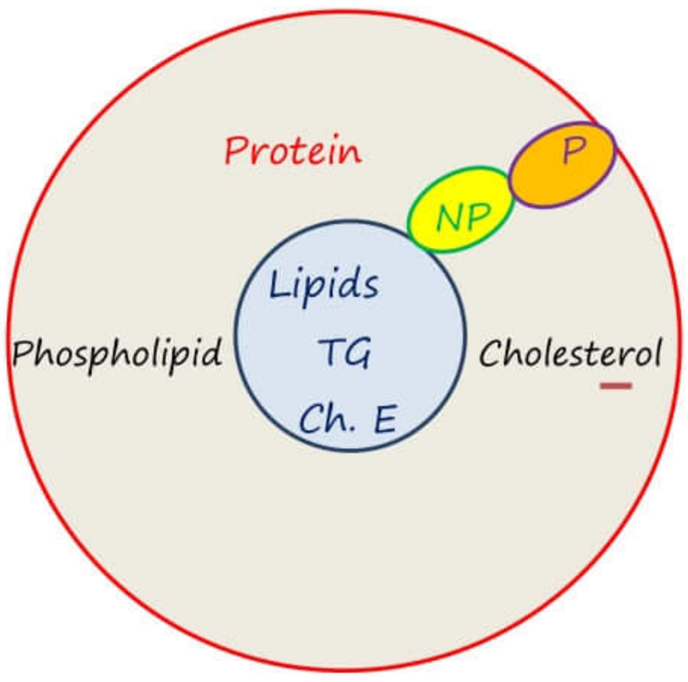
(1) Triglyceride → NP
(2) Phospholipid → Amphipathic
(3) Cholesterol → Amphipathic
(4) Cholesterol ester (cholesterol + FA) → NP

Lipids arranged in Structure of Lipoprotein

* Lipids which is non-polar → Present in core → TG, cholesterol ester

*Amphipathic Lipids → Polar portion will be towards outside
Non-polar portion will be towards inside

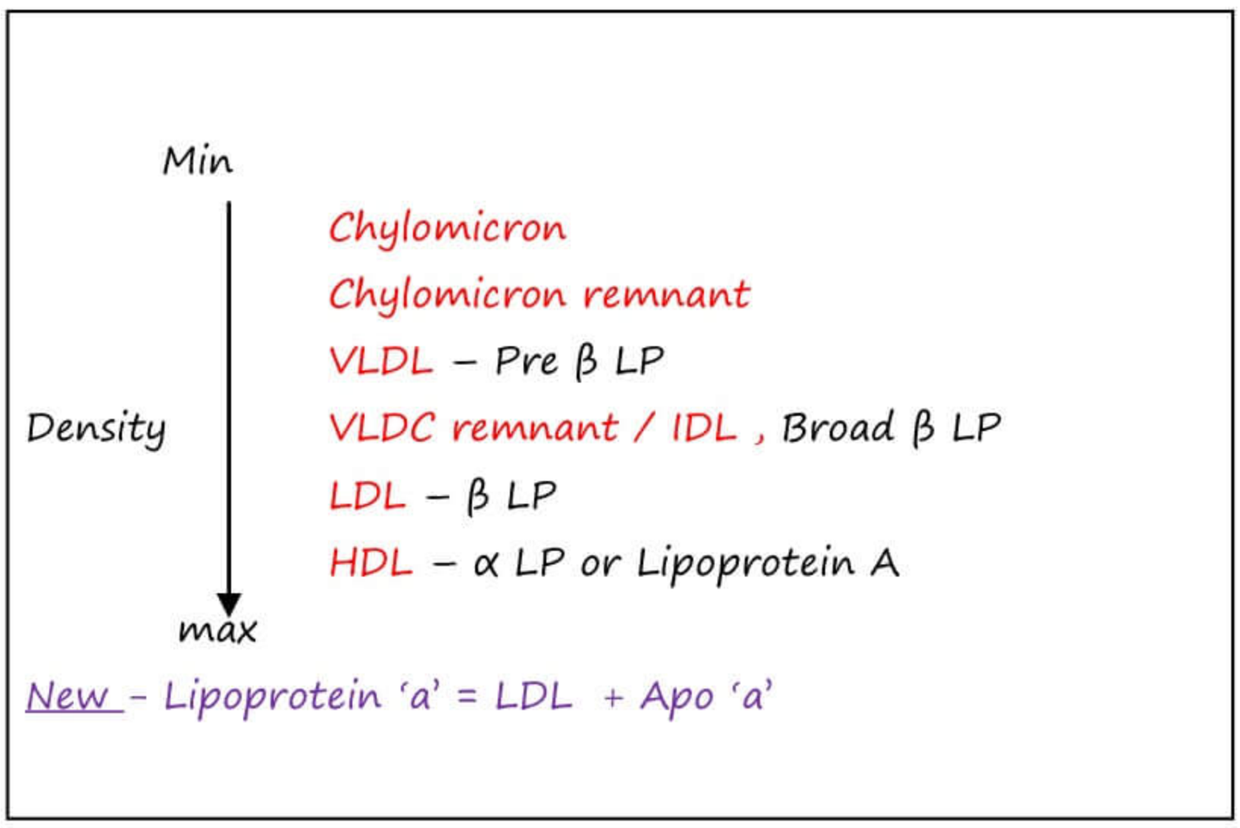
*E.g.: - Cholesterol → OH portion will be towards (Polar), Lipid portion towards inside (non-polar)
*Phospholipid - Phosphate portion (polar) towards outside Lipid portion towards inside



Density α $\frac{1}{\text{Size of LP}}$

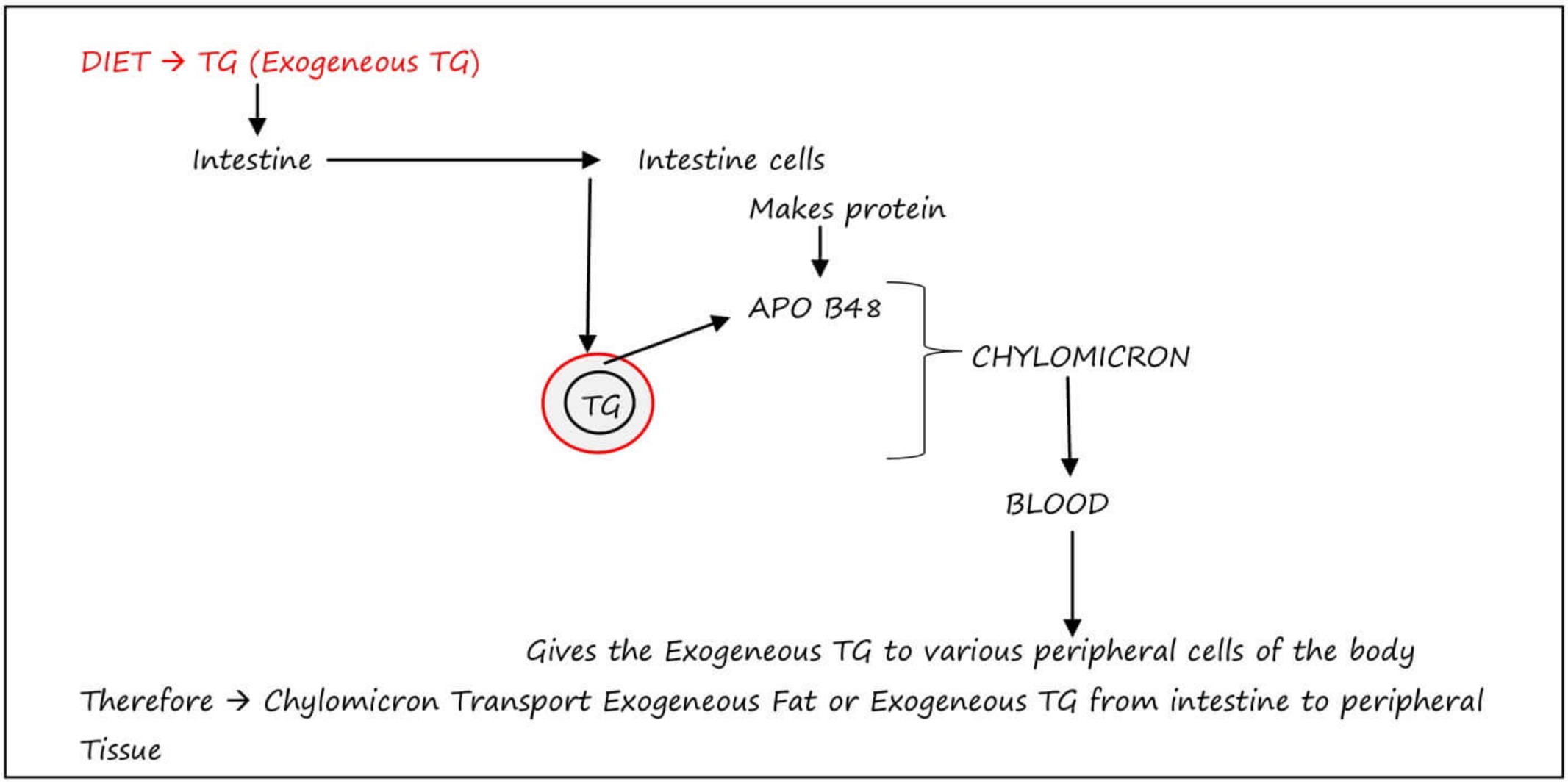
Density α $\frac{1}{\text{TG content}}$

Density α % proteins



New - Lipoprotein 'a' = LDL + Apo 'a'

	Lipoprotein	Lipid	Protein
Diet TG	(1) Chylomicron	TG	APO B48
Exogenous	(2) Chylomicron Remnant	TG + cholesterol	APO B48 + APOE
→Liver			
LIVER TG	(3) VLDL	TG	APO B100
Endogenous	(4) IDL / VLDL remnant	TG + cholesterol	APO B100 + APOE
Fat	(5) LDL	Cholesterol	APO B100 + APOE
	(6) HDL	Cholesterol ester	APO A, C, E.

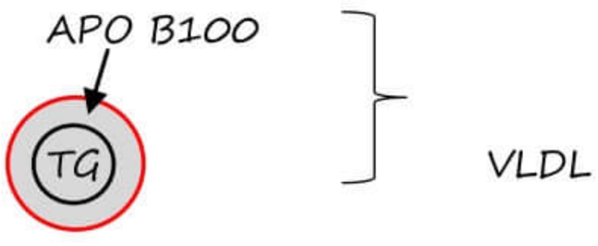


Therefore → Chylomicron Transport Exogeneous Fat or Exogeneous TG from intestine to peripheral Tissue

Endogenous Fat

- Liver makes Endogenous TG
- Liver also makes a protein APO B100

  And put at periphery of TG



* So VLDL Transport Endogenous TG from Liver to Peripheral Tissues

* VLDL moves in circulation keep losing TG

And makes IDL → IDL further moves in

Circulation Keep giving TG to peripheral Tissues

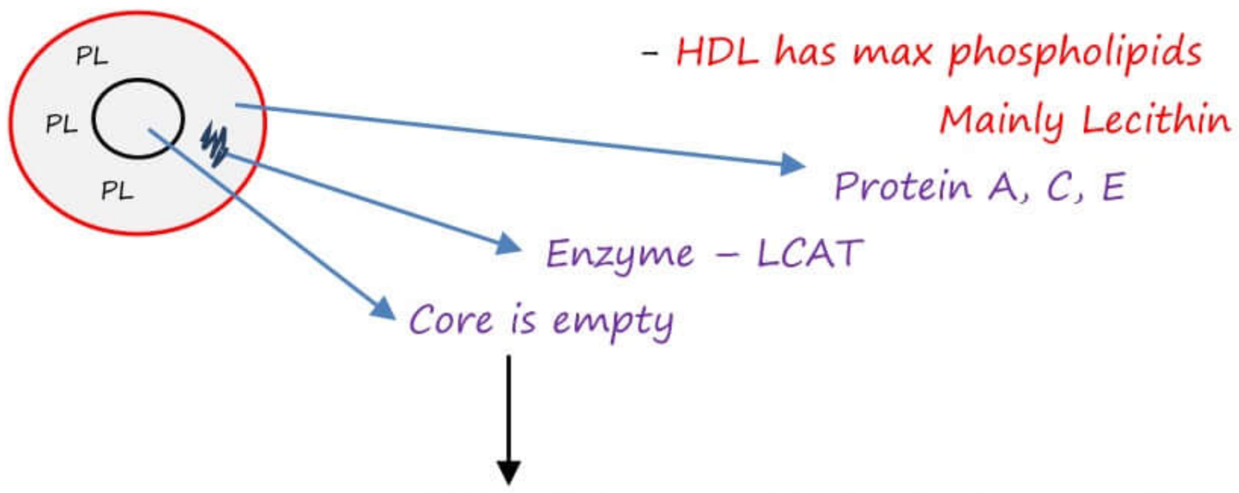
So that at Last only cholesterol is left

This structure is called LDL.

* LDL function is to give cholesterol to peripheral Tissues

Excess of LDL is bad cholesterol because it forms chain of oxidative free radical reaction.

HDL is Synthesized is Liver



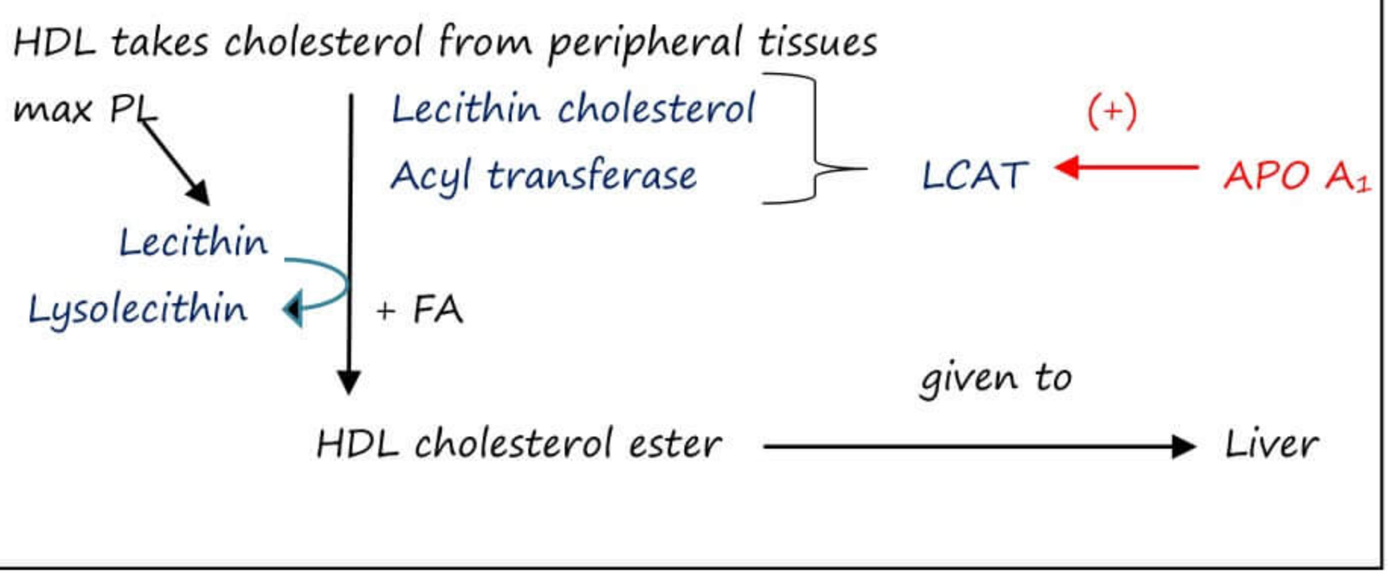
Because this structure can go to peripheral tissue and bring back extra cholesterol which are present in peripheral tissues / Blood vessels.

So it also called Reverse cholesterol Transport.

Lipid present is PL amd cholesterol ester and protein APO A, C, E are present

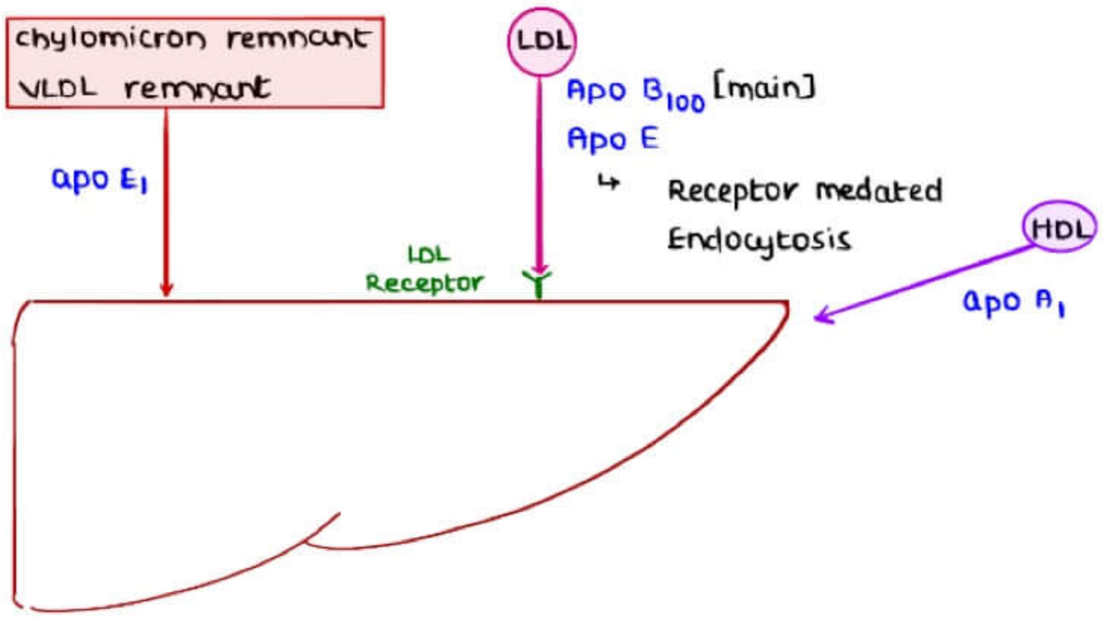
HDL is called good cholesterol because it has a special property that it takes cholesterol from tissue and immediately adds one FA to it making cholesterol ester.

How HDL adds Fatty acid



LIGANDS ON LIPOPROTEINS FOR UPTAKE BY LIVER

Ligand on Lipoprotein by which liver will recognise that Lipoprotein



ROLE OF APOPROTEINS

1. Structural Role → Ex: apo B
2. Enzyme activators or Inhibitor
   - Apo c_1 & c_2 → (+) Lipoprotein Lipase
   - Apo c_3 → (–) Lipoprotein Lipase
   - Apo A_1 → (+) LCAT
   - Apo A_2 → (–) LCAT
3. Ligand for the Receptors

HDL → α – Lipoprotein or Lipoprotein 'A' → Prevent atherosclerosis

Lipoprotein 'a' = LDL + apo 'a' → Cause atherosclerosis

Lipoprotein 'x'

→ Abnormal Lipoprotein

→ Found in 1. LCAT deficiency

2. Cholestatic states

Ex: 1° Biliary cirrhosis

1° Sclerosing cholangitis

→ Rich in amphipathic lipids [PL & cholesterol]

→ Poor in neutral lipids [TG & cholesterol ester]

HYPER LIPOPROTEINEMIA [FREDRICKSON]

TYPE	DEFECT	LP	TG	Cholesterol
I	Lipoprotein Lipase Or Apo C-II defect	Chylo > VLDL	↑	Normal
II a	LDL Receptor or Apo B_{100}	↑ LDL	N	↑
II b	Unknown	↑ VLDL ↑ LDL	↑	↑
III	apo E	↑ Chylo – remnant ↑ VLDL remnant	↑	↑

Type I → Familial Hyperchylomicronemia
→ Apo – C_2 defect ≈ Type 1

Type II a → Familial Hypercholesterolemia
II b → Familial combined hyper lipoproteinemia
III → Broad β diseases / Remnant removal disease | Dys β lipoproteinemia
TENDON XANTHOMA → ↑ Cholesterol

Eruptive Xanthoma → ↑ TG
Palmar & Tubero eruptive xanthoma → ↑ Chylo remnant
↑ VLDL remnant
Milky plasma → ↑ Chylomicrons
Acute pain abdomen [Acute pancreatitis] → ↑ TG

Friedwald's Equation (for calculating LDL)

Total chol = VLDL + HDL +LDL

LDL = Total cholesterol – HDL – VLDL

$$\text{LDL Cholesterol} = \text{Total cholesterol} - \text{HDL} - \frac{TG}{5} \quad \text{Mg/dl}$$

$$\text{LDL cholesterol} - \text{Total chol} - \text{HDL chol} - \frac{TG}{2.2} \quad \text{mmol/dl}$$

HDL (HIGH DENSITY LIPOPROTEIN)

SYNTHESIS

→ Synthesized in Liver (mainly) & small intestine

→ Has maximum phospholipids

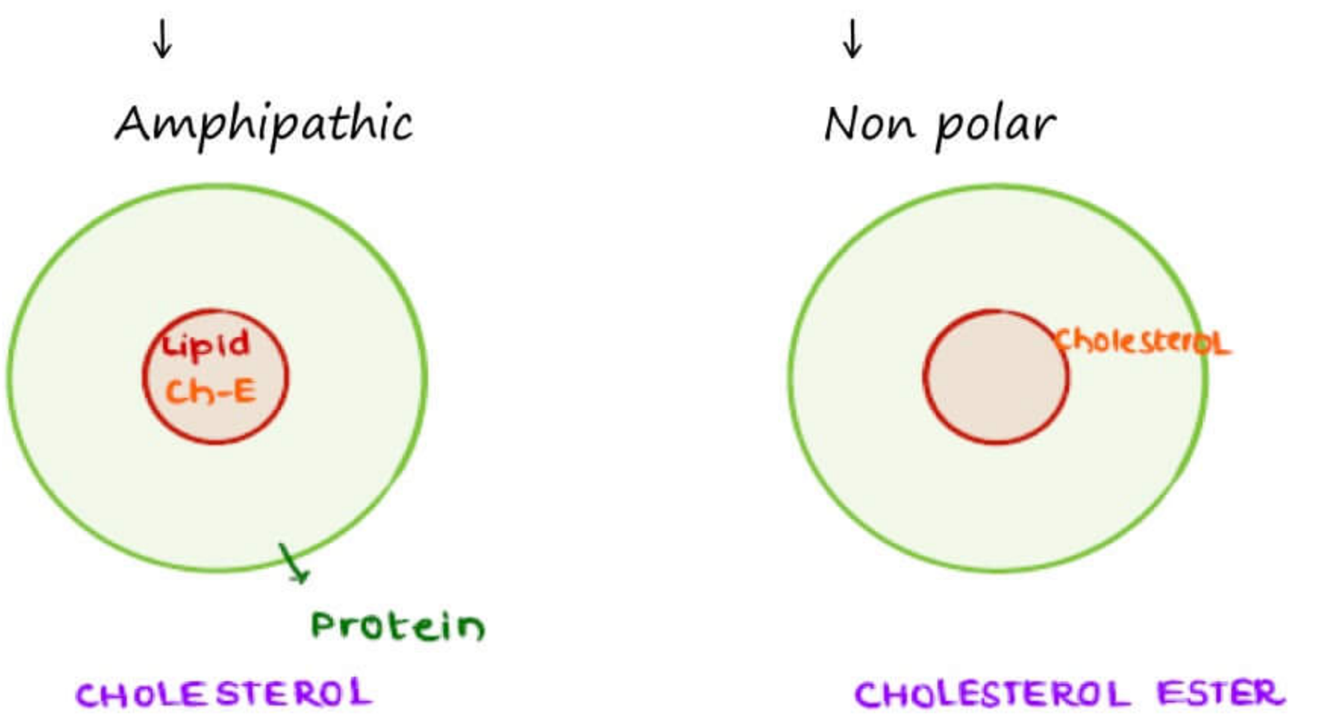
→ Proteins → Apo A, C, E

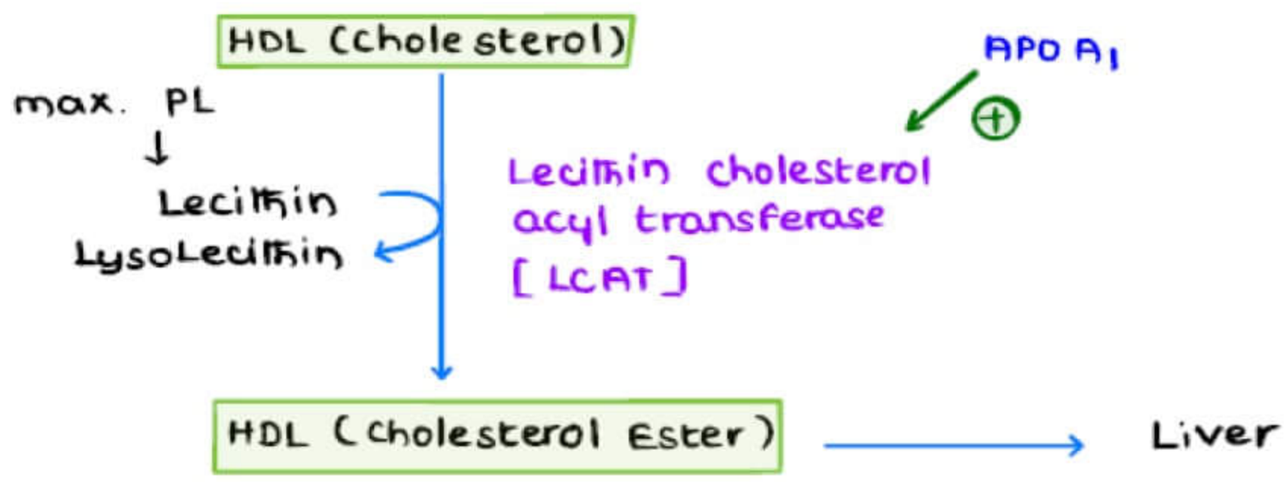
- Apo C & E are only synthesized in Liver

  They transfer from Liver to intestine

→ Contains LCAT enzyme

CHOLESTEROL TO CHOLESTEROL ESTER

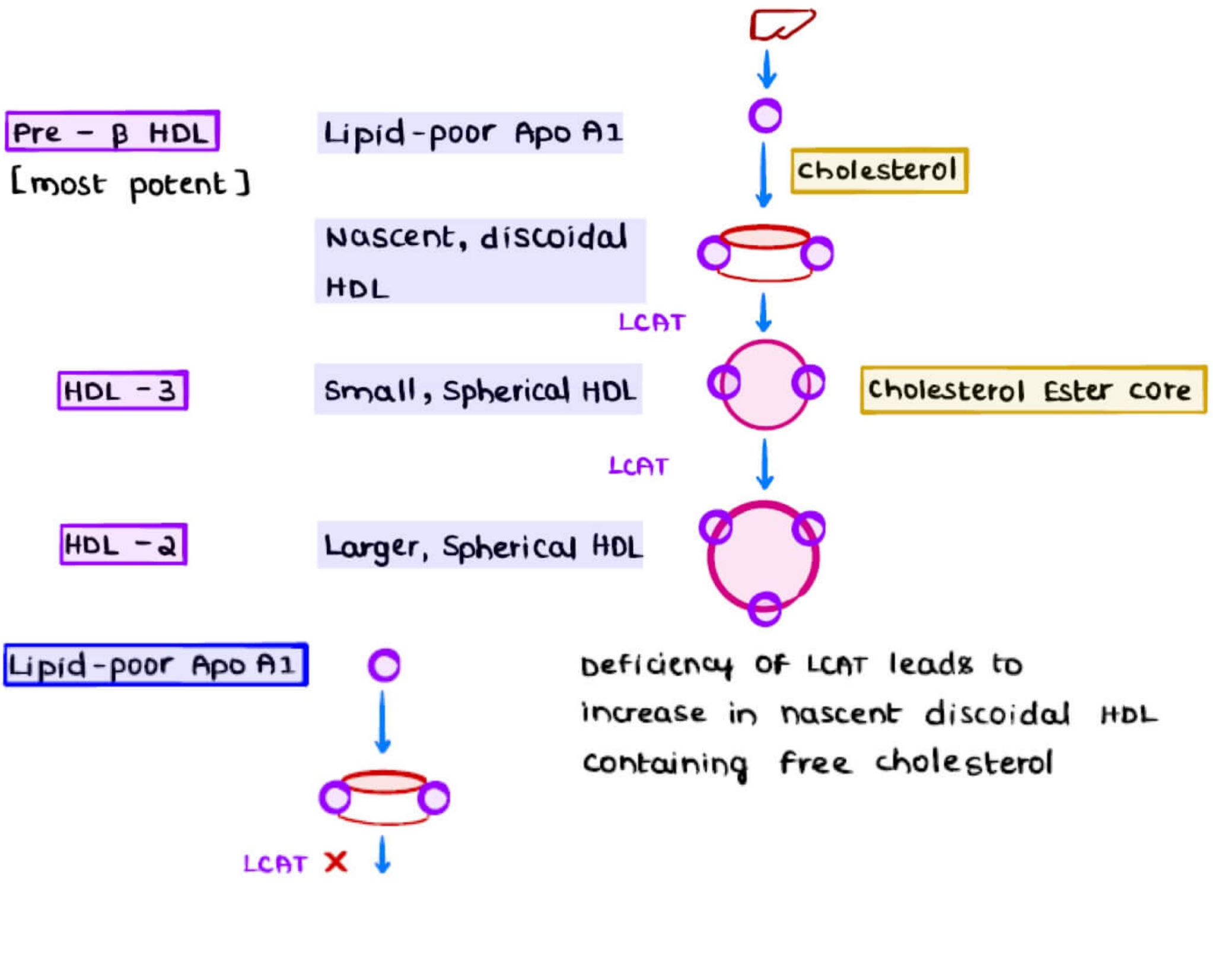


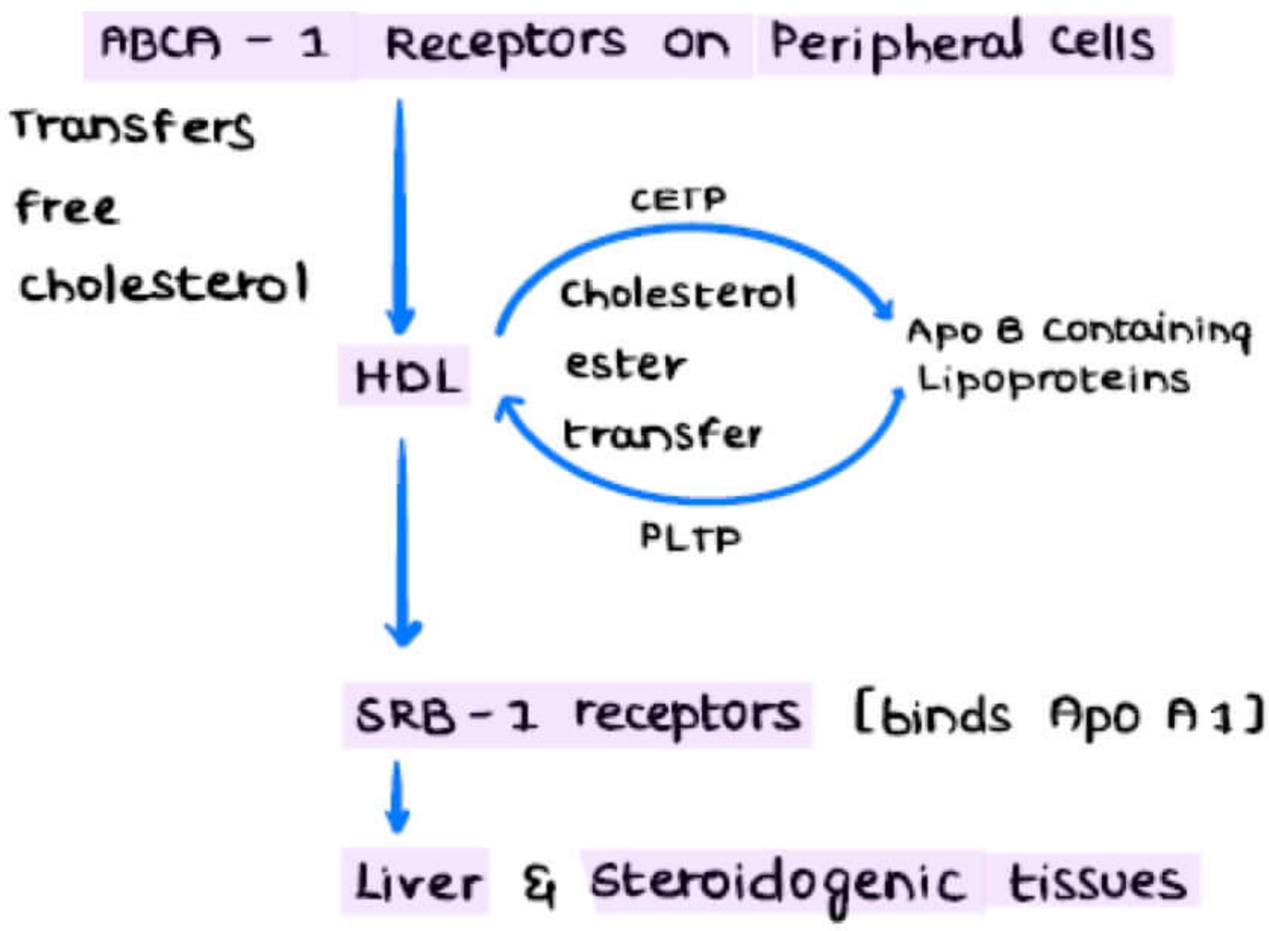


→ Addition of FA to cholesterol requires

1. Lecithin
2. LCAT
3. Apo A

} Lie within HDL

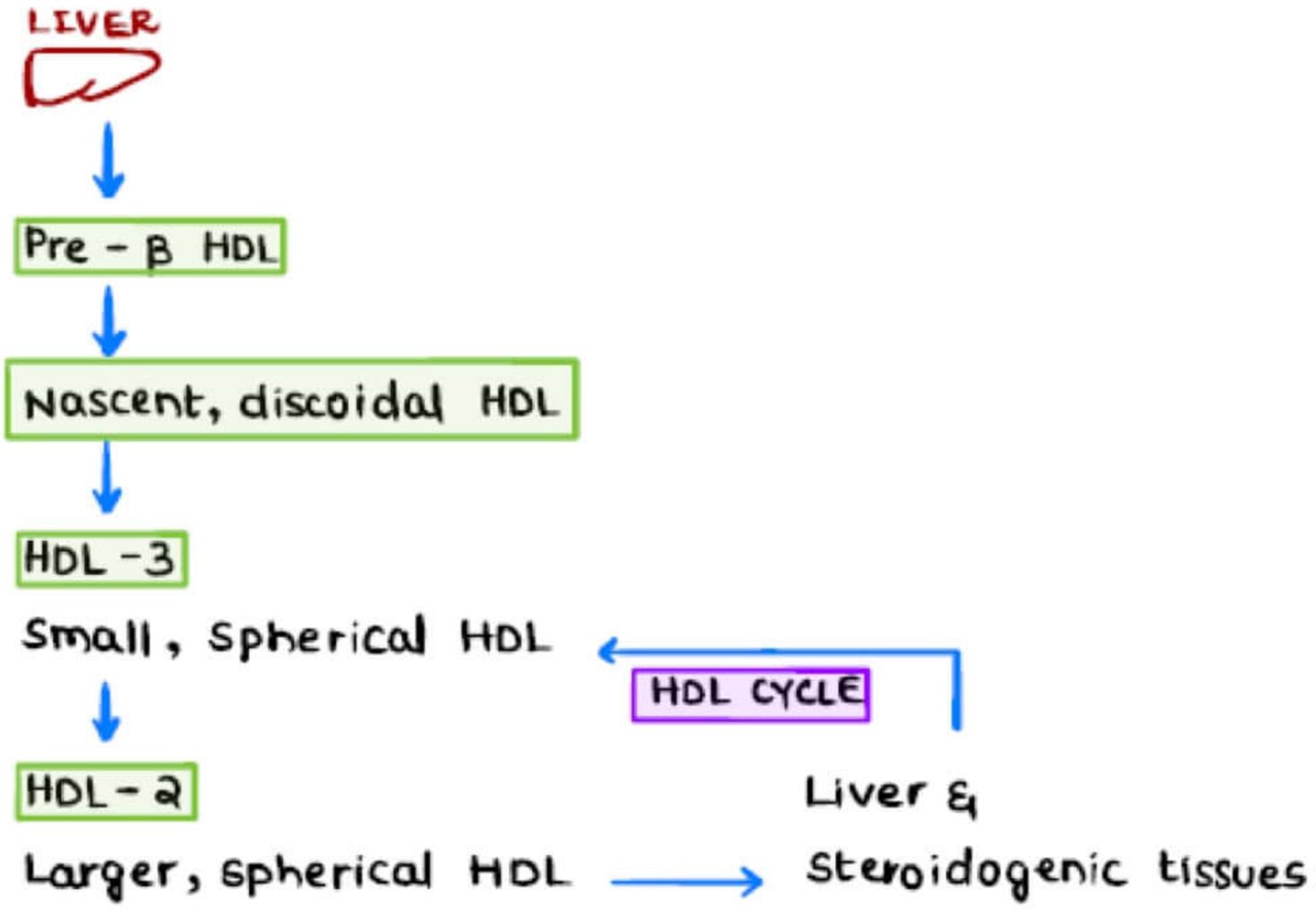

Pre - β HDL
[most potent]
Lipid-poor Apo A1
Cholesterol
Nascent, discoidal HDL
LCAT
HDL - 3
Small, Spherical HDL
Cholesterol Ester core
LCAT
HDL - 2
Larger, Spherical HDL
Lipid-poor Apo A1
LCAT
Deficiency of LCAT leads to increase in nascent discoidal HDL containing free cholesterol


ABCA - 1
Receptors on Peripheral cells
Transfers free cholesterol
HDL
CETP
Cholesterol ester transfer
PLTP
Apo B containing Lipoproteins
SRB - 1 receptors [binds Apo A1]
Liver & Steroidogenic tissues

ABCA – 1 RECEPTORS → ATP Binding casset A1 Protein

CETP → Cholesterol ester transfer protein

PLTP → Phospholipid transfer protein



FATTY ACID SYNTHESIS

→ Anabolic pathway

→ Occurs in cytoplasm

→ Activated by Insulin

1. Acetyl Co A (2c) —[+ CO_2; Acetyl Co A Carboxylase Or Malonyl Co A Synthetase [RLE]]→ Malonyl Co A (3c)

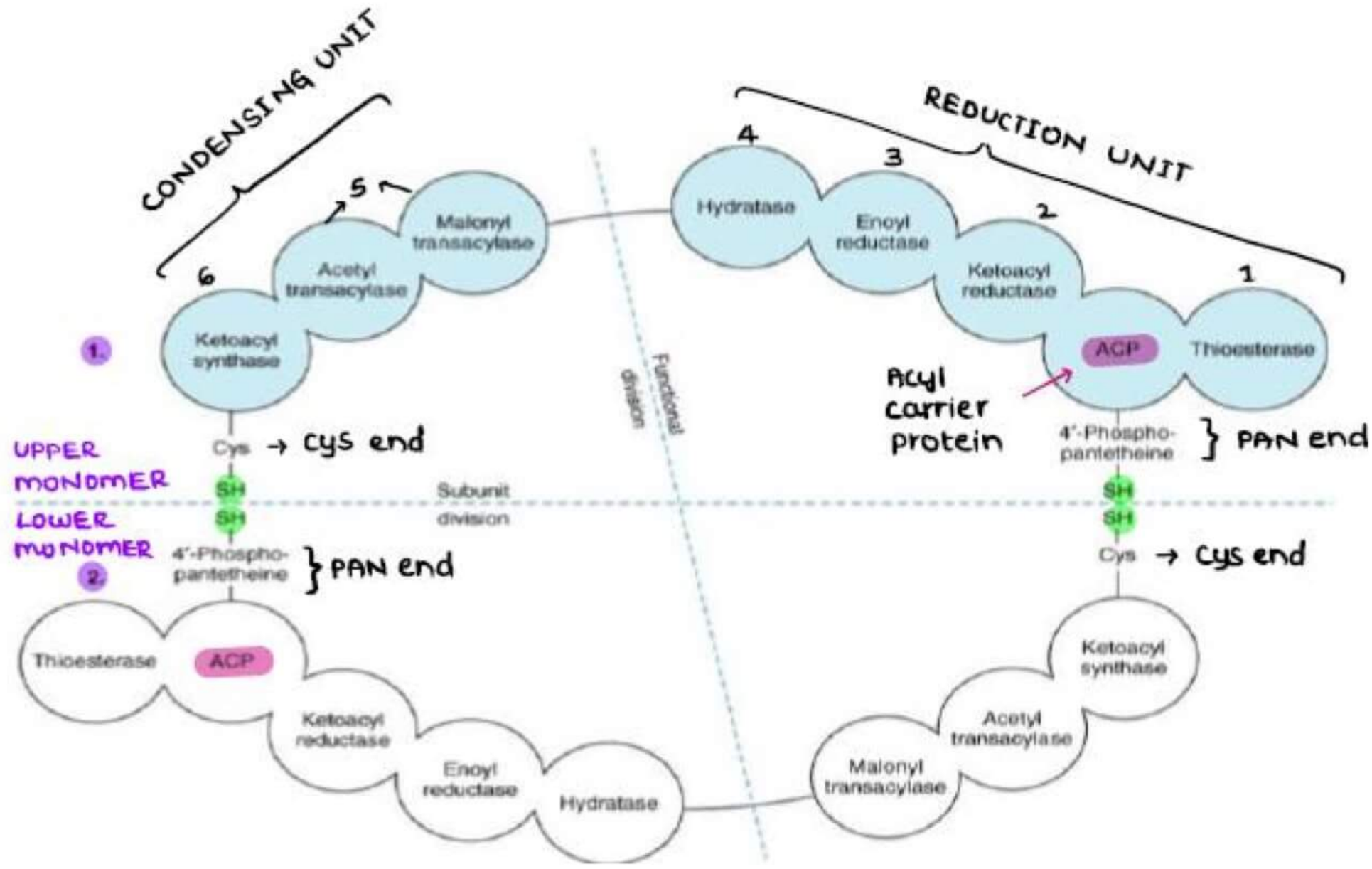
FA SYNTHASE COMPLEX

→ Main enzyme

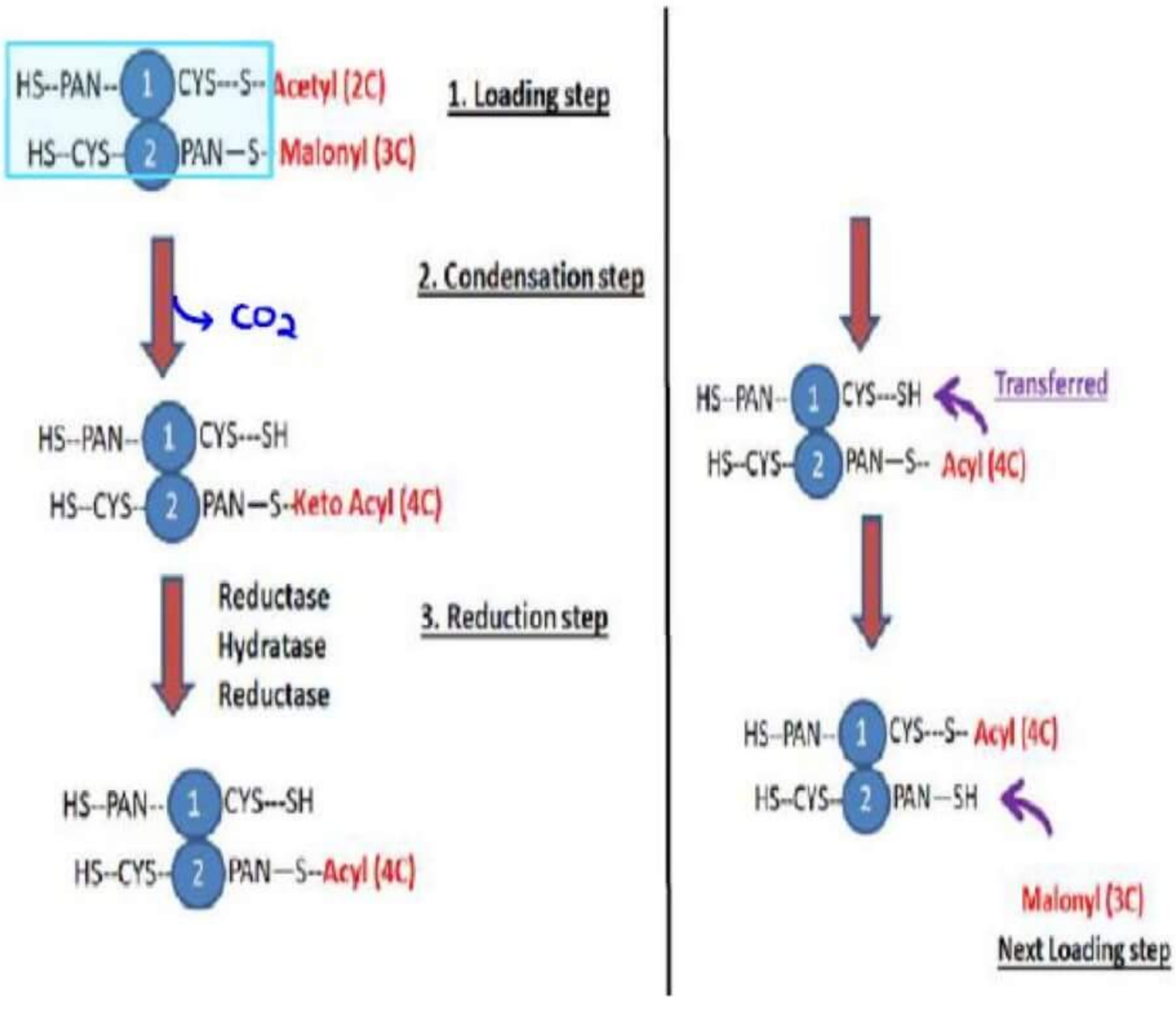
→ DIMER

X- shaped

Detected by X-Ray crystallography



➔ Two monomers are joined by disulphide bonds



LOADING STEP

→ Acetyl always loaded at Cys end

→ Malonyl always loaded at PAN end

CONDENSATION STEP

→ CO_2 is removed from Malonyl CoA

→ After this step, upper Monomer is empty

→ Lower monomer is → Keto Acyl [4c]

Acetyl (2c) + (Malonyl (3C)-(1C)

3. REDUCTION STEP

→ Removal of Keto group

→ Requires 3 enzymes

- Reductase → NADPH is used
- Hydratase
- Reductase → NADPH is used

→ 2 NADPH are used

→ In the product,

Upper monomer → empty

Lower monomer → Acyl (4C)

The above steps are repeated again & again to make Long chain FA

NEXT CYCLE

→ Acyl (4C) from the Lower monomer is transferred to upper monomer

→ Malonyl Co A (3C) comes at PAN end (next Loading Step)

→ Cycle repeats again & again

In 1st cycle, 5 carbons are loaded & only 4 carbons are added [-1C at condensation]

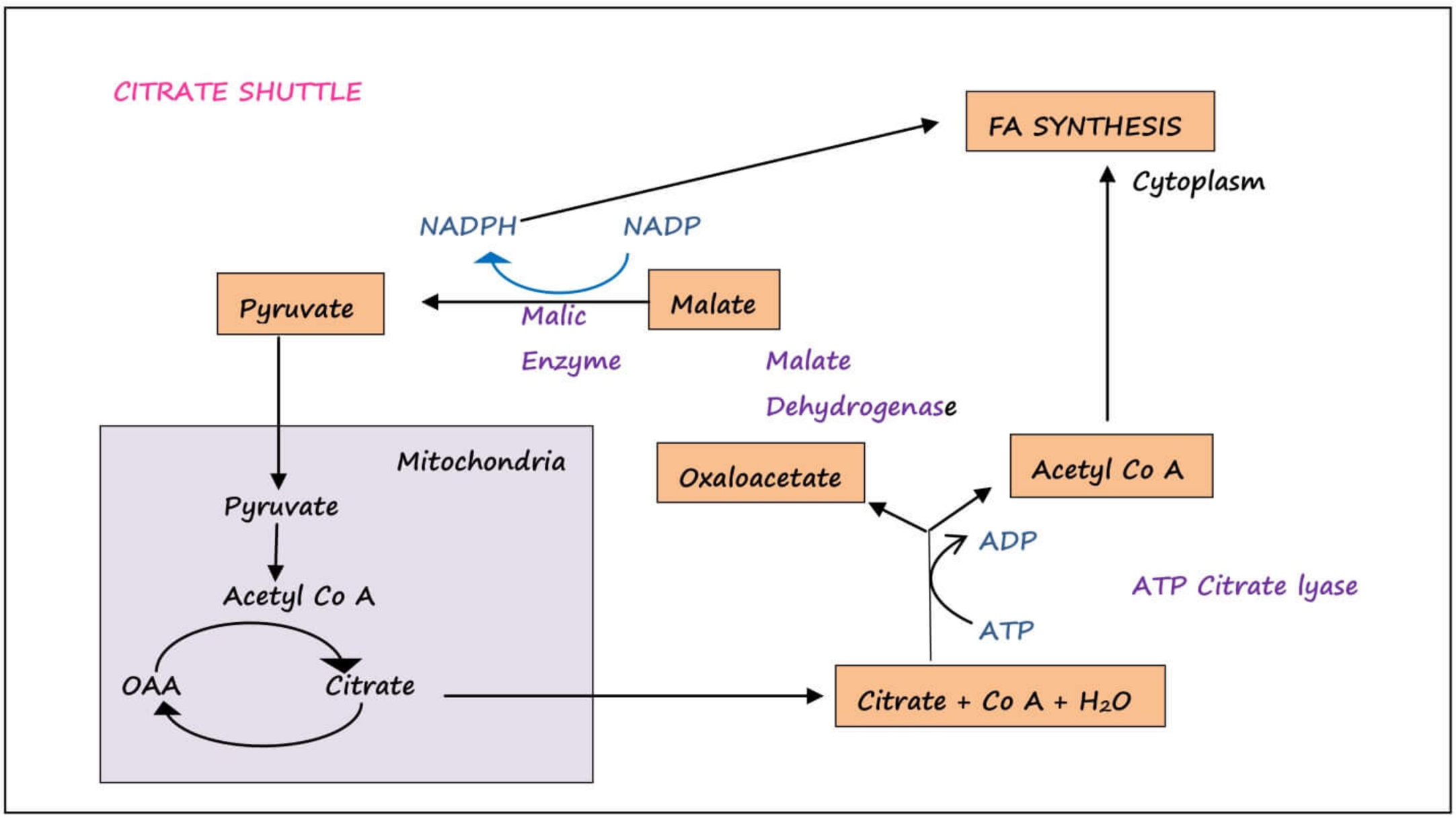
In next cycles, 3 carbons are loaded & only 2 carbons are added

Q FA is Synthesized from → Acetyl Co A

→ Carbon of Malonyl CoA not getting added in FA

- 1st enzyme carboxylase added one CO_2

  2nd enzyme FA synthase removed the CO_2



CITRATE SHUTLE → For the transport of Acetyl Co A from mitochondria to cytoplasm

ATP Citrate Lyase

→ Uses ATP

- Generally, Lyases do not use ATP

→ Anabolic enzyme but active in phosphorylated state.

- Generally anabolic enzymes are active in dephosphorylated state.

KETONE BODY PATHWAYS

KETONE BODY SYNTHESIS

→ Catabolic pathway
- Occurs in mitochondria
- Activated by glucagon

→ Occurs only in Liver

KETONE BODY UTILIZATION

→ Catabolic pathway
- Occurs in mitochondria
- Activated by glucagon

→ Occurs in brain, heart & muscles

KETONE BODY SYNTHESIS

(2C) Acetyl Co A
↓ Thiolase
(4c) Acetoacetyl Co A
↓ HMG Co A synthase (RLE)
(6c) HMG Co A
↓ HMG Co A lyase

(1°) Acetoacetate [1st KB to be formed]

Beta-hydroxy (2°) Butyrate

Acetone (2°)

- mc KB found in blood & Urine
- Do not produce energy
- Acetone like Smell indicates Ketoacidosis

KETONE BODY UTILIZATON

(4c) Acetoacetate
Thiophorase ← Co A added without using ATP
Rule of business not followed
Activation do not use ATP
Acetoacetyl Co A
Thiolase
Acetyl Co A → TCA

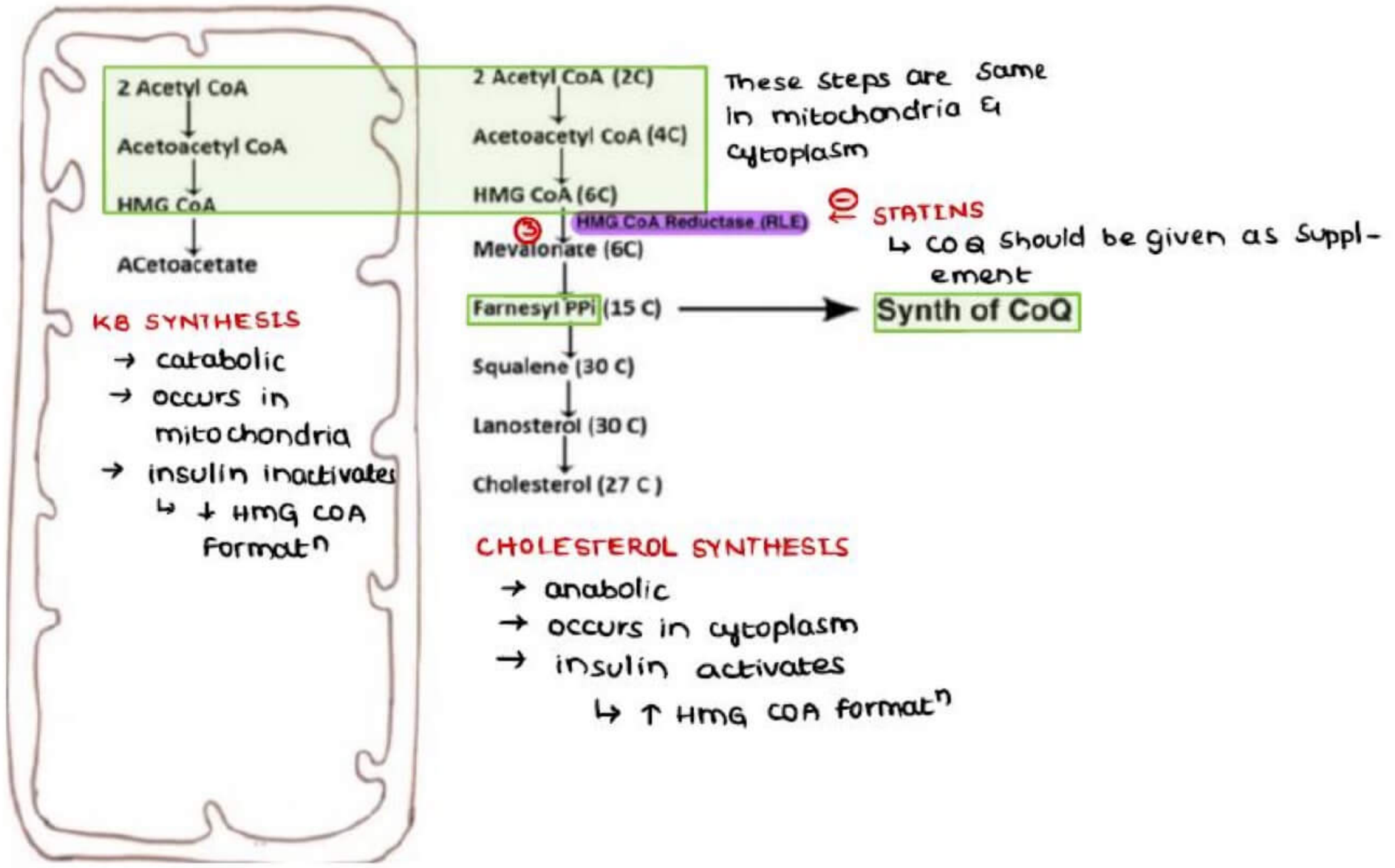
THIOPHORASE
→ Rule of business not followed
→ CO_2 is added without using ATP
→ CO_2 is absent in liver
- Liver never uses ketone bodies

TEST OF KETONE BODIES

1. ROTHERA'S TEST
   → Purple colour ring at junction of 2 liquids → POSITIVE
   → Positive for Acetoacetate & Acetone

2. GERHARDT'S TEST
   → Positive for Acetoacetate

Beta – OH – Butyrate —oxidized→ Aceto Acetate



BETA OXIDATION OF FATTY ACIDS

Catabolic pathway

- *Occurs in mitochondria*
- *Activated by glucagon*
- *Inhibited by insulin*

1. RULE OF BUSINESS / ACTIVATION OF FA

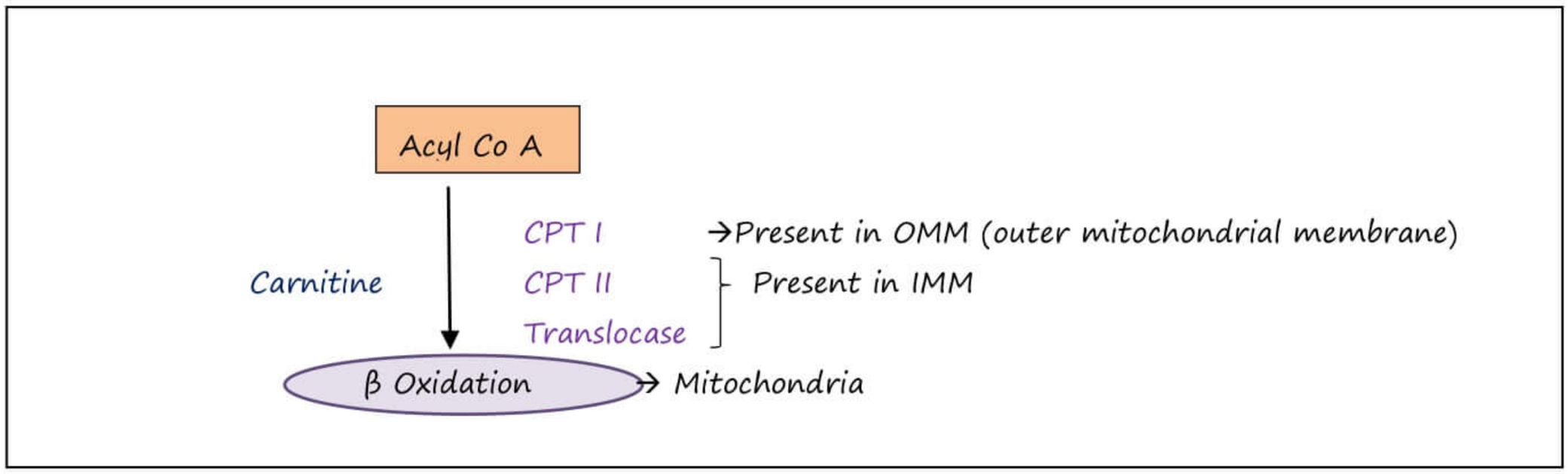
Acyl Co A Synthetase / Thiokinase (EC no 6)

FA ⟶ Acyl Co A

ATP ⟶ AMP (2 ATP used)

→ *Occurs in cytoplasm*

- *As it controls point of regulation to the body*
- *Only at necessity (starvation), Beta oxidation will be initiated by body*



CPT 1 (Carnitine Palmitoyl Transferase I)

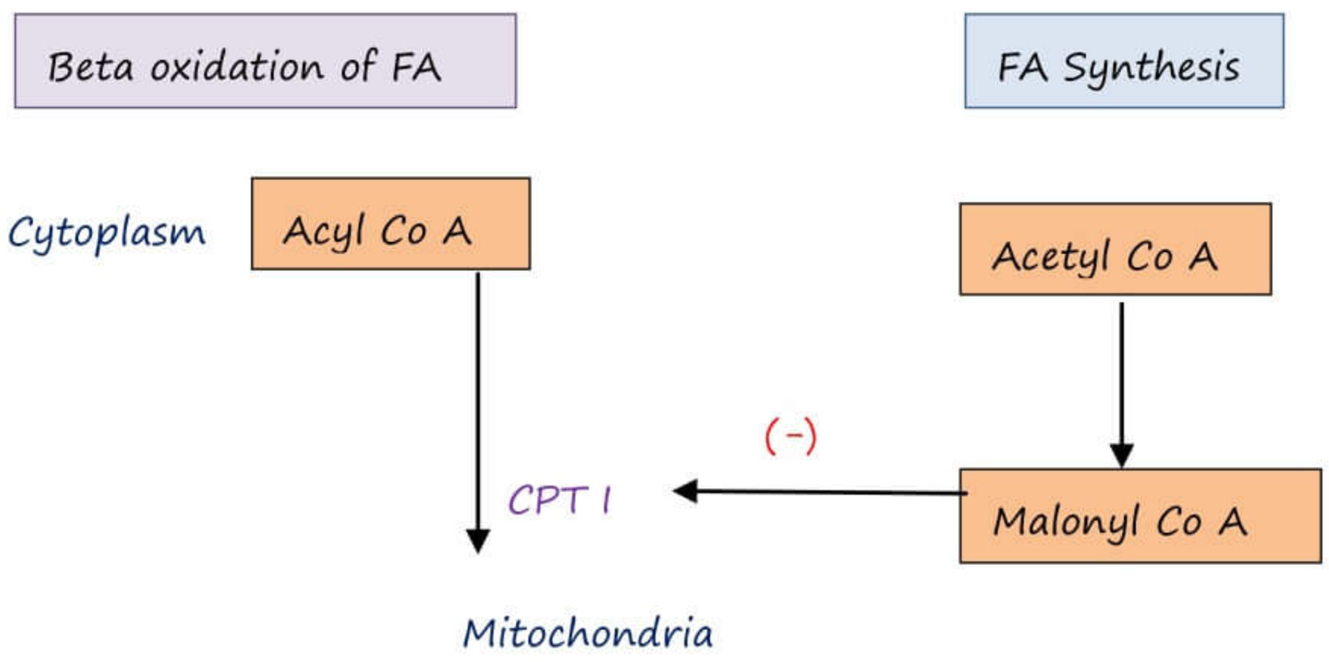
→ Outermost enzyme which will decide the entry of Acyl Co A into mitochondria

→ Has a role in reciprocal regulation

→ Only Long chain FA [14-20C] requires the carnitine system

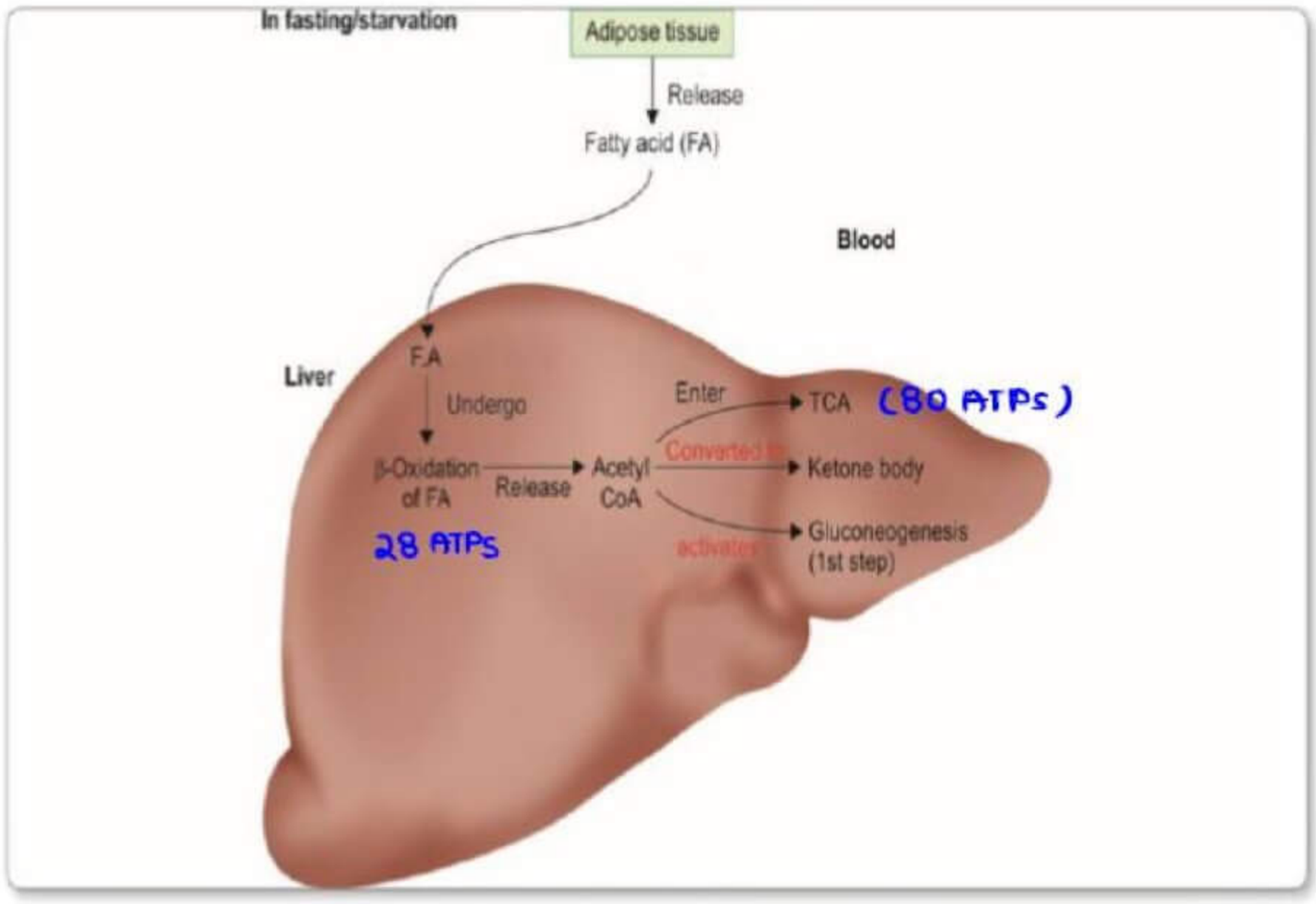
→ Medium chain (6-12C) , Short chain (2-4C) FA can directly enter into mitochondria

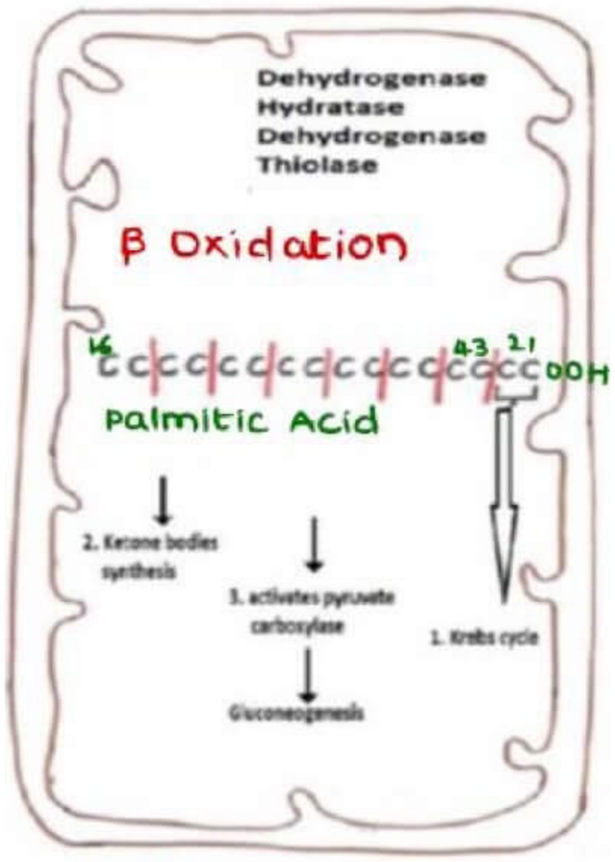
RECIPROCAL REGULATION



In fed state, Malonyl CoA is formed [FA Synthesis] → Inhibits CPT I [β oxidation of FA]

In starvation, Malonyl Co A is not formed → No inhibition on CPI





β OXIDATION

→ Cleavage occurs b/w c_2 (α) & c_3 (β)

→ For each cleavage 4 enzymes are required

1. Dehydrogenase → $FADH_2$ → 1.5 ATP
2. Hydratase
3. Dehydrogenase → NADH → 2.5 ATPs
4. Thiolase → 4 ATPs

Energetics for Palmitic acid (16c)

→ 7 cleavages → 8 Acetyl Co A releasing

→ 7 cleavages → 7 x 4 → 28 ATPs

→ 8 Acetyl Co A via Kreb's cycle → 8 x 10 → 80 ATPs

108

- 2 (Used for activation of FA)

106 ATPs

Energetics of (18C) Stearic Acid → 120 ATPs

DEHYDROGENASES

LCAD	MCAD
→ Long chain Acyl Co A Dehydrogenase → Breaks upto 12 C	→ Medium chain Acyl Co A dehydrogenase → breaks below 12c → MCAD Deficiency 1. Hypoglycemia 2. Low keto bodies

MCAD Deficiency leads to non-ketotic hypoglycemia during starvation

- Normal in fed state (as β oxidation not required)
- During fasting, only LCAD Functional releasing very less energy in Liver from TCA cycle
  - Ketone body Synthesis do not occur → Low ketone bodies
  - Gluconeogenesis do not occur → Hypoglycemia

- 12 C FA ↑↑ → Dicarboxylic acidosis occurs chronically

Odd Chain FA

Propionyl Co A (3c)

$+CO_2$ — Propionyl CoA Carboxylase (B_7)

D-Methyl Malonyl Co A (4c)

Racemase

L-Methyl Malonyl CoA (4c)

Mutase (B_{12})

(4c) Succinyl Co A → **TCA**

→ Propionyl CoA & Glycerol are Glucogenic
→ In B_{12} deficiency, L-methyl Malonic acid will come in Urine
→ In B_7 deficiency, Propionic acid will come in Urine

JAMAICAN VOMITING SICKNESS

→ d/t Toxin 'hypoglycin' from unripe fruit of akee tree
→ Inhibits Fatty Acyl CoA Dehydrogenase
→ Severe hypoglycemia occurs after ingestion
→ Sudden vomiting (2-6 hrs after ingestion)
→ Convulsions, coma & Death

Any defect in β oxidation will lead to non ketotic hypoglycemia

OXIDATION OF VLCFA (very long chain FA) (>20C)

→ Found in brain

→ Induced by high fat diet

→ Occurs in peroxisomes upto octanyl CoA & remaining occurs in mitochondria

➔ ZELLWEGER SYNDROME

- Defect in oxidation of VLCFA
- Defect in peroxisomes of all the body

α - OXIDATION

→ Occurs in peroxisomes & ER

→ For branched chain FA

→ Removal of 1 carbon from α carbon atom

→ NO ATPs produced

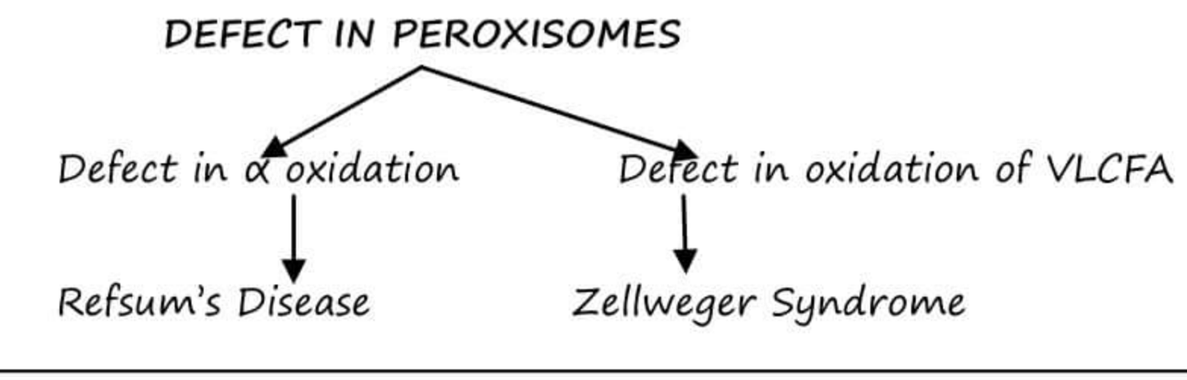
→ REFSUM's DISEASE

- Defect in α oxidation of peroxisomes
- Phytanic acid not oxidised
- Restrict dairy products & green leafy vegetables

ω - OXIDATION

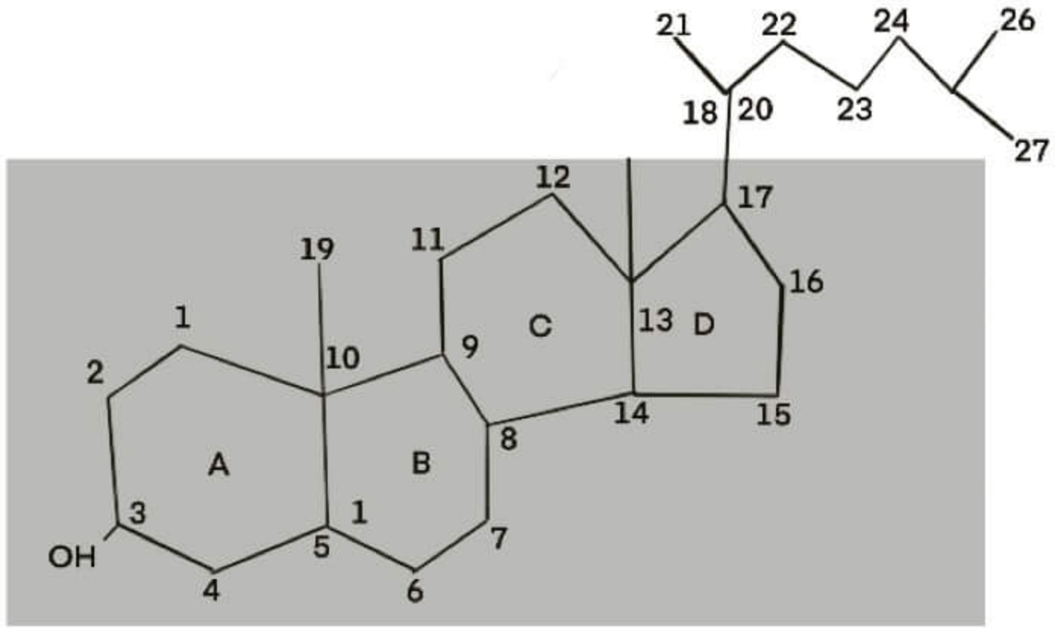
→ Dicarboxylic acids are formed [HOOC ——— COOH]

→ 12 carbon FA → ω oxidation → Dicarboxylic acids



CHOLESTEROL SYNTHESIS & BILE ACIDS

Cholesterol Structure – Cyclo Pentane Per hydro Phenanthrene ring



$C_{27}H_{45}OH$

Steroid + ≥ 1 Alcohol = Sterol

Sterol

→ Cholesterol (Chole – Bile) (Exclusively in animals)

→ Ergosterol (Ergus – fungus)

USES

→ Membrane Fluidity

→ Vit D Synthesis

→ Bile acids / Salts

→ Steroid hormone synthesis

De-novo synthesis

↓

DIET → Cholesterol → Uses

→ ½ Bile acids

→ ½ Coprostanol bacterial action, Faeces.

Cholesterol Synthesis

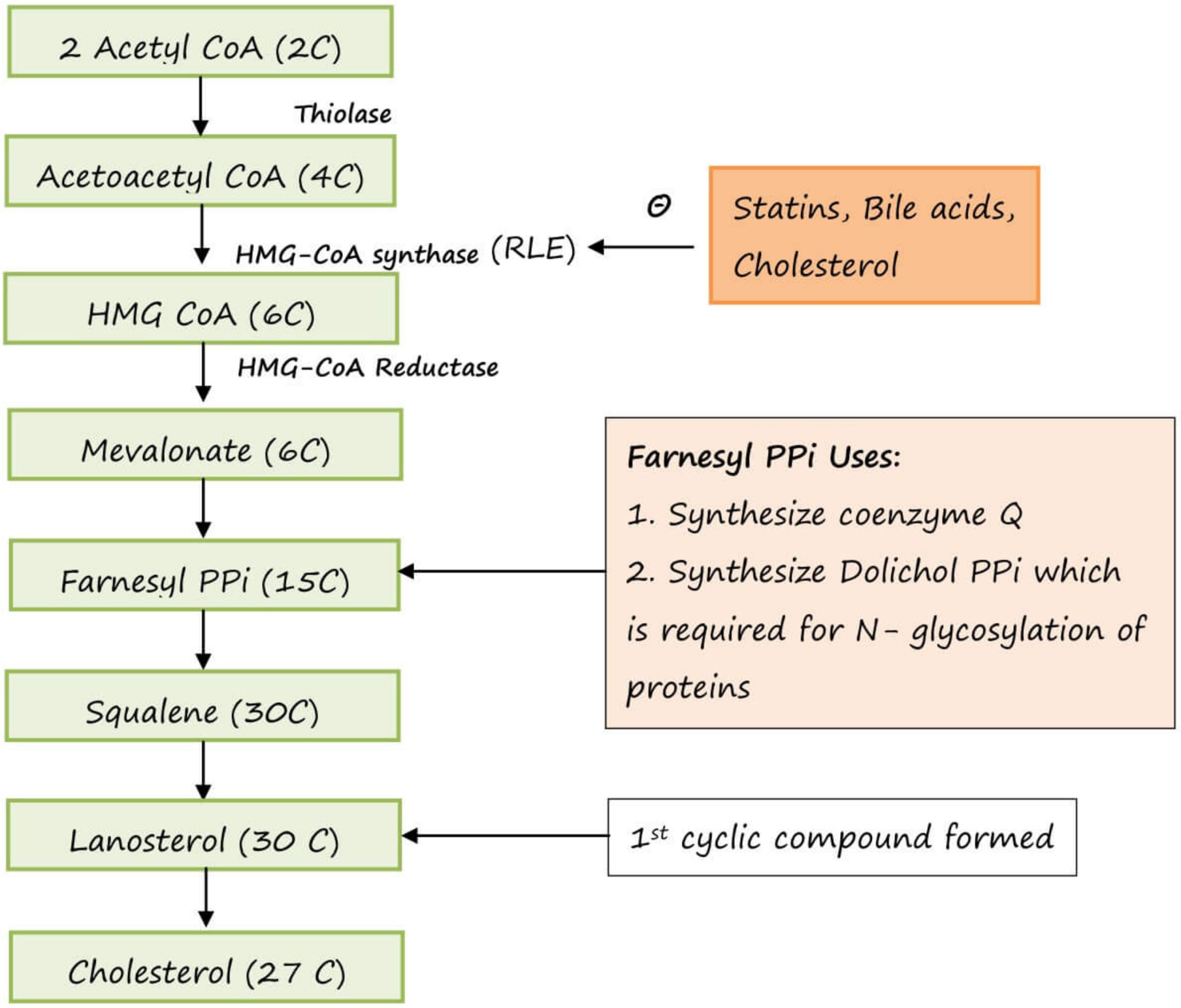
→ Anabolic, activated by Insulin and Dephosphorylation

Organ → Liver (mainly), Adipose Tissues, Adrenal cortex, Gonads, intestine & skin.

Compartment → Cytoplasm up to the formation of squalene

Smooth ER (Squalene to Cholesterol)

CHOLESTEROL SYNTHESIS



Changes from Squalene to Cholesterol

1. 30 C → 27 C
2. 6 double bonds – 1 double bond
3. OH – position 3
4. Cyclization

KB synthesis → Catabolic → Mitochondria

Cholesterol synthesis → Anabolic → Cytoplasm.

HMG CoA → β 13-OH, 3 Methyl / Glutaryl CoA

↓

- Cholesterol synthesis
- KB synthesis
- Leucine catabolism

Statins → Few patients → Red colored urine

Deficiency of CoQ

Muscle ATP → Lack of energy → Muscle cell damage → Myoglobin

BILE ACIDS

Cholesterol to Bile acids changes

1. 7 α Hydroxylation
2. Reduction of B ring of cholesterol
3. 27 C → 24 C
4. Oxidation of the terminal C → COOH Acids

USES

→ Bile Acid formation is the only way of cholesterol excretion from body.

→ Digestion/ Emulsification of lipids.

Bile Acids are synthesized → Liver

Liver Cholesterol —RLE (7–α–Hydroxylase)→ 1°BA —Conjugation (Glycine or Taurine)→ Conjugated Bile Acids

→ A cytochrome P450 enzyme

→ Vit C, O_2, NADPH

Primary Bile Acids

Cholic Acid – More abundant

Chenodeoxycholic acid

Primary Bile Acid → Secondary Bile Acid

7 α – OH group removed

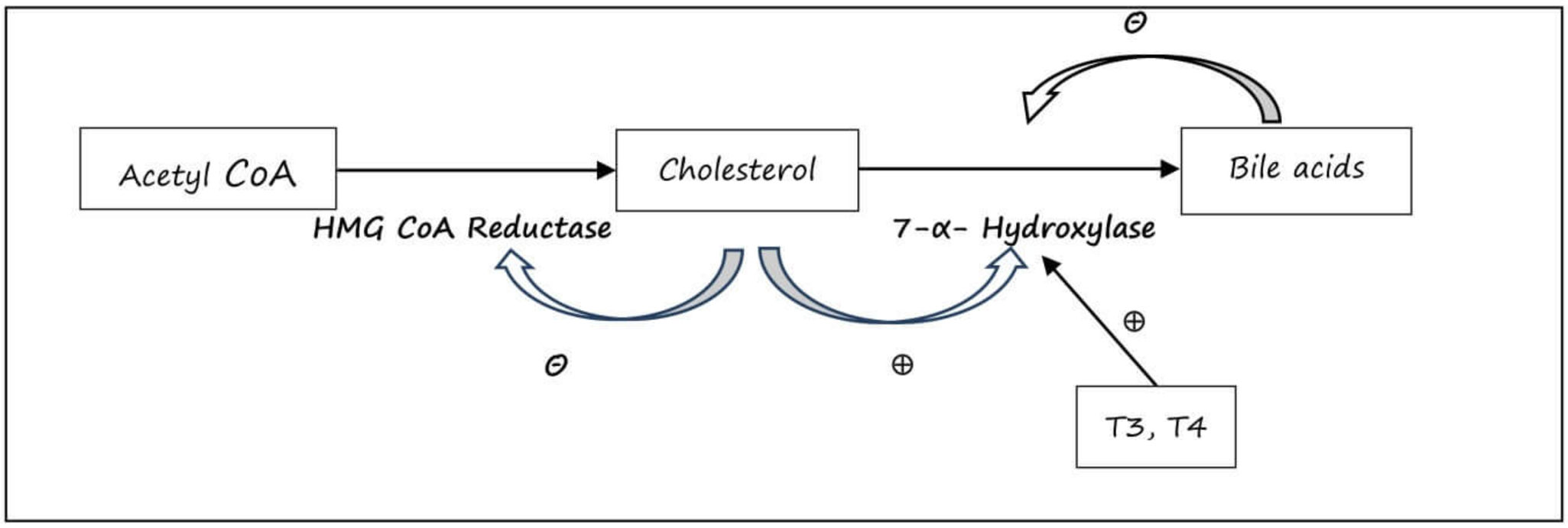
Cholic acid → Deoxycholic acid

Chenodeoxycholic acid → Lithocholic acid

2°BA → 98% - 99% Enterohepatic recirculation.

→ Bile released from liver contains both 1°BA and 2°BA

→ Least Enterohepatic recirculation → Lithocholic acid



Hypothyroidism = Chol level ↑

Ursodeoxycholic acids (2° BA)

'Ursus' – Bear.

Cheno deoxycholic acid ⟶ Ursodeoxycholic acid	
1°BA	

→ Hepato protective
→ Modify BA pool, ↑ Hydrophilic BA pool, ↓ Hydrophobic BA pool
→ Immuno modulatory
→ Cyto protective (Delay Gastroesophagial Varices)

Can be used in Treatment of:

→ Primary Biliary Cirrhosis
→ Obstetric Cholestatis – To relieve itching
→ Gall Stones medically (non-surgically)
→ Cystic Fibrosis associated liver disease
→ Non-alcoholic fatty liver disease.

MOLECULAR BIOLOGY

NUCLEOTIDES

Nucleic Acid => Polymers of nucleotides (DNA / RNA)

COMPONENTS

1. Nitrogenase base
2. Sugar

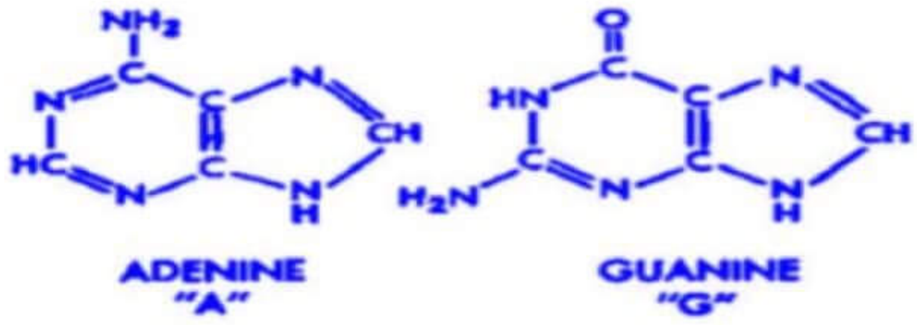
→ **NUCLEOSIDE**

3. Phosphate

NITROGENOUS BASES

PURINES

1. Adenine [2 rings + amino]
2. Guanine [2 rings, no amino]



PYRIMIDINES

1. Uracil [1 ring, no amino, no methyl]
2. Thymine [1 ring, no amino, methyl]
3. Cytosine [1 ring + amino]

PURINE

N1	→	Aspartate
N_3 N_9	→	Glutamine
C_4 N_7 C_5	→	Glycine
C_6	→	CO_2
C_2 C_8	→	THF

PYRIMIDINE

N_1	→	Aspartate
N_3	→	Glutamine
C_4 C_5 C_6	→	Aspartate
C_2	→	CO_2

THYMINE

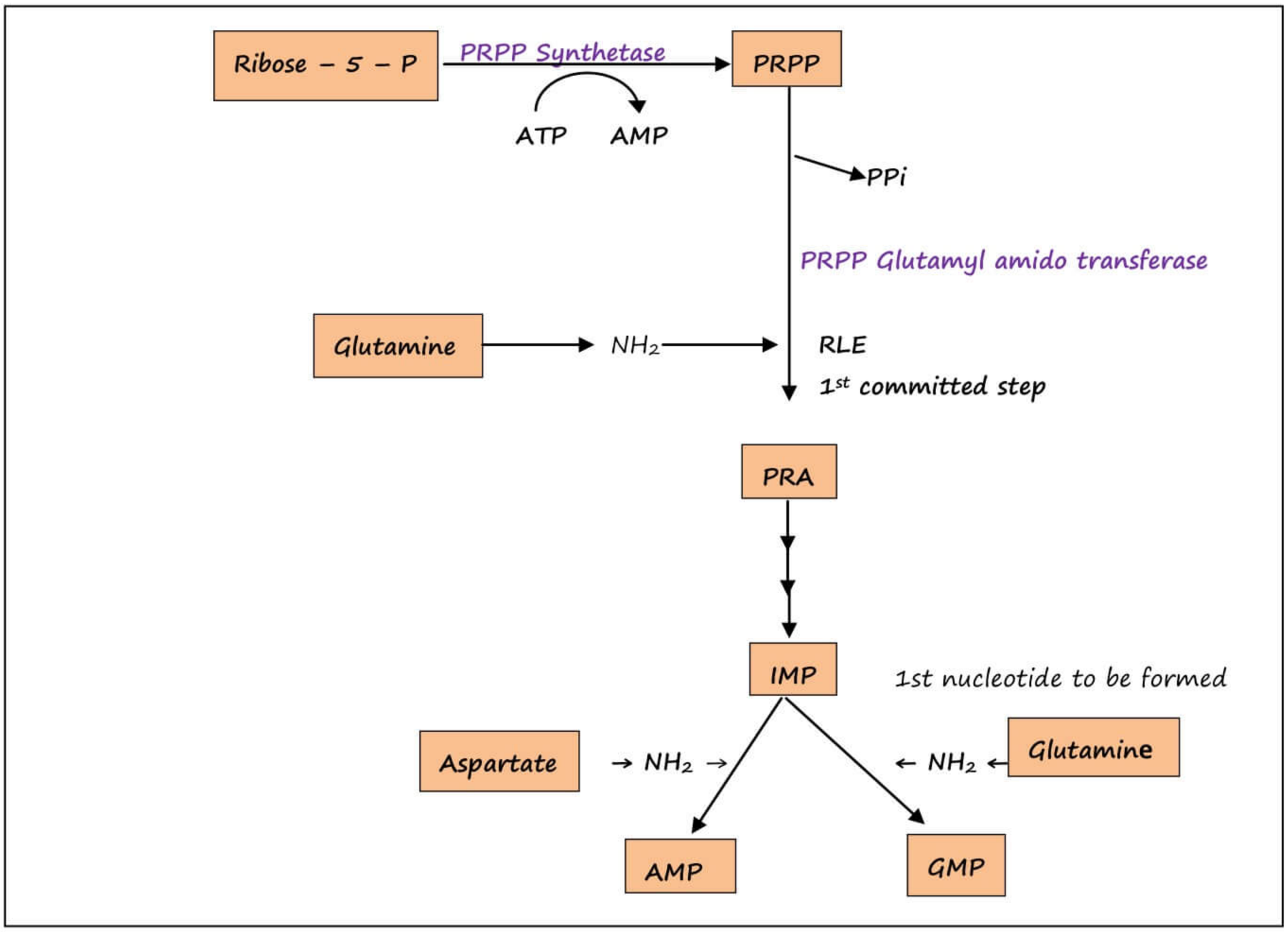
Extra CH_3	→	THF

PRODUCT OF CATABOLISM

Purines	→	Uric Acid (Soluble)
Pyrimidines	→	CO_2, NH_3, β Alanine
Thymine	→	CO_2, NH_3, β Aminoisobutyrate

PURINE SYNTHESIS

1. DENOVO PATHWAY



Ribose – 5 – P

→ Act as primer

→ Only source → HMP

N – BASE	NUCLEOSIDE	NUCLEOTIDE
Adenine	Adenosine	AMP, ADP, ATP
Guanine	Guanosine	GMP, GDP, GTP
Cytosine	Cytidine	CMP, CDP, CTP
Uracil	Uridine	UMP, UDP, UTP
Thymine	Thymidine	TMP, TDP, TTP

PRPP (Phosphoribosyl pyrophosphate)

IMP (Inosine mono phosphate)

→ 1st nucleotide to be formed

→ Nitrogenous base → Hypoxanthine

→ Parent nucleotide to be formed

- Further give rise to AMP & GMP

AMP (Adenosine monophosphate)

CMP (Cytidine monophosphate)

TMP (Thymidine monophosphate)

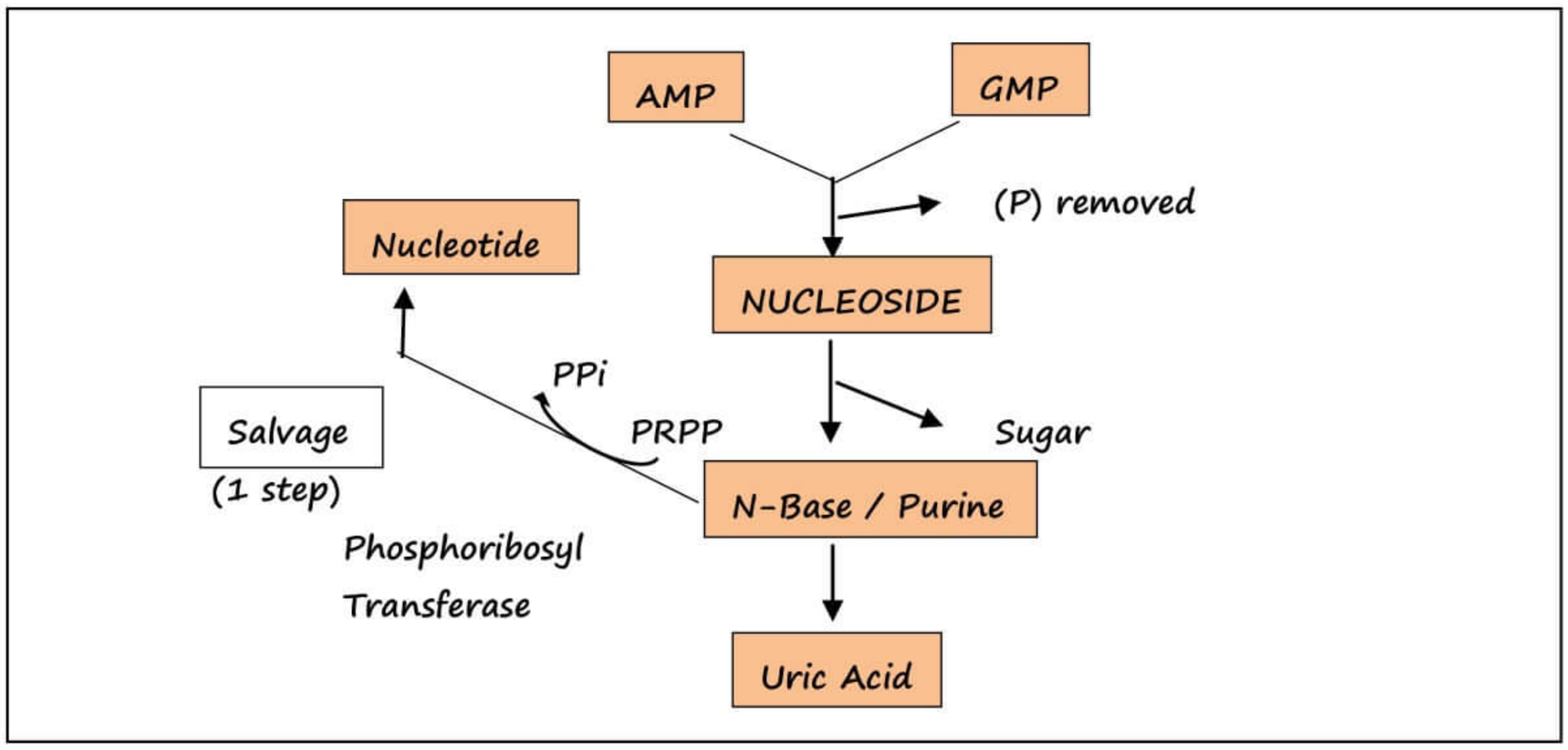
II SALVAGE PATHWAY

→ Less energy consuming pathway

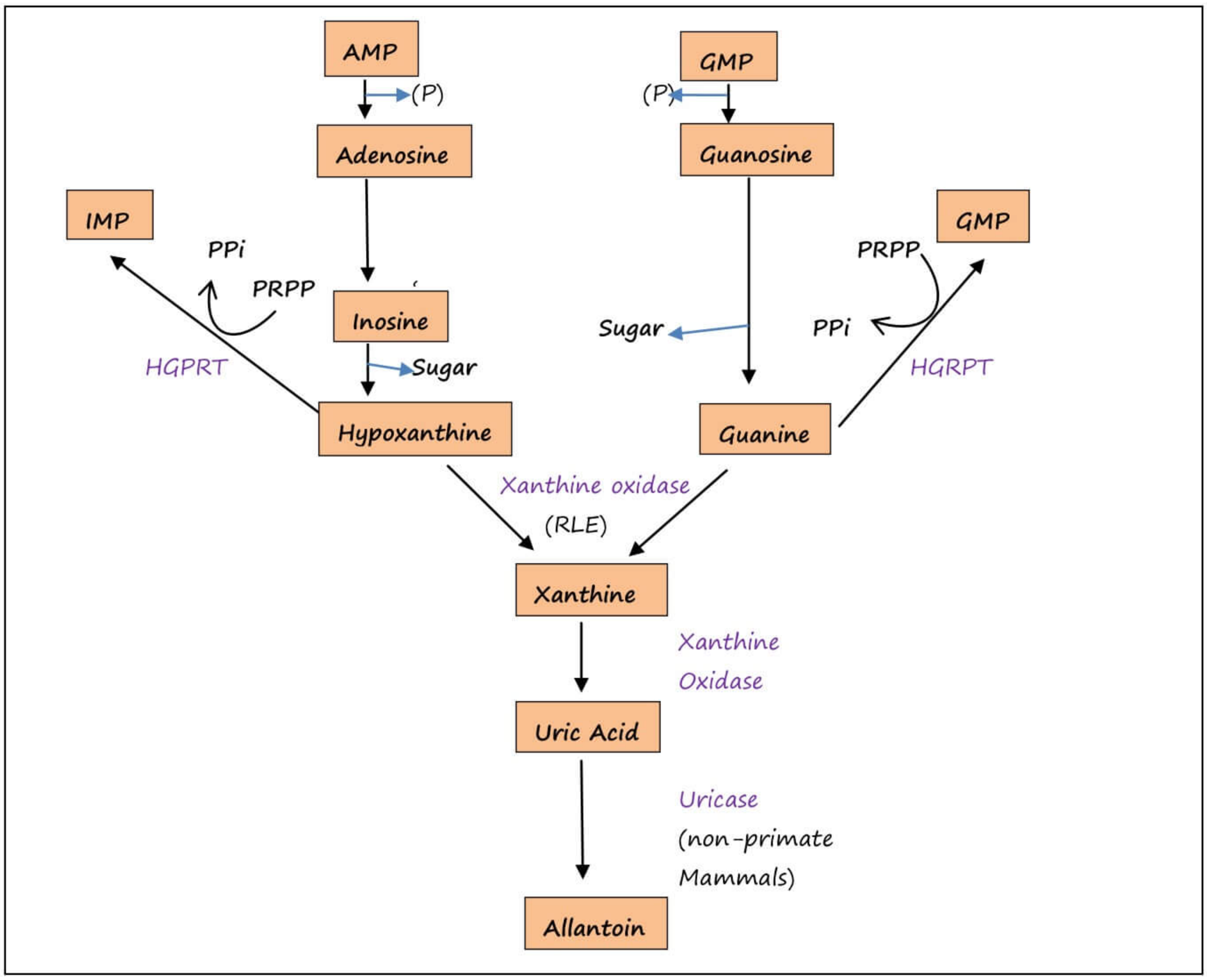
→ Occurs in RBC, WBC, brain & bone marrow

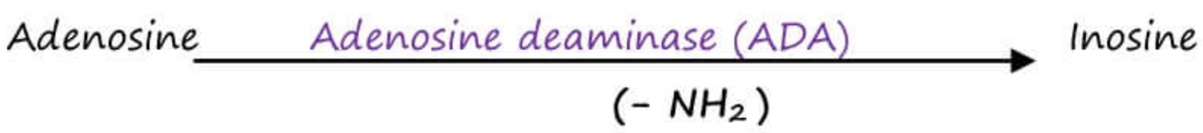
→ Salvage → Saved from degradation

CATABOLISM OF PURINES



PURINE CATABOLISM





Adenosine Deaminase (ADA)

→ Important in B & T Lymphocytes

→ Easily measured in any fluid of the body

→ ↑ ADA → Suggestive of TB

→ ↓ ADA

- Both B & T Lymphocytes affected
- Leads to severe combined Immunodeficiency

Xanthine Oxidase

→ RLE

→ inhibited by ALLOPURINOL (Suicidal inhibitor of Xanthine Oxidase)

End product in Primates → URIC ACID

End product in non-primates → ALLANTOIN

HGPRT

LESCH NYHAN SYNDROME

→ Complete deficiency of HGPRT

→ Gout dlt ↑ ↑ Nitrogenous bases → ↑ ↑ Uric acid

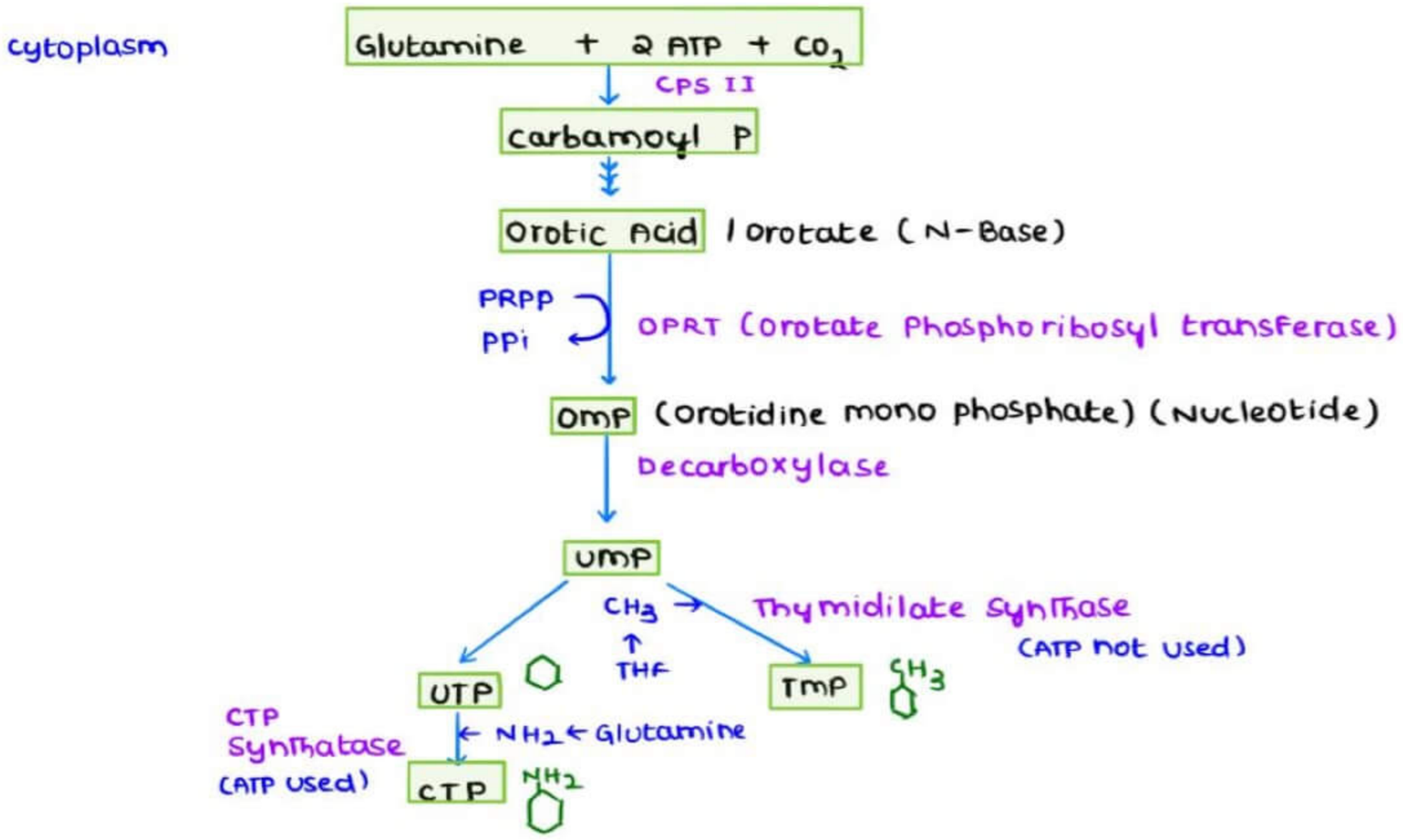
→ Self-mutilation dlt ↑ PRPP (neurotoxic)

KELLY SEEGMILLER SYNDROME

→ Partial deficiency of HGPRT

→ Only gout present

PYRIMIDINE SYNTHESIS



PRPP

→ Used in
- Purine synthesis
- Pyrimidine synthesis
- Histidine synthesis
- In conversion Tryptophan → Niacin

OMP

→ 1st Pyrimidine Nucleotide to form

Conversion of U to C [UTP to CTP] occurs at the level of Triphosphate

Conversion of U to T [UTP to TMP] occurs at the level of Monophosphate

OPRT, Decarboxylase } Bi functional enzymes (single protein with 2 enzymatic activities)

→ Deficiency leads to OROTIC ACIDURIA

- TYPE I → both enzymes are deficient
- TYPE II → only one enzyme is deficient (mostly Decarboxylase)

- C/F
  - → Megaloblastic anaemia, non-responsive to B_{12} or folic Acid R_1
  - → Growth Retardation

- R_1 → only URDINE (others Synthesized from this)

SUGAR

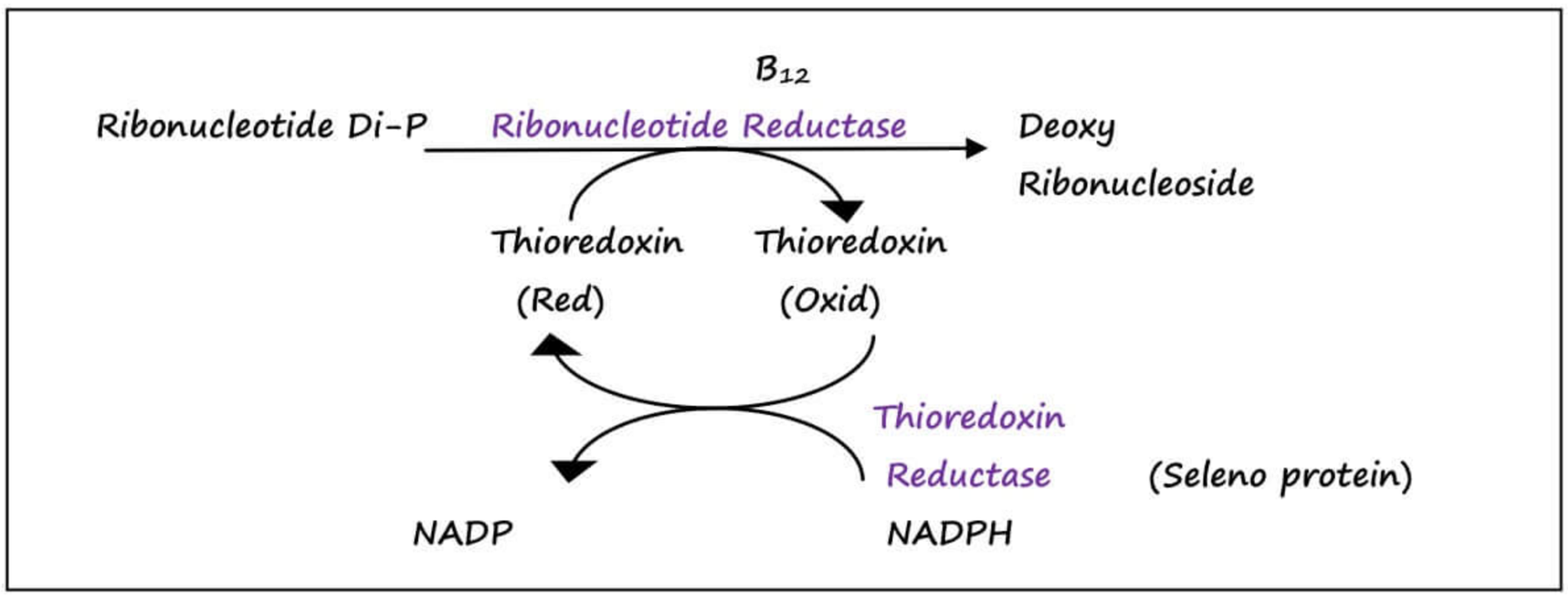
→ Present in the form of PENTOSE SUGAR { Deoxyribose [DNA] / Ribose [RNA]

RIBOSE

→ 5C, 4 OH → Furanose form (Aldehyde)

→ O_2 is shared b/w functional & second last carbon

DEOXYRIBOSE => remove O_2 from position

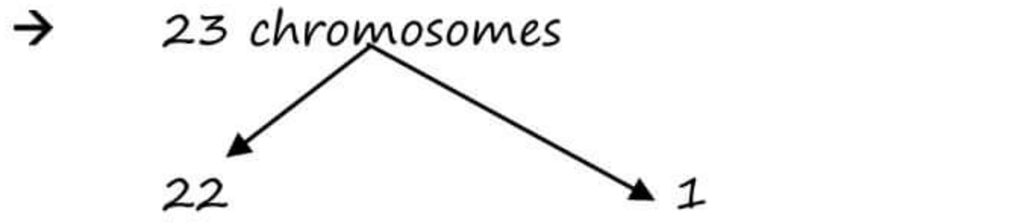


Ultimate donor of hydrogens for the conversion of Ribonucleotide Di-P to Deoxyribonucleoside is NADPH

BASICS OF GENETICS

Chromosomes

Haploid state (n) → 23 chromosomes



Diploid state (2n) → 23 pairs

Two arms → Short – p

Long – q

Homologous Chromosomes

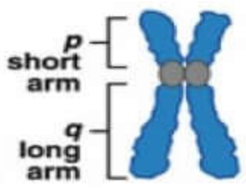
AA aa — Homozygous

Aa — Heterozyogous

→ Dominant allele (A) → Homo/Hetero

→ Recessive allele (a) → Homo

Types of chromosome–Depends upon position of centromere

Position	Chromosome		
1. Centre	Metacentric		Primitive Chr 1,3
2 Near the centre	Submetacentric		most of autosome & X chromosome
3 Close to end	Acrocentric		Y autosome 13,14,15,21,22
4 At the end	Telocentric		Not present in human

Barr Bodies → Inactive condensed X chromosome

Number of Barr Bodies = (No of X chromosomes -1)

In a case of (N) female → XX → No, of Barr Bodies → (1)
Male → XY → No of Barr Bodies → 0

Klinefelter's Syndrome → XXY →No of Barr Bodies → (1)

One gene one protein theory

20,000 gene → 2.5 lakh

→ **Exception to one gene Protein theory**

1. Alternate splicing
2. RNA Editing

NUCLEIC ACID

DNA	RNA
→ Double stranded (ds)	→ Single stranded (ss)
→ Has A T C G	→ Has A U C G
→ Has Deoxyribose	→ Has ribose

Q Main difference b/w DNA & RNA → SUGAR

DNA

→ ds in both Prokaryotes (circular,) Eukaryotes [linear]

→ Right helical

→ 2 strands are Anti parallel

CHARGAFF's RULE

A = T

G = C

A + G = T + C

No. of Purines = No. of Pyrimidines

TYPES

B DNA	A DNA	Z DNA
mc type	Present in RNA DNA duplex	Has zig zag backbone
10 bp / turn	11 bp | turn	12 bp / turn
Right-handed	Right-handed	Left-handed
Low salt concentration Hydrated environment Most stable from	High salt concentration Dehydrated environment	In the area where Purines alternate with pyrimidines & Regulation of gene expression

NUCLEOSOMES

→ DNA + HISTONE PROTEINS

HISTONE PROTEINS

→ Basic AA

→ Has positive charge

DNA

→ Has Po_4^{2-} → Negative charge

→ Not free → Inactive

Euchromatin

→ Loose DNA

→ Genes are active

Heterochromatin

→ Tightly packed DNA

→ Genes are inactive

PTM's of HISTONE

→ PTM → Post translational modifications

→ Helps in regulation of gene expression

VARIOUS WAYS OF HISTONE MODIFICATIONS ARE

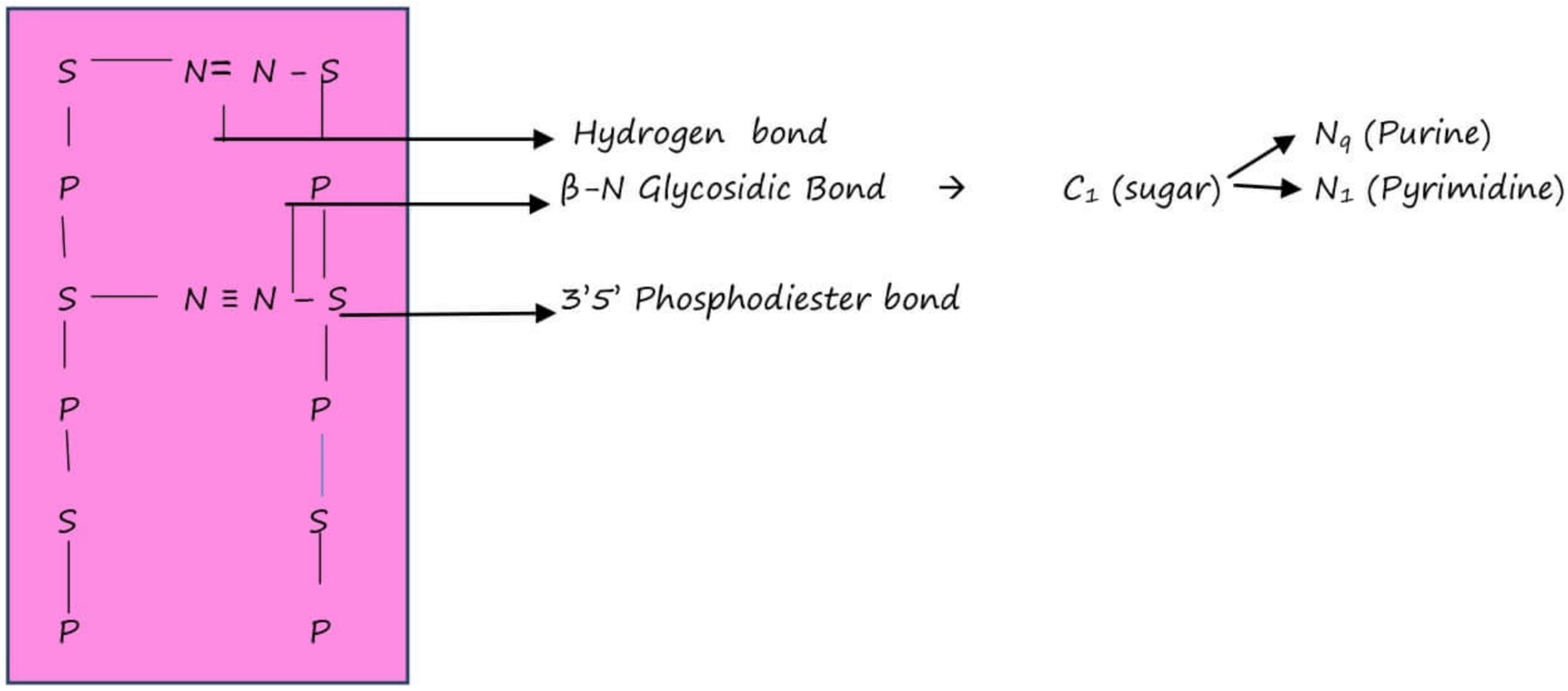
1. Acetylation
2. Phosphorylation
3. Methylation
4. ADP Ribosylation
5. Mono ubiquitylation
6. Sumoylation

Acetylation → + $CH_3 COO^-$ } addition of H^- & DNA^-
Phosphorylation → + PO_4^{2-} } - repel each other → free
- Gene activation occurs

Acetylation & Phosphorylation of Histones → Gene activation
→ ↑ Euchromatin formation

Deacetylation & Dephosphorylation of Histones → Genes inactivation

BONDS IN DNA



β-N Glycosidic bond
3'5' Phosphodiester bond } Covalent bond

Q	Which is correct
a	5' AG 3'
b	3' GA 5'
c	both
Ans c	

Q	Which is correct	
a	AG	→ if the direction
b	GA	not given, then
c	Both	Left → A
Ans a		Right → G

NUCLEASES

→ Breaks the covalent bonds present in nucleic acid

→ EC no. 3 (Hydrolases)

→ TYPES

1. EXONUCLEASES
   - Cutting from sides
   - 5' → 3' Exonucleases
   - 3' → 5' Exonucleases

2. ENDONUCLEASES
   - Cut anywhere in between
   - aka Exonuclease
   - Restriction endonuclease
     - cut at a specific site → PALINDROMES

Q which of the following is a palindrome

a GGCC

b GACC

c TAAT

→ GGCC

5' GGCC 3' → Read in 5' to 3' direction

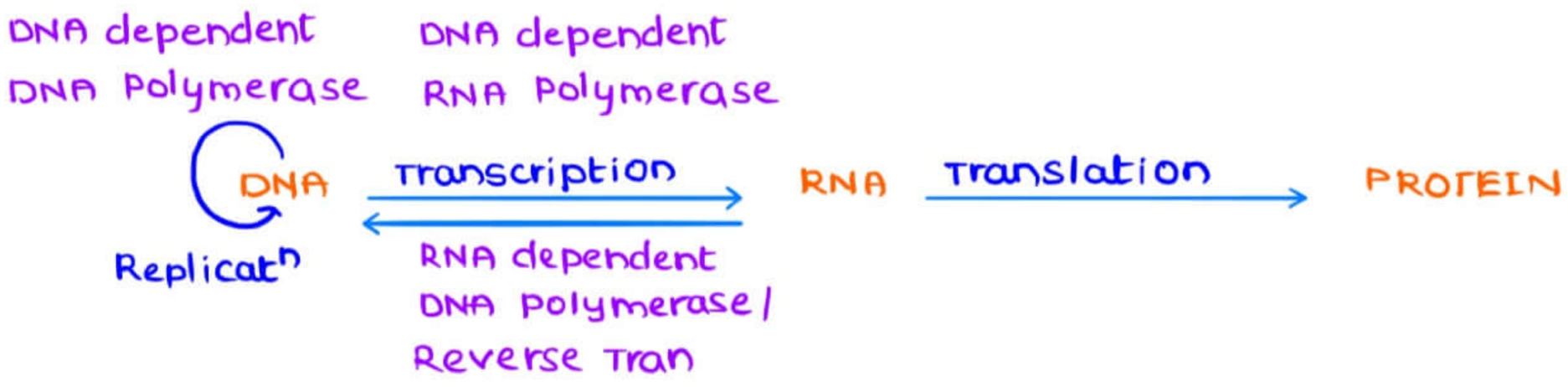
3' CCGG 5' → Palindrome

→ TAAT

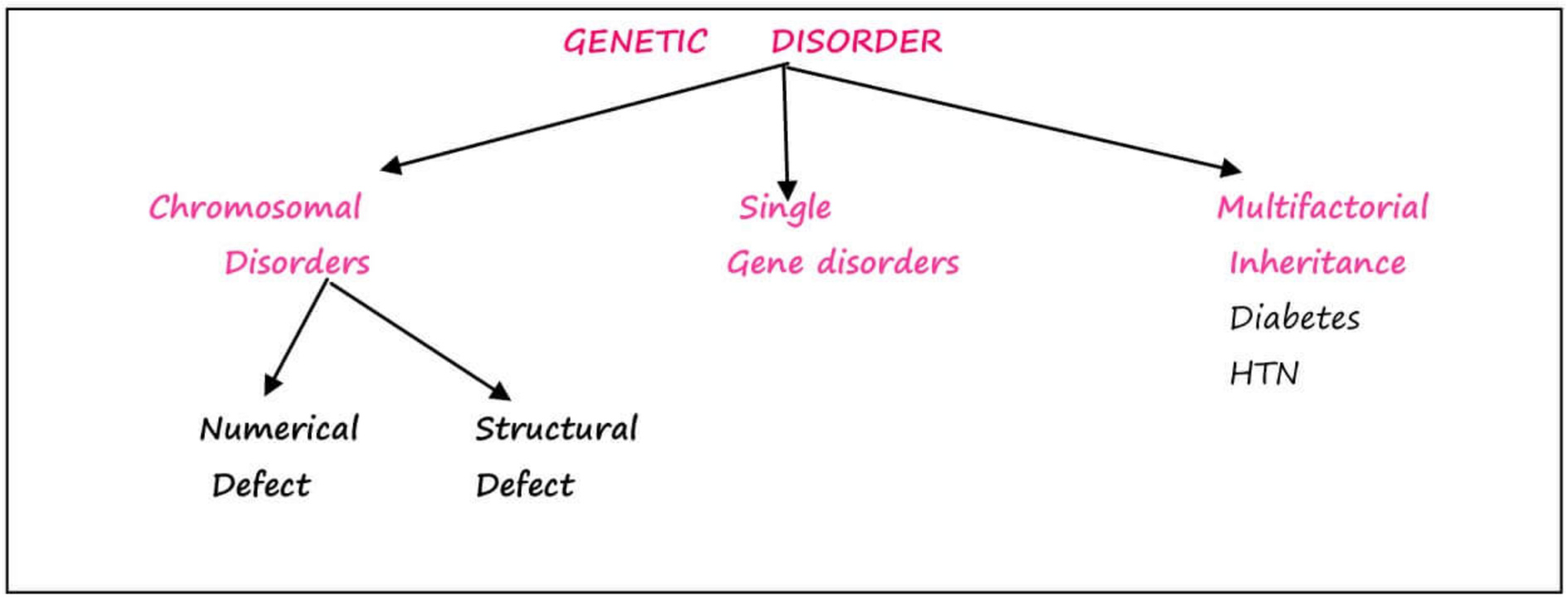
5' TAAT 3' → not a palindrome

3' ATTA 5'

CENTRAL DOGMA of MOLECULAR BIOLOGY



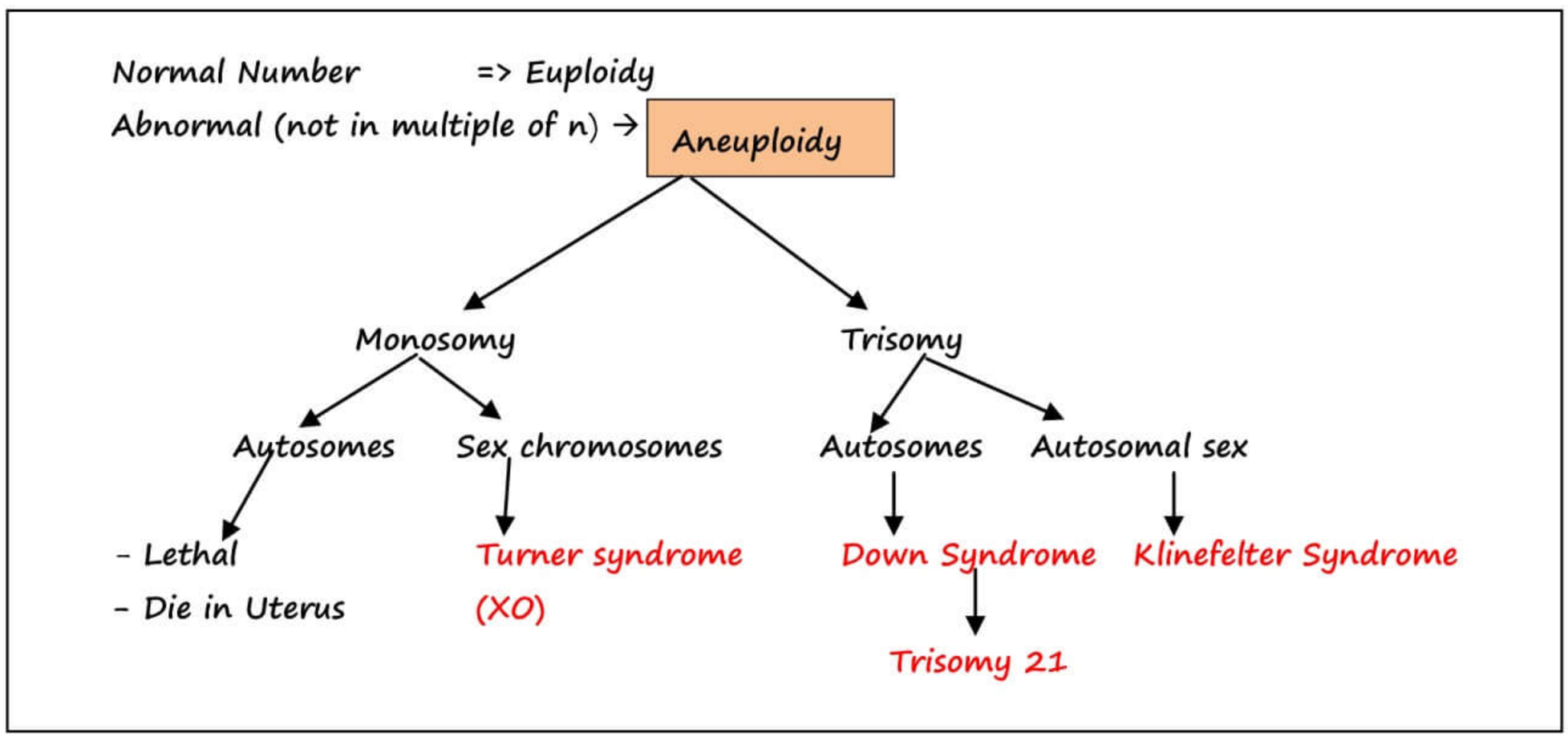
GENETIC DISORDER



(1) Chromosomal defect

Numerical Defect ⇨ 23 pairs of chromosomes

- Haploid = (N)
- Diploid = 2N



Trisomy

Autosome

Down Syndrome

-Trisomy 21

-M/C chromosomal Disorder of human

Sex

* **Klinefelter Syndrome** (XXY)

* Super female (XXX)

* Super male (XYY)

Structural Defect

Large / Macro Scale Alteration

Chromosome

(1) Insertion
(2) Deletion
(3) Inversion → same chromosome
(4) Translocation → different chromosome

Small / Micro Scale Alteration

Gene

(1) Insertion
(2) Deletion
→ Not in multiple of three

(1) Frame shift mutation

Single Gene disorder

Classical /Mendelian inheritance

- Autosomal dominant
- Autosomal recessive
- X inked dominant and recessive
- Y linked

Non Mendelian inheritance

- Mitochondrial Disorders
- Genomic imprinting
- Trinucleotide repeat expansion disorders

Practical Tips

If one person has the disease

AA	Aa	aa
Homozygous	**heterozygous**	**homozygous**

	Dominant allele		Recessive allele
	↓	↓	↓
	AA	Aa	aa
Express	↓	↓	↓
	A	A	a

→ If neither parent are affected - Recessive

→ If there is no male to male transmission - X linked

→ If both male & female are affected with equal frequency - AD

→ More males are affected
- Affected son are born to Unaffected mother → XR

→ If father _____ all sons → Y linked

→ If mother _____ all off springs → Mitochondrial Inheritance

AD

- Familial hyper Cholesterolemia
- Huntington's disease

AR → Most biochemical defects

- Amino Acid disorders
- All sphingolipidosis - except – Fabry's disease

→ All urea cycle disorder's except → OTC

→ All MPs disorder's except → Hunter

→ All glycogen storage disorders

→ Wilson's disease, Hemochromatosis

XR

- Fabry's disease
- Hunter's disease
- G6PD deficiency
- Lesch Nyhan Syndrome
- OTC deficiency

XD → Vit D Resistant Rickets

MITOCHONDRIAL DNA

→ 1% of total cellular DNA
→ Only present in Eukaryotes
→ Resembles Prokaryotes
  - Circular dsDNA
  - No Introns'
    - Introns prevents mutations
    - No Introns → More chances of mutations

→ Contains around 16,000 bp, 37 genes
→ No histone → 19 % of protein of ETC are derived from mitochondria DNA.
  - High rate of mutation due to no intron, no repair enzyme.
→ RESPIRATORY CHAIN
  - Requires 67 proteins
  - 13/67 → **19% of proteins of ETC are derived from mitochondrial DNA**

High rate of mutation –
- No intron
- Hydrocarbons → O_2 radicals
- No repair enzymes

DISEASE RELATED TO MUTATIONS IN MITOCHONDRIAL DNA

Mostly ETC affected → ↓ ATP → Lactic Acidosis (Brain / CNS affected)

1. **MELAS** – Mitochondrial encephalopathy lactic acidosis and stroke like episode
2. **LEBER HERIDITARY OPTIC NEUROPATHY**
3. **LEIGH SYNDROME**
4. **KEARNS SAYRE SYNDROME**
5. **NARP SYNDROME**- Neuropathy ataxia retinitis pigmentosa
6. **MERRF**- Myoclonic Epilepsy, Ragged red fibres in muscles
7. **CPEO**- Chronic Progressive External Ophthalmoplegia
8. **PEARSON SYNDROME**- Lactic Acidosis, Pancytopenia, Pancreatic Insufficiency

REPLICATION

ENZYMES

1. **HELICASE** → Causes strand separation
   → Use ATP
   → Create supercoils

2. **TOPOISOMERASE** → Relieve supercoils
   → Do not use ATP
   → TYPES → I & II

Helicase & topoisomerase work in tandem

3. **SINGLE STRAND DNA BINDING PROTEINS (SSBs)**

→ Prevents reannealing

→ In Eukaryotes, Replication Protein A will do this function

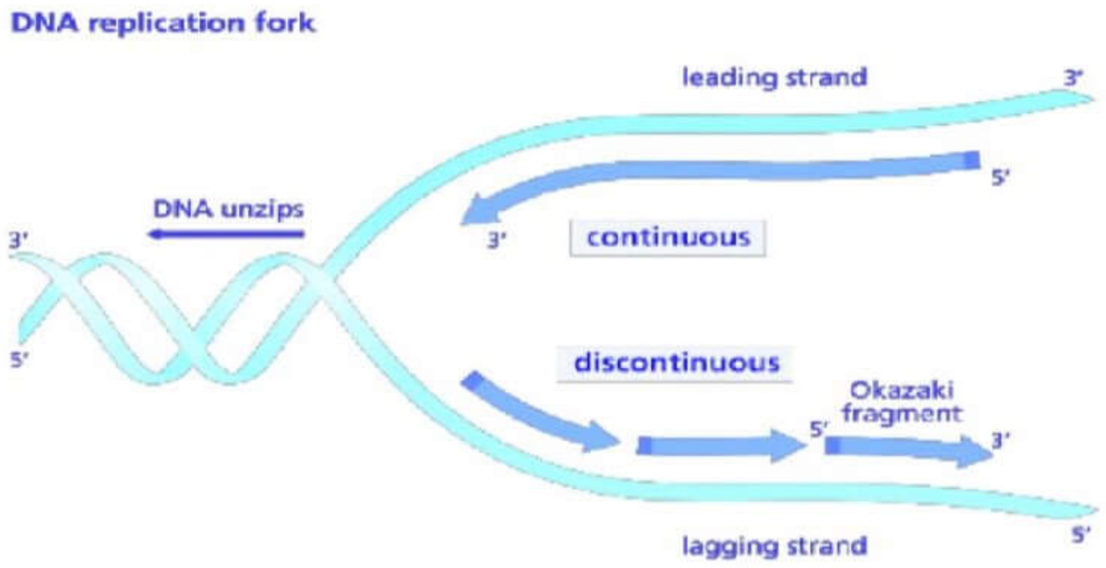
Helicase, Topoisomerase & SSBs → UNWINDING PROTEINS

4. **PRIMASES**

→ Synthesizes the primers (RNA primer)
   - Template taken is DNA

→ DNA dependent RNA Polymerase

→ In Eukaryotes → α – polymerase will act as primase
   In Prokaryotes → DNA G protein will act as primase



→ 1 primer is required for leading strand

Multiple primers required for lagging strand

5. DNA POLMERASE III

→ Synthesize both leading & lagging strands

→ DNA Dependent DNA Polymerase

6. DNA POLYMERASE I

→ Removes RNA primers from both leading & lagging strands → gap created

→ Gap in lagging strand is filled

7. DNA LIGASE

→ creates 3' 5' Phosphodiester bond

→ uses ATP

→ acts only on lagging strand

→ The above 7 enzymes are present in most of cells of our body (of which most are somatic cells)

SOMATIC CELLS

→ Have limited no. of divisions

- dlt gap present in leading strand
- The gap left is called TELOMERE SHORTENING
- With further divisions, telomere shortening increases & cell division stops after some divisions

→ TELOMERE

- Ends of chromosome → Telomere
- Has $(TTA\ GGG)_n$ Sequence repeated 'n' no. of times
  - Telomere Shortening occurs at this area

- responsible for aging & death

GERM CELLS | STEM CELLS

→ Have infinite no. of divisions

→ Gap left in leading strand is filled by TELOMERASE

→ TELOMERASE

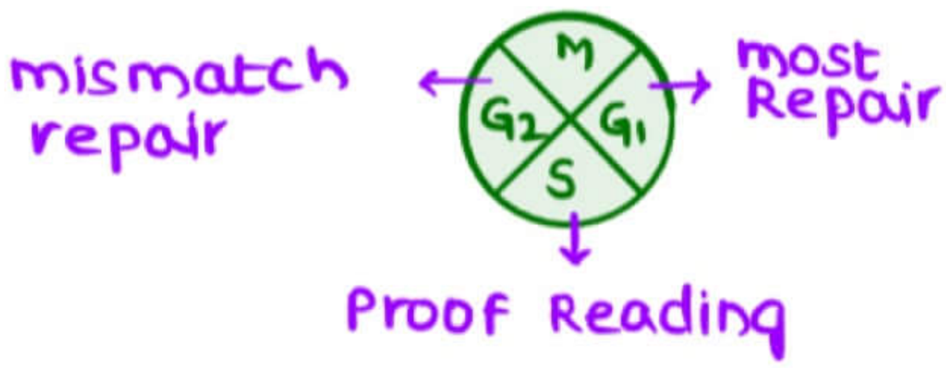
- Protein with RNA attached to it
- RNA acts as template on which DNA is synthesized
- RNA dependent DNA polymerase
- Not a Ribozyme as RNA do not act as enzyme
- Activity increases in cancer
- Activity decreases with aging
- Germ cells have more telomerase compared to stem cells

PROOF READING	REPAIR
→ correction during synthesis	→ correction after Synthesis
→ 3' → 5' Exonuclease activity	→ endonuclease activity mostly 5' → 3' Exonuclease activity sometimes
→ In Prokaryotes, DNA Polymerase I, II, III	→ IN Prokaryotes DNA Polymerase II, I
→ In Eukaryotes, All Polymerase except α & β polymerase	→ In Eukaryotes, β polymerase (mainly) ε polymerases (sometimes)

→ Proof reading occurs in S phase of cell cycle

→ most of Repair occurs in Late G_1 phase

→ mismatch Repair occurs in G_2 Phase



KLENOW FRAGMENT

DNA POLYMERASE

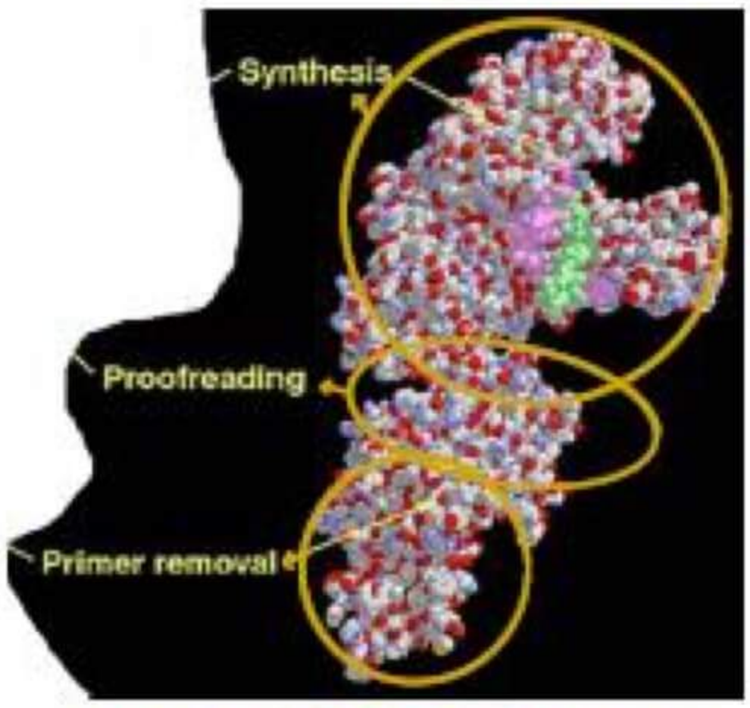
→ present in prokaryotes [I, II, III] & Eukaryotes [α, β, γ, δ]

→ All the DNA polymerases (I, II, III) have 2 activities

1. Synthesis → 5′ → 3′ polymerase activity
2. Proof Reading → 3′ → 5′ Exonuclease activity

(exonuclease means cutting from one side of DNA

3′ → 5′ means from 3' end towards 5' end of DNA)



DNA POLYMERASE I

DNA polymerase I ACTIVITY

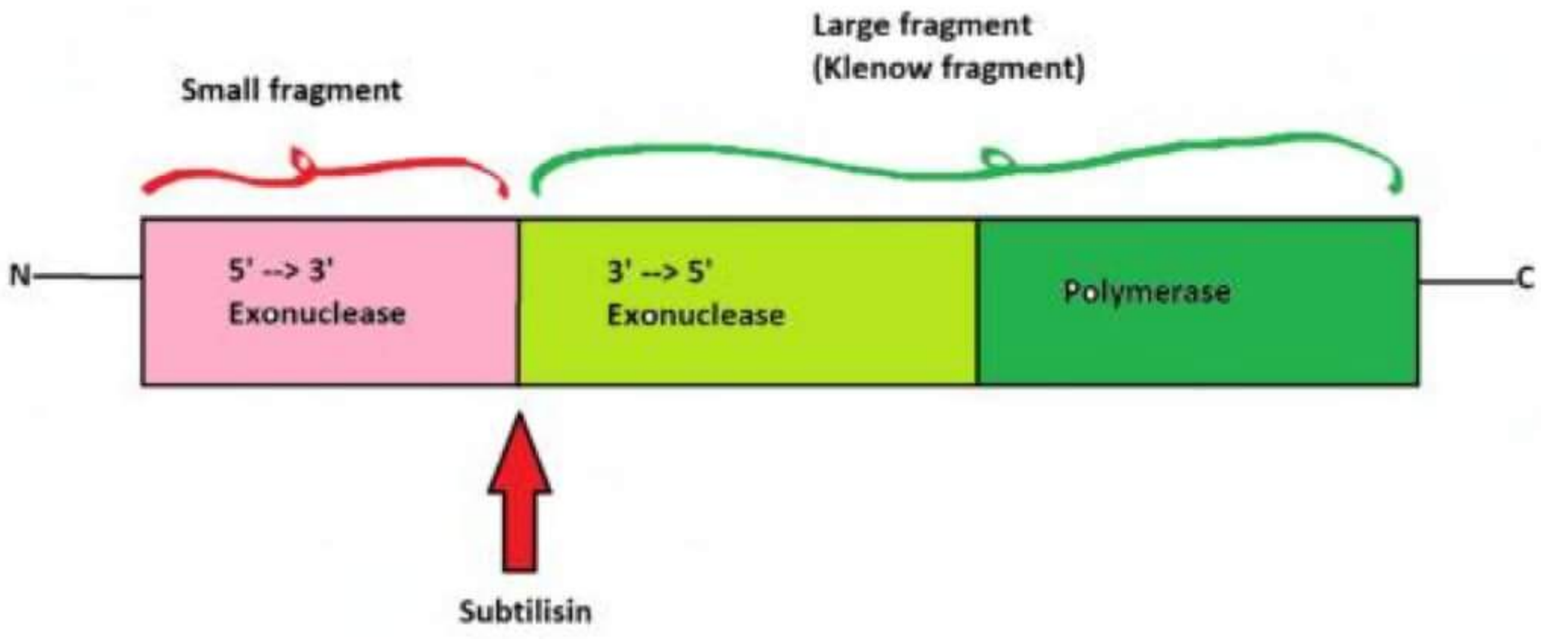
1. 5′ → 3′ polymerase activity for synthesis

2. 3′ → 5′ exonuclease activity for proof reading

3. 5′ → 3′ exonuclease activity for RNA primer removal [Extra activity]

RNA primers are short RNA fragments formed during replication. They are removed by DNA polymerase I by 5′ → 3′ exonuclease activity and gaps get replaced with DNA.

1° structure



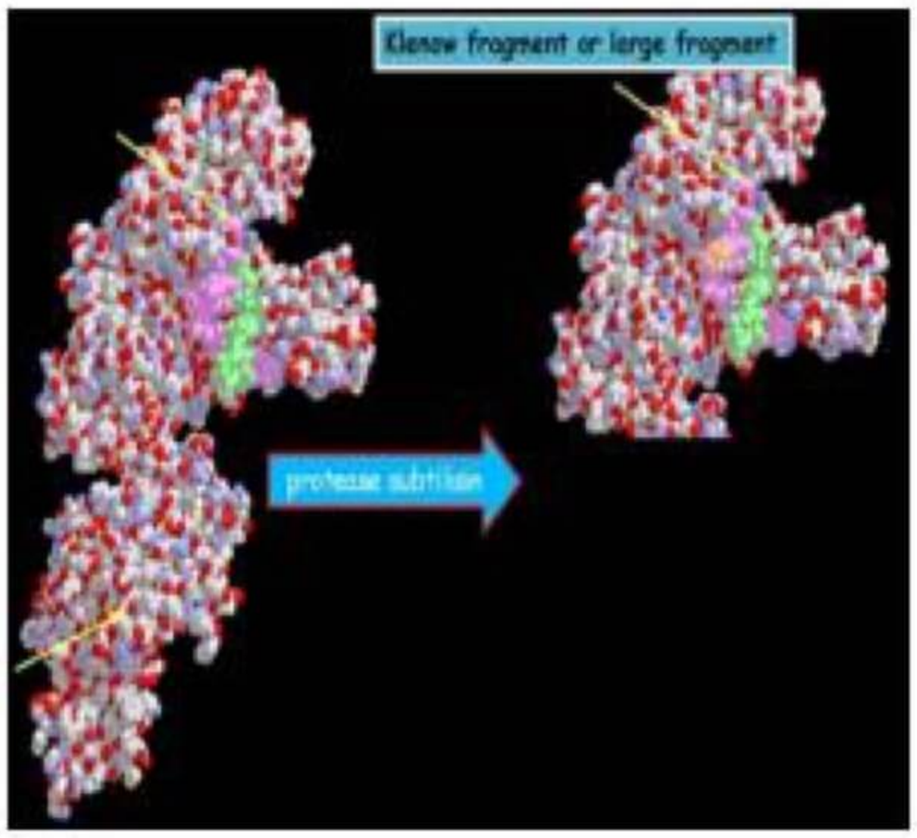
SUBTILISIN

→ Derived from Bacillus subtilis

→Endopeptidase enzyme

→Releases

- Smaller fragment with 5' → 3' exonuclease activity
- Larger fragment



KLENOW FRAGMENT

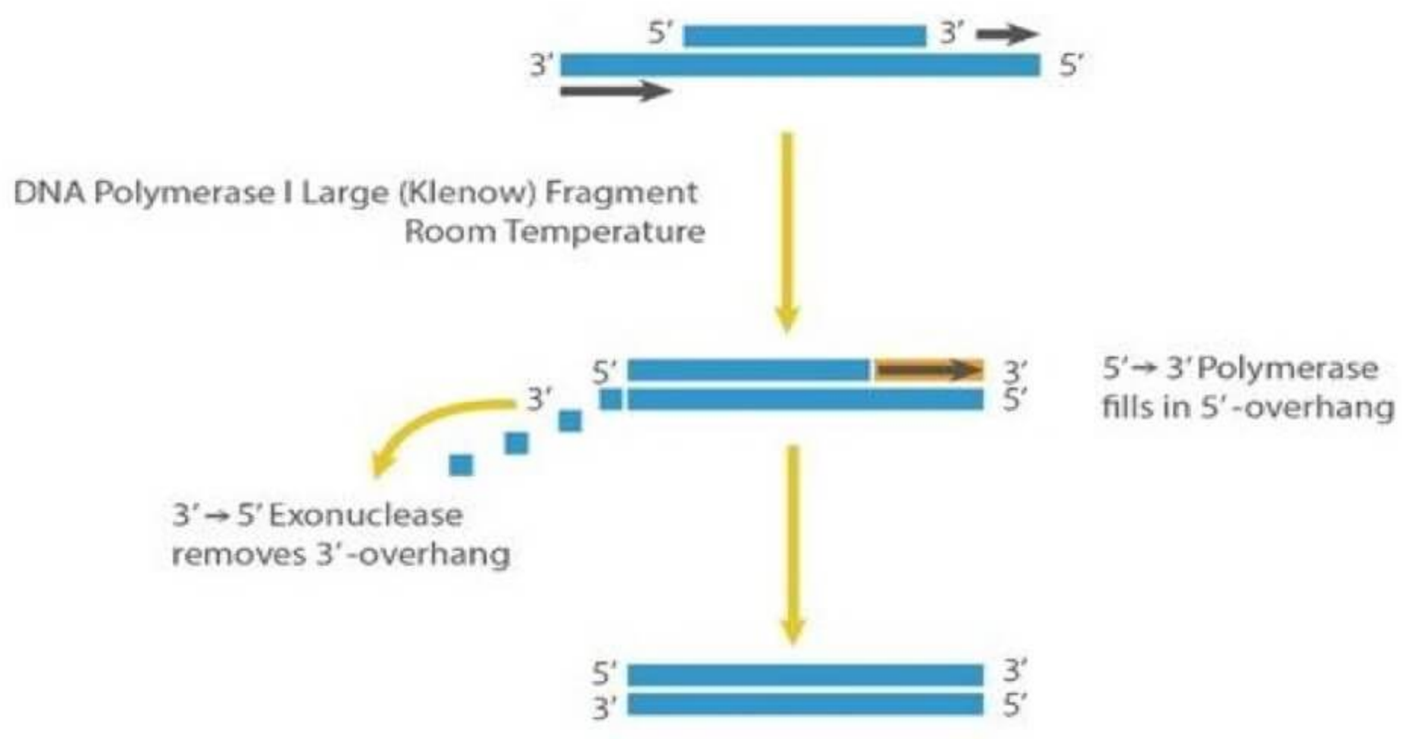
→Larger fragment towards the 'C' terminal which is lacking 5' → 3' exonuclease activity

USES

→ Used to remove 3' overhang by 3' → 5' exonuclease

→ Can fill 5' overhang by 5' → 3' polymerase activity

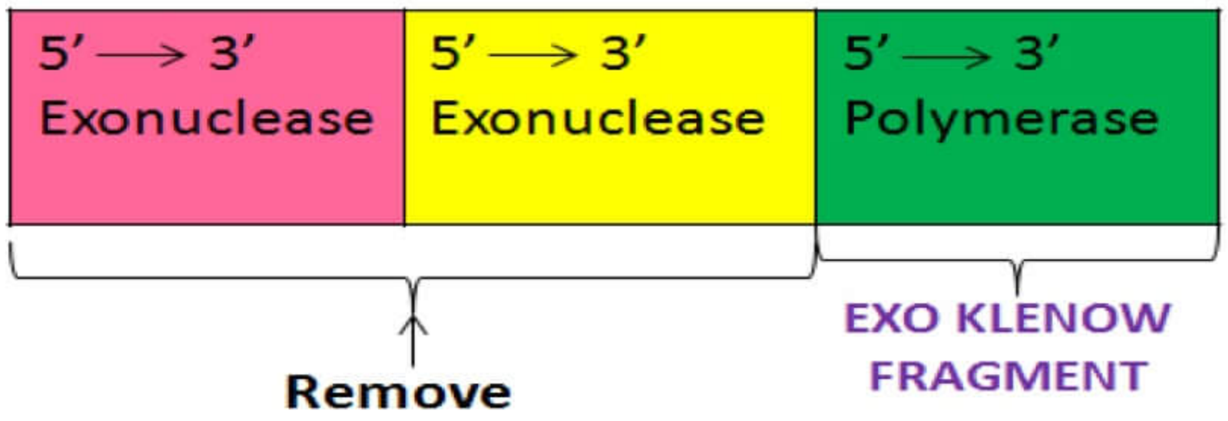
→Sticky or overhanging ends of DNA can be converted to blunt end



→ used earlier in PCR before the discovery of thermostable taq polymerase

→ used to convert ssDNA to dsDNA

→ used to produce Radioactive DNA probes



EXO - KLENOW FRAGMENT USES

1. Microarray for creating fluorescent probes
2. deoxy adenine (dA) & deoxy thymine (dT) tailing
3. To prepare gene libraries for next generation sequencing techniques

DNA REPAIR

Type of repair

1 Single strand break

- Nucleotide excision repair
- Corrects damage caused by UV radiation which leads to TT dimer formation
    - Defect in this repair leads to Xeroderma pigmentosa
- Base excision -
    - In DNA Cytosine get deaminated to form uracil spontaneously or by heat, infra red rays, viral infection, nitrous oxide
    - This repair corrects this change
    - defect in this repair leads to MUTYH associated polyposis

- Mismatch repair –
    - Corrects damage caused by Mismatched base due to proofreading error
    - Defect in this repair leads to hereditary non polyposis colon cancer (HNPCC)

2 Double strand break

- Homologous repair
- Non-homologous repair

COMPARISION OF PROKAROTIC AND EUKARYOTIC DNA POLYMERASE

E. Coli	Eukaryotic	Function
I		Remove primer & fill the gap
II		DNA proof reading & repair
	B	DNA Repair
	Y	Mitochondrial DNA Synthesis
III	ε	Leading strand synthesis
	δ	Lagging strand synthesis
DNA - G	α	Primase

Primer Removal

Prokaryotic	Eukaryotic	Eukaryote
	Nucleus	Mitochondria
	RNASE H FEN – 1 δ polymerase (minor role)	RNASE H FEN – 1

FEN → Flap Endonuclease

TRANSCRIPTION

TYPES OF RNA	TRANSCRIPTION SITE
1. r RNA → 80% → most abundant	Nucleus
2. t RNA → 15% → smallest has maximum modified bases	Nucleus
3. m RNA → most heterogenous	Nucleus

r RNA [RIBOSOMAL RNA]

→ present in RIBOSOME

→ **RIBOSOMES**

- made up of

1. r RNA (2/3) → MARKER for Ribosome
2. Proteins (1/3)

- 70s
  → in prokaryotes

	70s	80s
	→ in prokaryotes	→ In Eukaryotes
Larger Subunit	50 S subunit 23S + 5S rRNA	60S subunit 28S + 5.8S + 5S rRNA
Smaller Subunit	30S subunit 16S rRNA	40S subunit 18S rRNA

RIBOSYME

→ 23s rRNA of Prokaryotes
28s rRNA of Eukaryotes } RIBOZYME

→ has peptidyl transferase activity

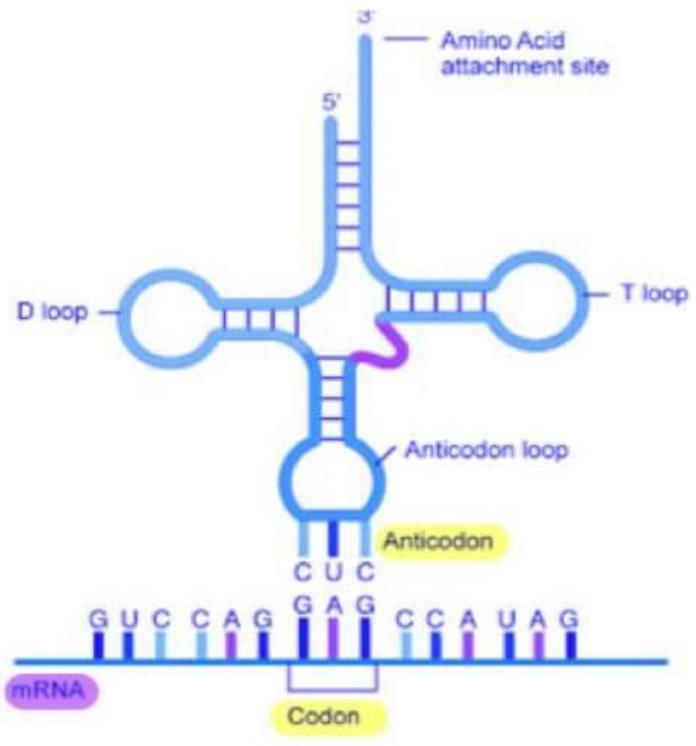
- 16S rRNA sequence of prokaryotes is complimentary to **SHINE DALGARNO SEQUENCE (SD sequence)**

t RNA [transfer RNA]

→ clover leaf like shape

→ **CODON & ANTI CODON**

- Codon present on mRNA
- Anticodon present on tRNA
- They have complimentary base pairing & they bind & help in translation
- During translation, codon can see anticodon but it can't see which amino acid is attached. Ex: codon is for cysteine, then anticodon is also for cysteine



If by chance, tRNA brings a wrong AA, it is added & it will be a mutation

CODING RNA → m RNA (RNA with codons)

NON-CODING RNA (nc RNA)

1. **LARGE nc RNA**
    1. T RNA
    2. r RNA
    3. lnc RNA (long non-coding RNA)
    4. linc RNA (Long intervening non Coding RNA)
2. **SMALL nc RNA**
    1. Pi RNA (Piwi-interacting RNA)
    2. sn RNA (Small nuclear RNA)
    3. sno RNA (Small nucleolar RNA)
    4. si RNA (Small interfering RNA)
    5. mi RNA (micro RNA)

TRANSCRIPTION

→ Synthesis of RNA from DNA

→ Enzyme involved → DNA dependent RNA Polymerase

RNA POLYMERASE

→ do not require primer

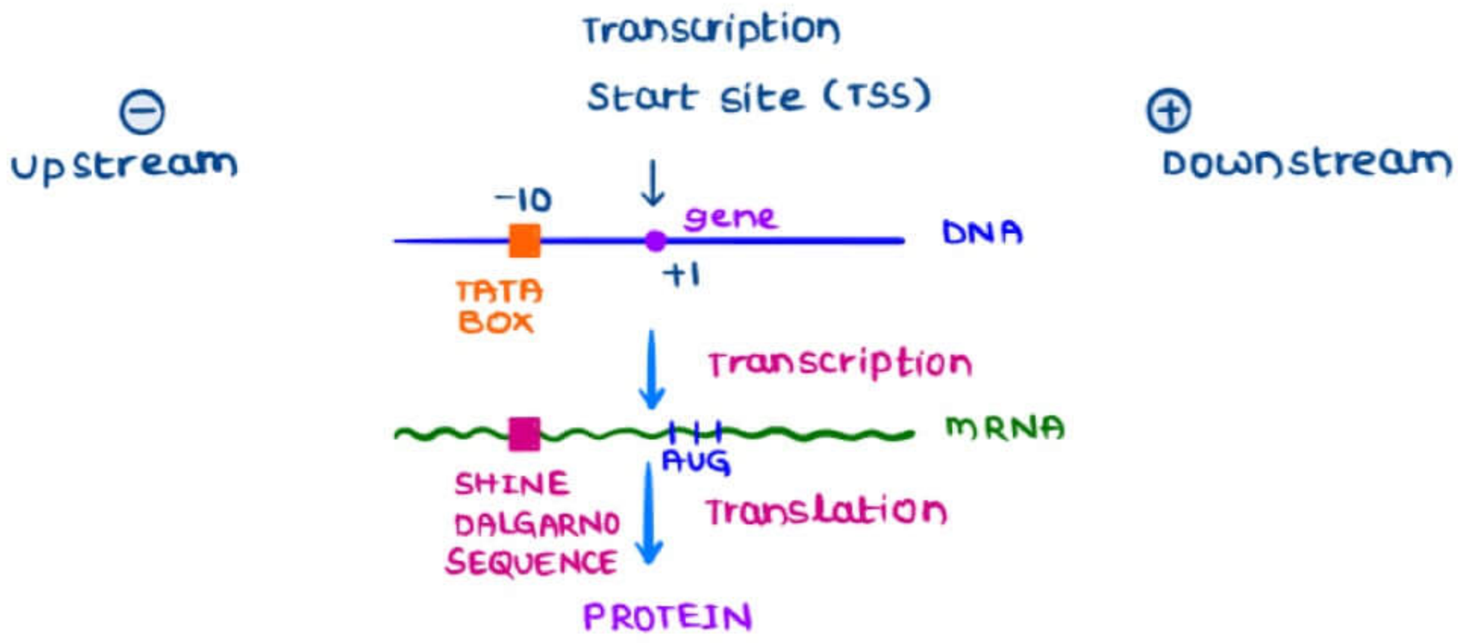
→ cannot do proof Reading

→ TYPES

- In prokaryotes, only single type present
- In Eukaryotes

Eukaryotic RNA POLYMERASE TYPE	RNA SYNTHESIZED
Type I	All rRNA except 5s RNA
Type II	mRNA, miRNA, lncRNA few snRNA & snoRNA
Type III	5s rRNA, tRNA Few snRNA & snoRNA
Mitochondrial RNA Polymerase	Mitochondrial RNA

TATA BOX
SHINE DALGARNO SEQUENCE
} present at - 10 position



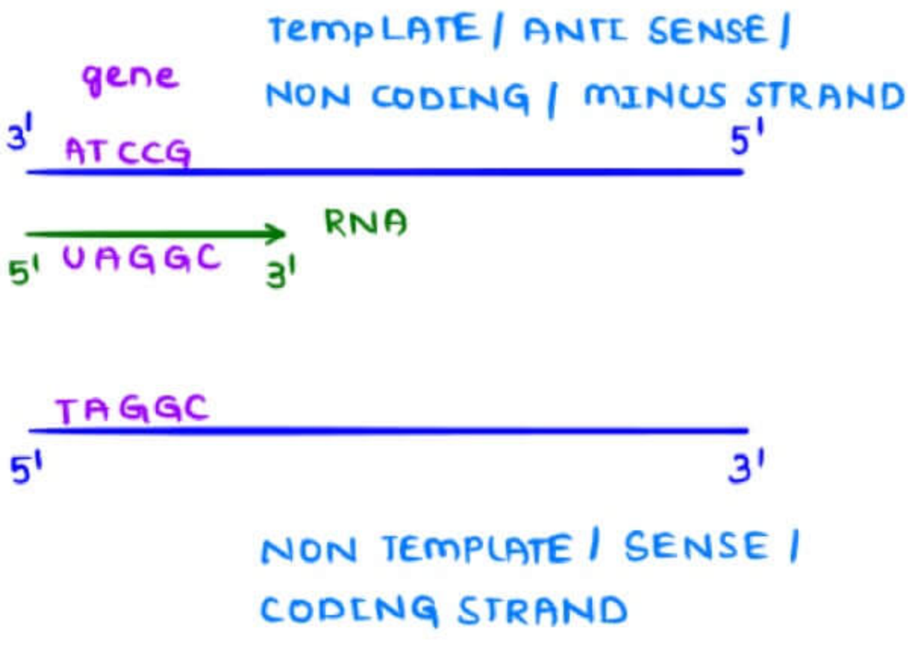
TATA BOX

→ Present at -10 position upstream to TSS on DNA
→ Helps in initiation of transcription
→ Has many T & A
→ Present in both Prokaryotes & Eukaryotes
- Name in Prokaryotes → PRIBNOW BOX
- Name in Eukaryotes → HOGNESS BOX

SHINE DALGARNO SEQUENCE

→ Present at -10 position upstream to AUG codon on mRNA
→ Only present in prokaryotes
→ Helps in initiation of translation
→ Purine rich sequence (A + G)

TEMPLATE & NON-TEMPLATE STRANDS



TEMPLATE / ANTI SENSE / NON-CODING / MINUS STRAND

→ RNA getting synthesized taking this strand as template

NON-TEMPLATE / SENSE / CODING / PLUS STRAND

→ also called Sense strand as RNA & non-template strands have same sense of direction
→ CODING STRAND → Has the same codons like new RNA

INTRONS → Intervening sequences between Exons
→ ≥ 98%
→ Only present in eukaryotes
→ Prevent mutations
→ Can be transcribed, but not translated

EXONS → Genes which give rise to proteins
→ only 1-2%

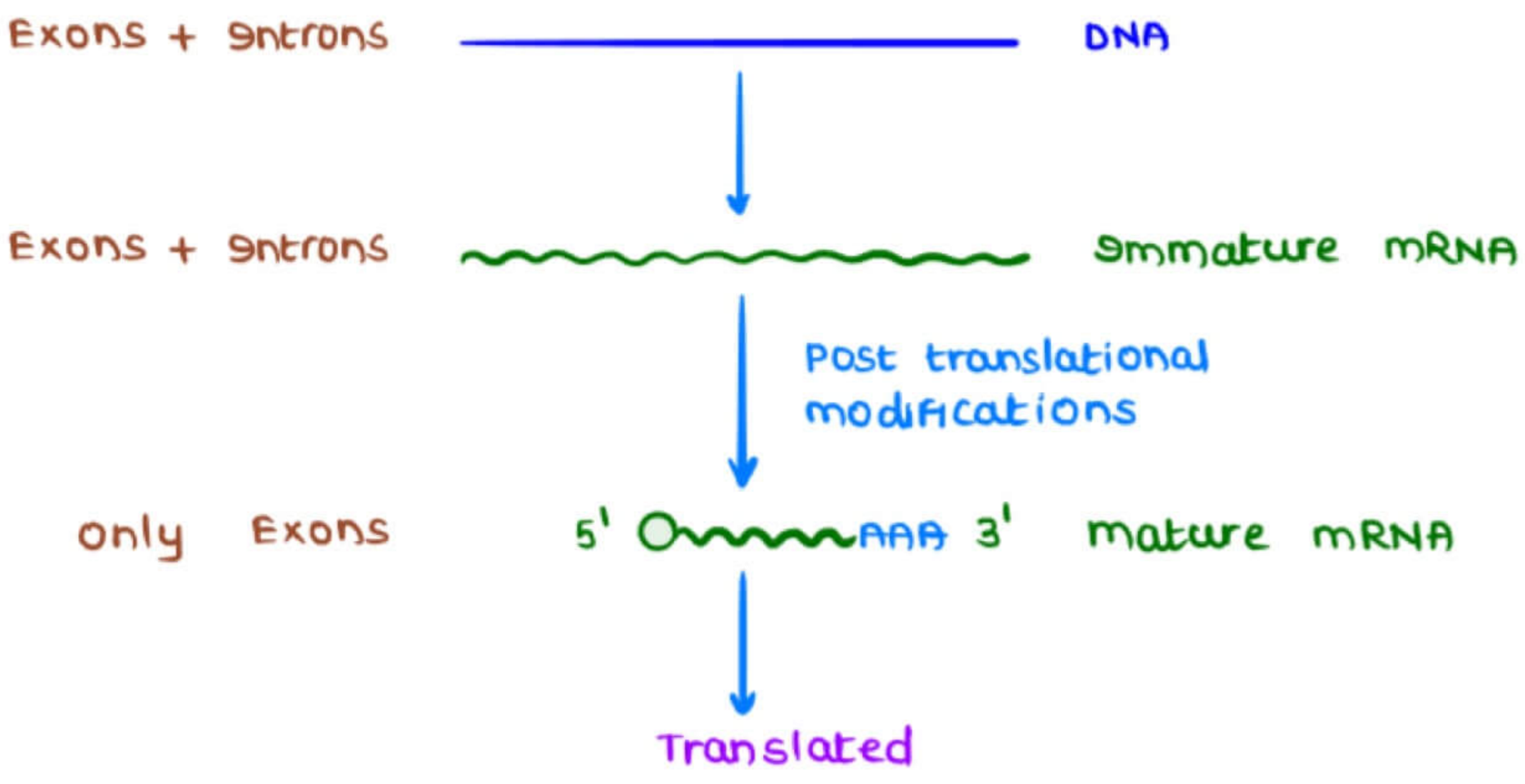
EUKARYOTIC NUCLEAR DNA
→ Have introns
→ Prevented from mutations

PROKARYOTES
→ Do not have introns
→ Mutations occurs easily

EUKARYOTIC MITOCHONDRIAL DNA
→ Do not have introns
→ High rate of mutations occur as compared to nuclear DNA

	NUCLEAR DNA	MITOCHONDRIAL DNA
REPLICATION	Nucleus	mitochondria
TRANSCRIPTION	Nucleus	mitochondria
TRANSLATION	cytoplasm	Mitochondria

INTRONS & MUTATIONS



→ Introns consists of 98%
→ Whenever mutation occurs, it most probably occurs in introns
→ Introns are excised in post translational modification
→ Incidence of mutation is diminished

POST TRANSCRIPTIONAL MODIFICATION

→ Occur in nucleus

1. **5' cap Addition**
   → 7 methyl guanosine cap
   → Methyl group is donated by SAM (in cytoplasm)
   → Prevents the attack from 5'exonuclease

2. **3' Poly A tall Addition**
   → added by Poly Adenylate Polymerase
      - Uses ATP as SUBSATRATE (AAAA---)
      - no. of AAAAAs added → 40 – 200

   → Added in all mRNA **Except** in mRNA for histone proteins

   → USES
      1. Help the RNA to exit from nucleus
      2. Prevent attack from 3' exonucleases

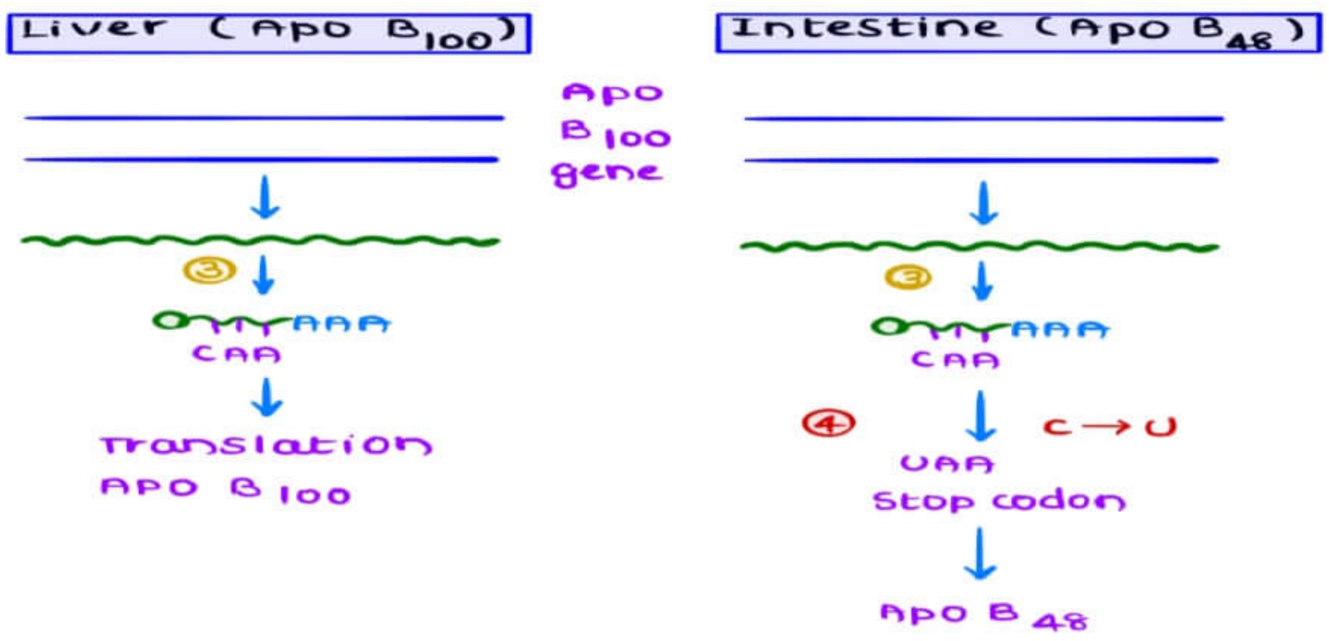
3. SPLICING

→ Done by sn RNA

These (3) modifications occur in all cells & called as RNA PROCESSING

4. DIFFERENTIAL RNA PROCESSING / RNA EDITING / CHEMICAL MODIFICATIONS OF RNA

→ Occurs in few cells



RIBOZYMES

Ribozyme means RNA acts as enzymes

Substrates of Ribosome → is mostly RNA

No ATP is used.

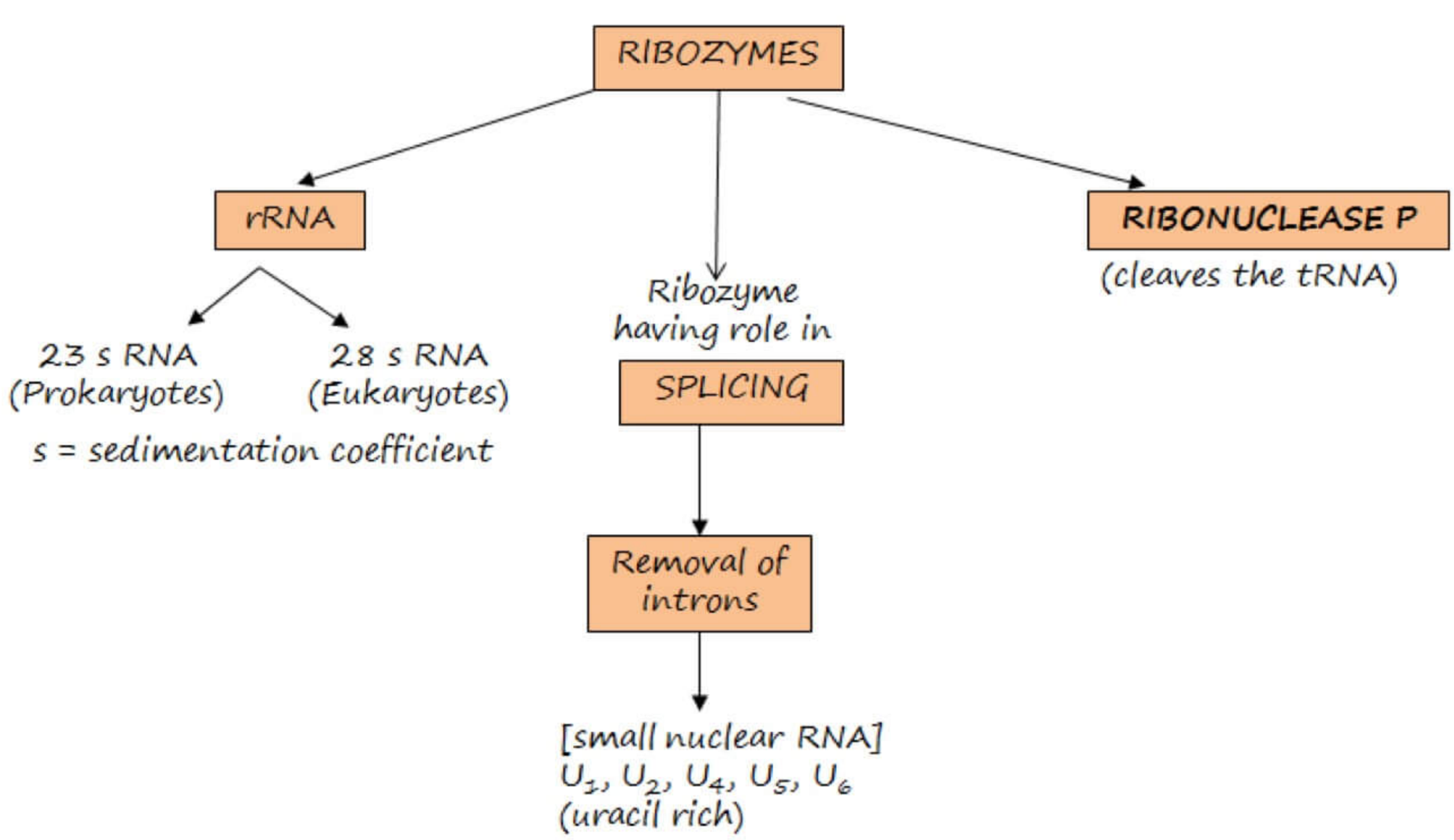
Similarity of Protein enzymes & Ribozymes

→ Specificity

→ Accerlate Rate of Reaction

→ Kinetic Behaviour

→ Can be Competitively Inhibited



Telomerase is not a Ribozyme

RNA ase H is not a Ribozyme

rRNA

→ 23S rRNA in Prokaryotes

→ 28S rRNA in Prokaryotes

→ Peptidyl Transferase

→ Elongation and Termination of Translation

Ribonuclease P

→ Cleaves the tRNA

→ An endonuclease

→ Structurally a Ribonucleoprotein

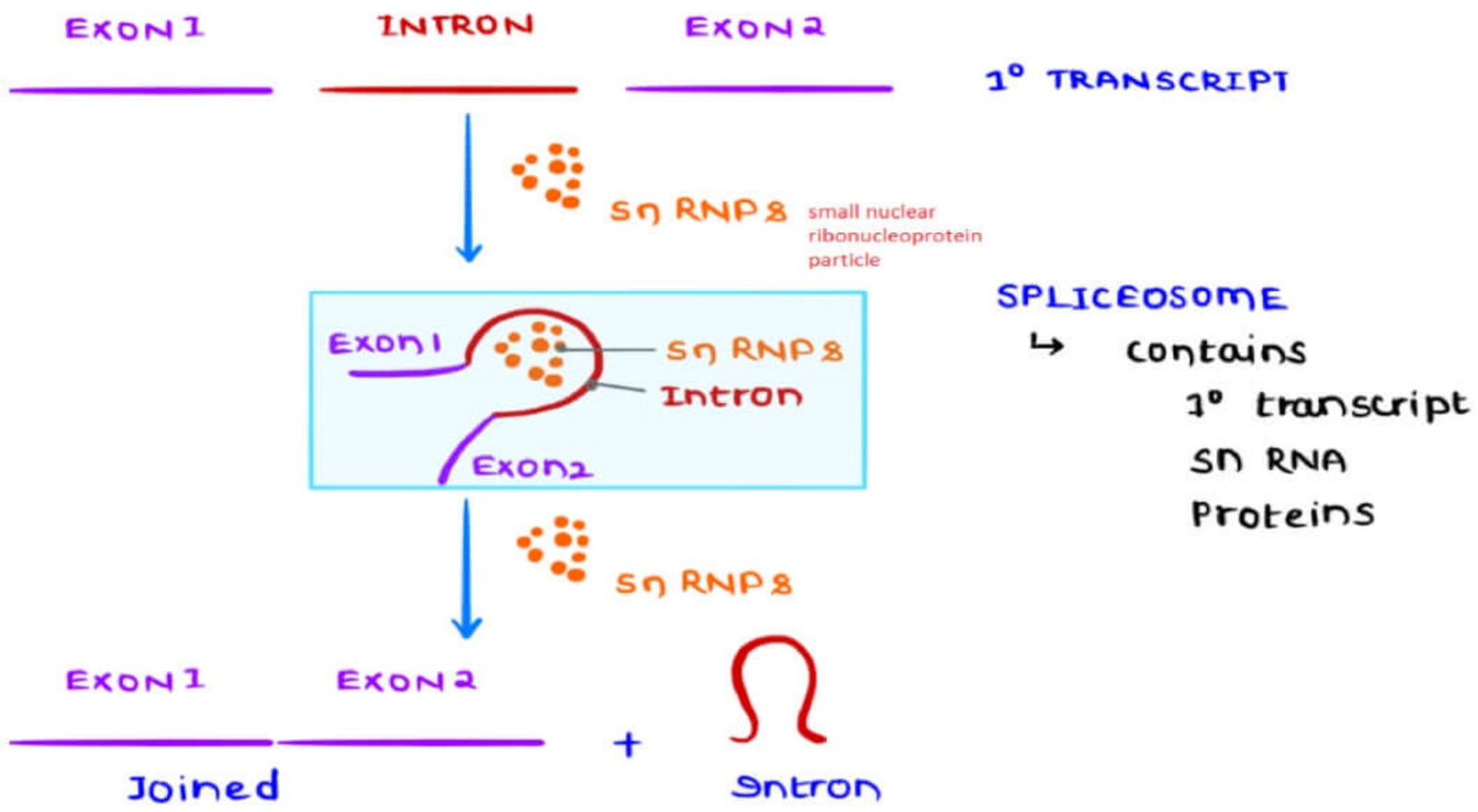
→ Create a native 5' end of tRNA

→ Ubiquitous

Splicing – snRNA (small nuclear)
Rich in Uracil

(1-6) U1 U2 U4 U5 U6

X 3



Telomerase

→ RNA + Protein

→ But RNA acts as a template for the synthesis of DNA

→ RNA dependent DNA Polymerase

→ Reverse Transcriptase

RNAase H

→ It is an endonuclease

→ Non Sequence specific

→ Cleaves RNA in RNA-DNA duplex

→ And produce ss DNA

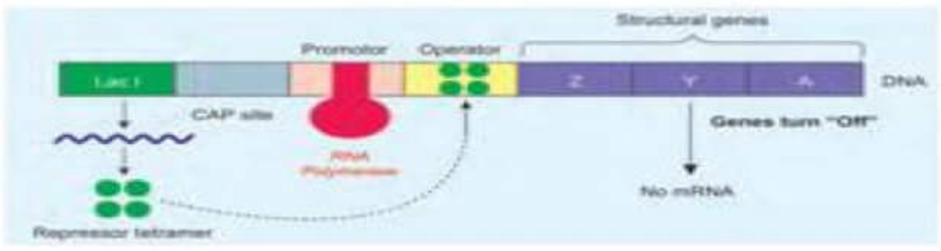
→ Used in Synthesis of cDNA by reverse transcriptase.

OPERON MODEL

LACTOSE OPERON IN E.COLI

→ In Normal State

- The Z, Y, A genes of operon model
  Kept in inhibited State



INHIBITION Of Z, Y, A genes (NORMAL STATE)

Lac I GENE

→ I – Inhibitory

→ Housekeeping / constitutive gene

- Always active
- Forms REPRESSOR TETRAMER (protein)
- Repressor tetramer binds to OPERATOR SITE
- PROMOTER is present upstream to operator
  - RNA Polymerase is present bindng to promoter
- Repressor Tetramer by binding to operator inhibits RNA Polymerase

ACTIVATION of Z, Y, A GENES

1. ↓ Glucose in environment of E. coli
2. Presence of Lactose in environment of E. coli

1. ↓ Glucose → ↑ cAMP → cAMP + CAP complex → Binds to CAP site → RNA Polymerase activation

CAP → Catabolic Activator Protein

2. Lactose → Binds to repressor tetramer → Removes it from operator → Hinderance is gone

CASE 1

→ ↓ Glucose → enzyme active
→ Lactose (+) → Repressor removed
} Transcription occurs
Genes are strongly expressed

CASE 2

→ ↓ Glucose → enzyme active
→ Lactose (-) → Repressor not removed
} Transcription does not occur
Genes are not expressed

CASE 3

→ Glucose (+) → enzyme very very slow
→ Lactose (+) → repressor removed
} Transcription occurs [basal level]
very very slow

CASE 4

→ Glucose (+) → enzyme very very slow
→ Lactose (-) → Repressor not removed
} Transcription does not occur

CODON & MUTATION

→ Nucleotide Triplets
→ ACG → 3 bases make 1 codon
→ 4 bases are used to make codons → AUCG
→ 4^3 → 64 codon combinations are possible
- If 4 bases make 1 codon, 4^4 → 256 codons are possible

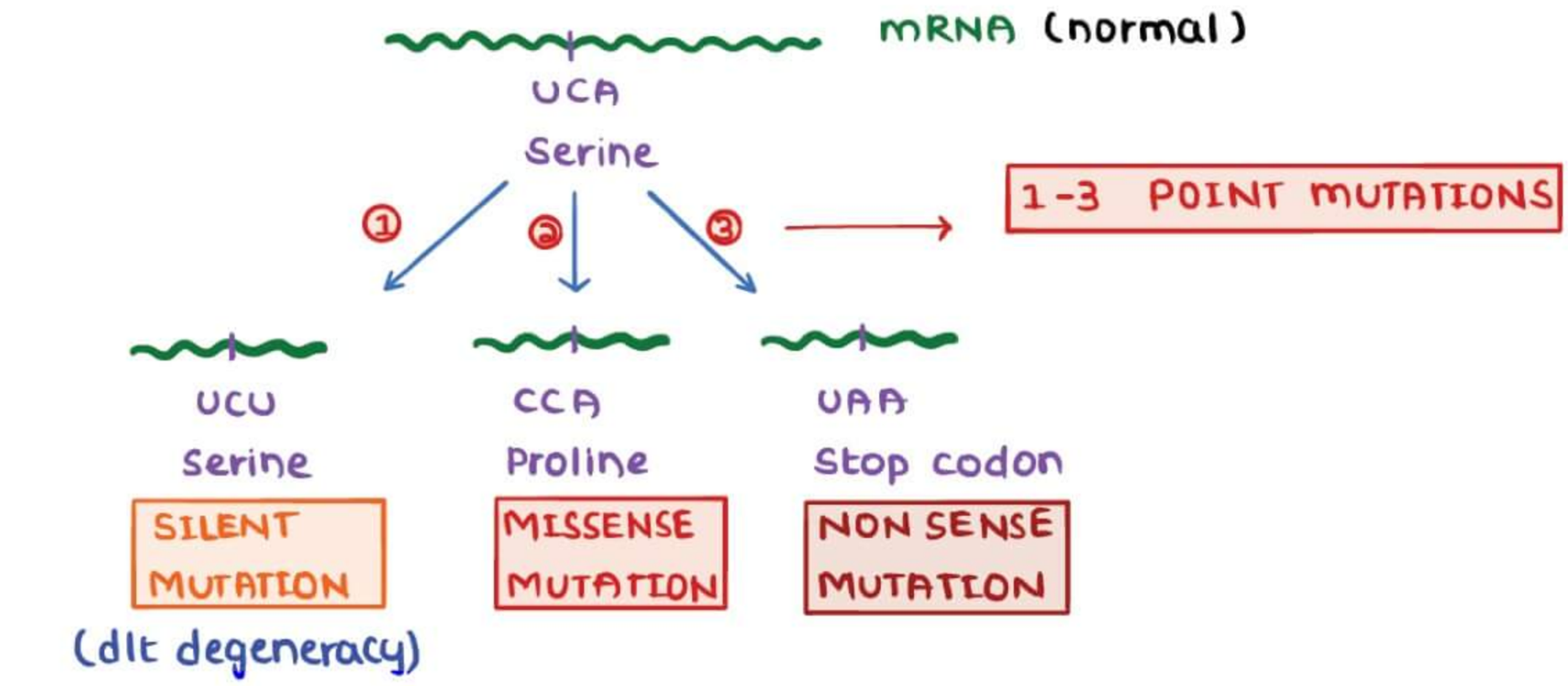
→ 3 STOP CODONS

UAA
UAG
UGA
} do not give any amino acids
EXCEPT Selenocysteine & Pyrrolysine

→ for 20 Amino Acids, 61 codons are present
On an average, for each amino acids 3 codons are present
- **DEGENERACY / REDUNDANCY of CODON**
→ Each amino acid have more than 1 codon
→ Methionine & Tryptophan do not show degeneracy (only 1 codon)

→ Degeneracy prevent us from mutations

MUTATIONS



(dIt degeneracy)

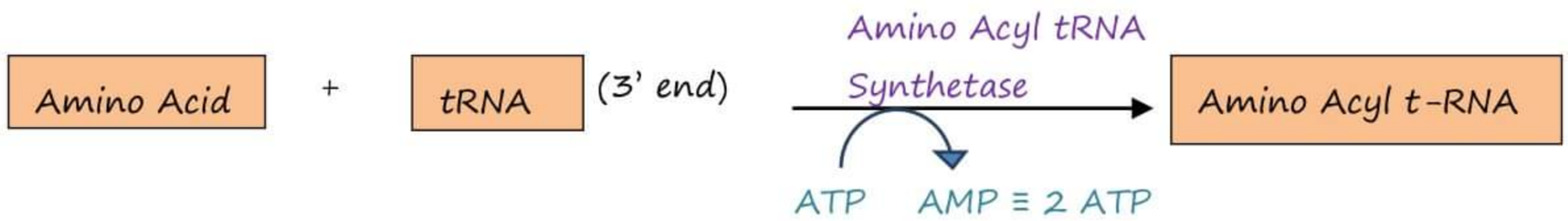
TRANSLATION

1. ACTIVATION OF AA/ CHARGING OF tRNA
2. INITIATION
3. ELONGATION
4. TERMINATION

} TRANSLATION

1. ACTIVATION of AA

→ occurs before all the 3 steps of translation



Amino Acyl tRNA Synthetase

→ Only point of proof reading in translation

→ Responsible for fidelity / Accuracy of protein Synthesis

→ Have 20 iso enzymes (one for each AA)

Q Which of the following does not require amino acyl tRNA Synthetase?

a Proline

b Lysine

c Hydroxy proline → Derived AA

d Methionine

INITIATION

→ First AA

- In Eukaryotes → Methionine
- In Prokaryotes → formyl Methionine

→ Initiation codon → AUG (codes for methionine)

INITIATION IN EUKARYOTES

mRNA + 40s + methionine-tRNA

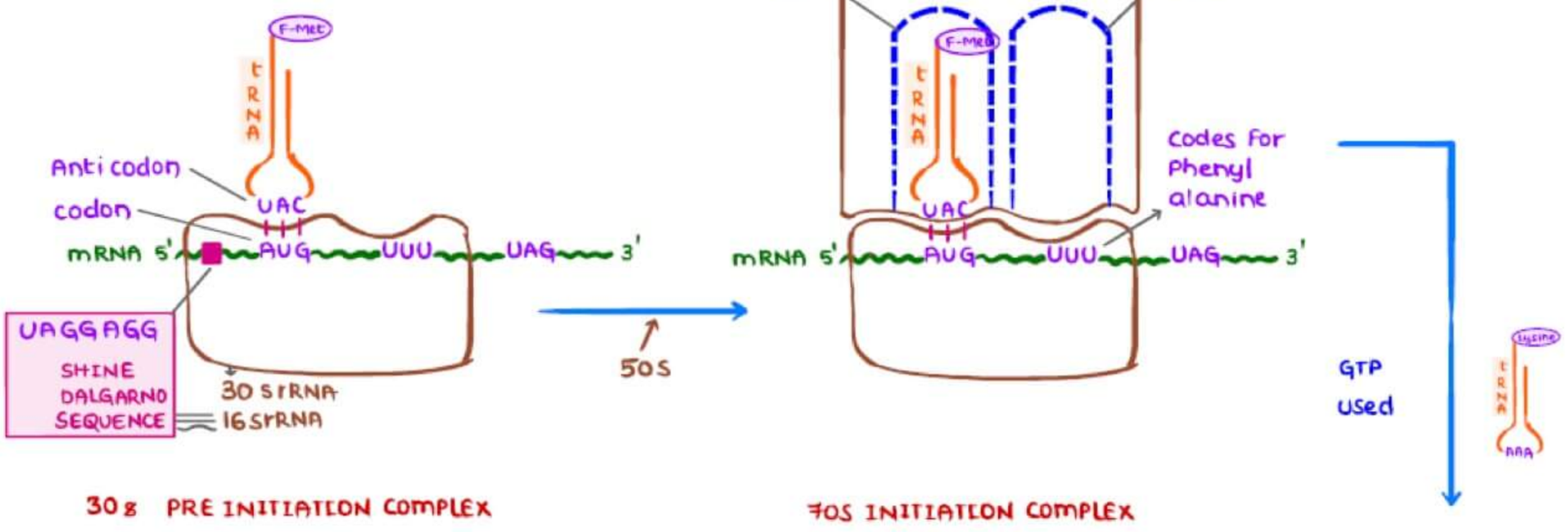
↓ eukaryotic Initiation factor [eIf] 1,2,3

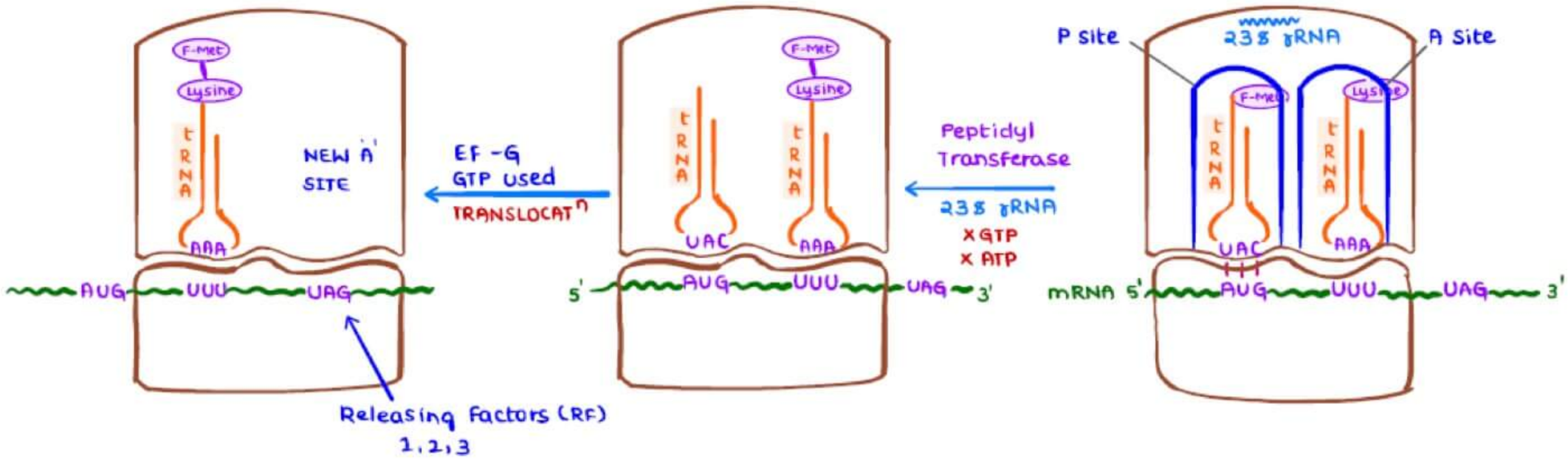
PRE INITIATION COMPLEX

60s → ↓ eIF 4f (4E + 4G + 4A) (Rate limiting factor)

80s INITIATION COMPLEX

INITIATION IN EUKARYOTES





'P' SITE (Polypeptide site)'

→ Polypeptide is released from this site at the end of translation

'A' SITE (Acceptor Site)

→ All AA except first Methionine are accepted here

Codon UUU
Anticodon AAA
} Codes for phenyl alaine

TRANSLOCATION → Movement or ribosome on mRNA

RELEASING FACTOR

→ Misnomer → Do not release

→ Only recognises the stop codon

Peptidyl transferase will release the polypeptide from P site

Q How many ATP & GTP & are used to add one AA in the growing polypeptide chain

A 2 ATPs → for the activation of AA

2 GTPs → 1 GTP used for entry at A site

1 GTP used for translocation

4 high energy phosphates are used to add one AA in the growing polpeptide chain

FACTORS IN TRANSLATION IN PROKARYOTES & EUKARYOTES

	PROKARYOTES	EUKARYOTES
Initiation	If 1, 2, 3	e1F 1, 2, 3, 4f
Elongation	EF – Tu, Ts, G	eEF 1α, 1β, 1r, 2
Termination	RF – 1, 2, 3	eRF

IF → Initiation factor

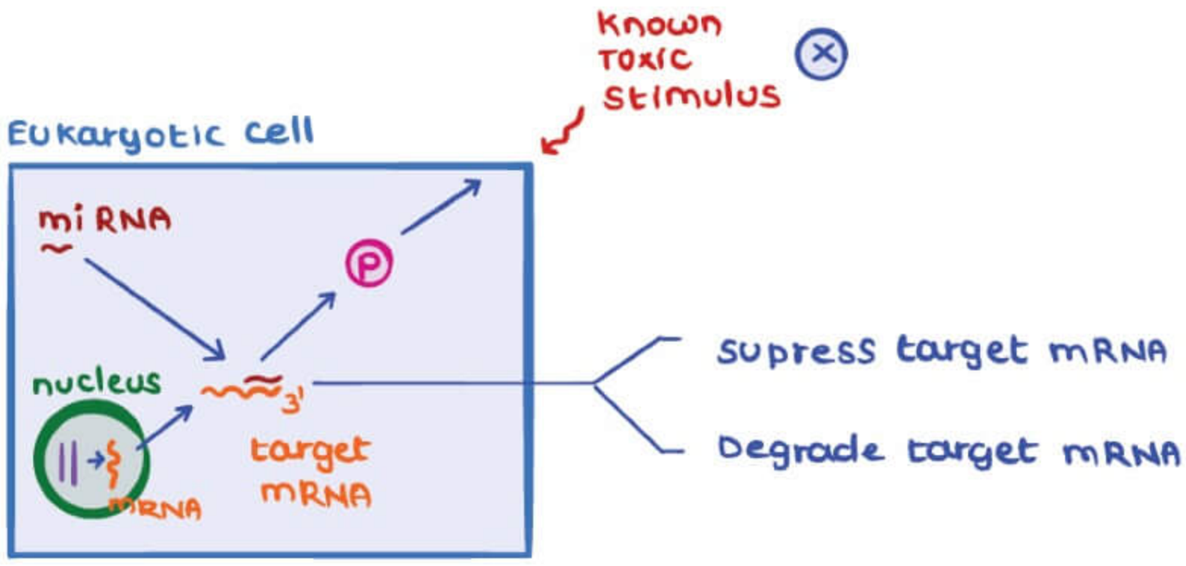
EF → Elongation factor

RF → Releasing factor

e → Eukaryotic

TECHINQUES IN MOLECULER BIOLOGY

BASICS



ABOVE REGULATION IS REQUIRED FOR

1. To stop energy wastage
2. Autoimmunity prevention

GENE KNOCK OUT TECHINQUE → Gene deleted
→ Not successful

RNA INTERFERENCE / SILENCING TECHINQUE / GENE KNOCKDOWN TECHIQUE

→ **RNA INTERFERENCE** → Interferes / inhibits mRNA by miRNA

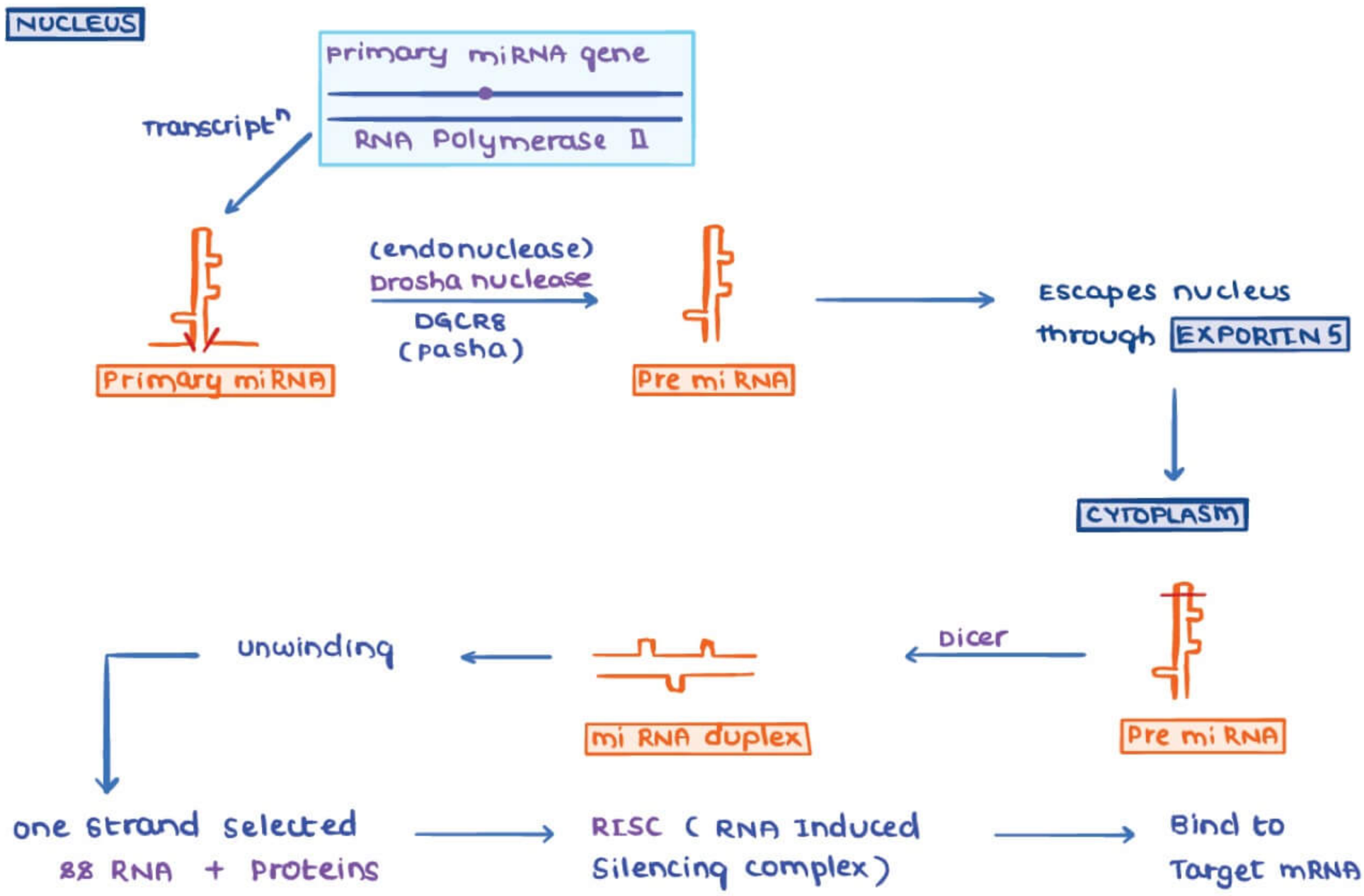
→ **SILENCING TECHINQUE** → Gene is present but function is suppressed

micro RNA

TYPES (based on source of Synthesis)

1. mi RNA
   - Synthesized from DNA [RNA Polymerase II]
   - Can only bind to 3' end of mRNA

2. si RNA – Small interfering RNA / Silencing RNA
   - Synthesized from cytoplasmic RNA
   - Ex: tRNA, Viral RNA
   - Can bind anywhere on target mRNA



→ Inhibition at the level of translation

GENOMIC IMPRINTING

→ Regulation of gene expression

→ Inhibition at the level of transcription

→ Imprinting = Inhibiting

PCR [POLYMERASE CHAIN REACTION]

→ Amplification of DNA

→ Heat is used for strand separation

COMPONENTS of PCR

1. ds DNA
2. 2 primers (1 for each strand)
3. Enzyme for polymerisation → Taq polymerase [derived from Thermus aquaticus bacteria]
4. Substrates → Deoxy ribonucleotides
5. Mg^{2+}

Dideoxy Ribonucleotide is never the component of PCR

STEPS

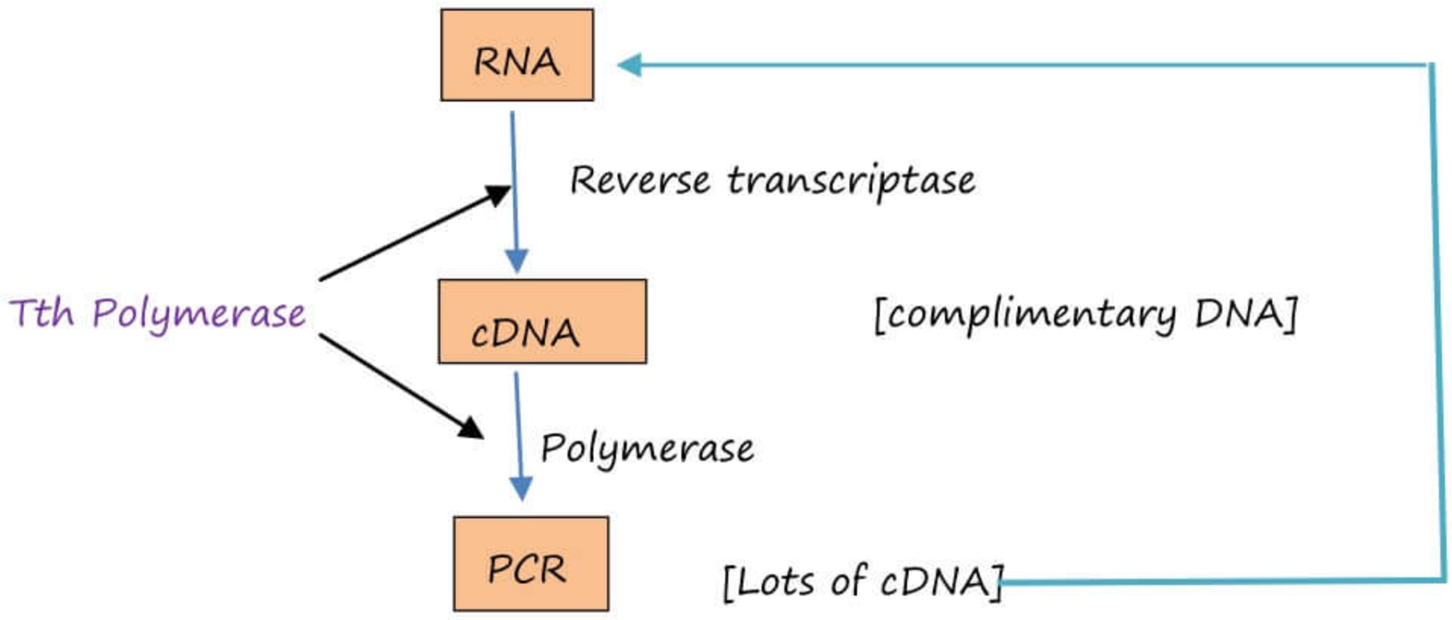
1. Denaturation → Two strands get separated
2. Annealing → Primers get attached
3. Extension → Polymerization

REAL TIME PCR / QUANTITATIVE PCR

→ 5 components + SYBR Green dye (Fluorescent when bound to ds DNA)

→ Monitors the amplification of target DNA

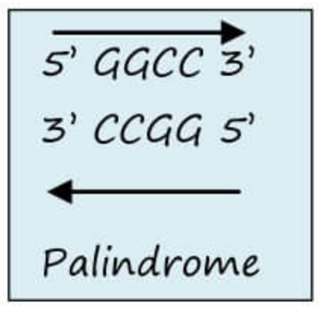
REVERSE TRANSCRIPTION PCR

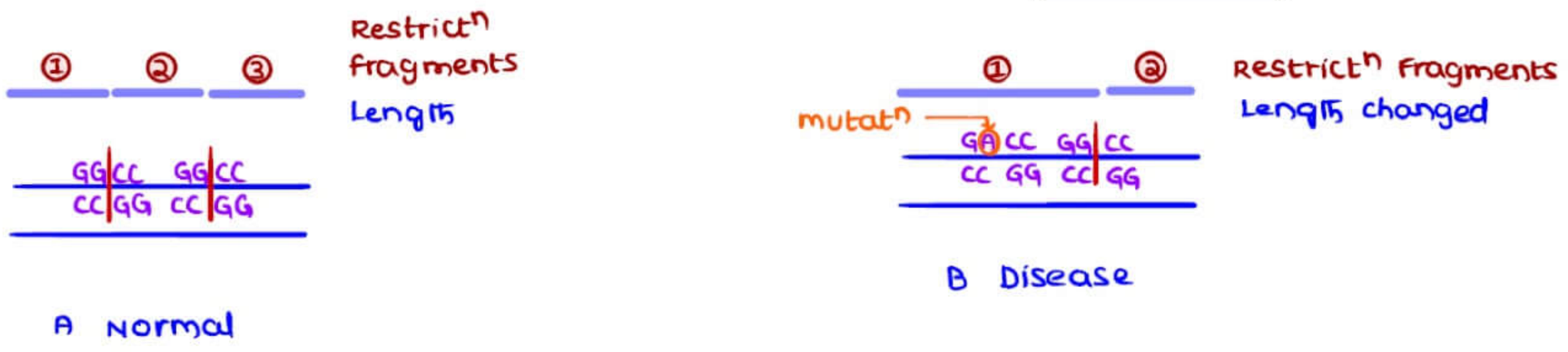


RFLP [Restriction Fragment Length Polymorphism]

→ RESTRICTION FRAGMENTS → The fragments got after digestion with restriction endonuclease enzyme

→ Restriction endonuclease cut at PALINDROME





LIMITATIONS

→ Detect only single mutation at a time

→ Can detect only those mutation which affect palindrome

→ Lengthy procedure

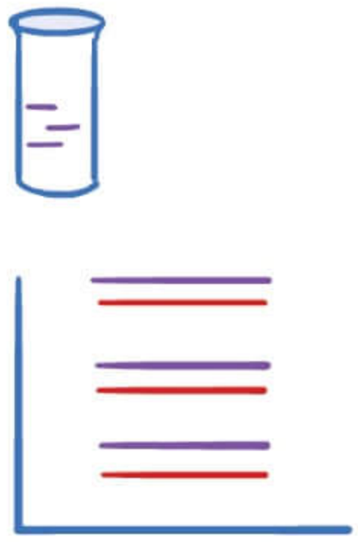
RFLP

↓

Electrophoresis (For separation of fragments)

↓

Blotting



- Done for Visualization
- Probe (complementary DNA) is added
- Probe is complementary to sequence of β globin gene
- Probe is labelled

BLOTTING / HYBRIDIZATION

	From Patient Sample	PROBE
1. SOUTHERN BLOTTING DNA DNA HYBRIDIZATION	DNA detected	DNA
2. NORHTERN BLOTTING RNA DNA HYBRIDIZATION	RNA detected	DNA
3. WESTERN BLOTTING IMMUNO BLOT	Protein [antigen] detected	ANTIBODY

Q Single gene expression analysis is done by

a Northern blotting

b Western blotting

c BOTH

MICRO ARRAY / Chip

→ Can detect multiple mutations

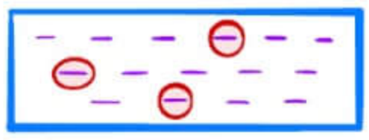
→ Multiple gene expression analysis can be done

→ Can do comparative genomic hybridisation

→ Can detect SNPs (single nucleotide polymorphisms)

→ Can detect genetic transfer of the disease

→ Can do Global pattern of gene expression



Patient sample

LIMITATION

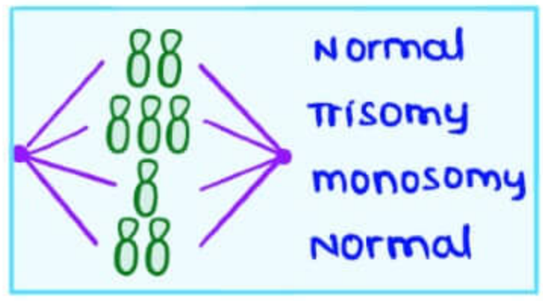
→ Can't detect monosomy & Trisomy

KARYOTYPING

→ Best technique for detecting monosomy & trisomy

→ Metaphase arrest

- All chromosome lie at equator



LIMITATIONS

→ Can not be done in any phase of cell cycle

→ Lengthy

→ Can not detect micro deletions, amplifications

FLUORESCENT INSITU HYBRIDIZATION [FISH]

→ IN SITU → done in morphologically intact cell, tissue or organ

→ Done in any phase of cell cycle

→ Rapid technique (< 24hrs)

→ Can detect microdeletions, amplifications, monosomy & trisomy

→ Can detect gene location on chromosome

FRAP (Fluorescence Recovery after Photobleaching)

→ Used to detect movement of proteins from one compartment of cell to another

DNA MARKERS

1. SNPs (single Nucleotide Polymorphisms)

   → ~ 10 millions

   - mc polymorphism

2. Repeat length polymorphism

STR	VNTR
Short Tandem Repeat	Variable number Tandem Repeat
Micro Satellite	Mini satellite
Repeat size 2-6 bp	15-70 bp

EX: STR

3. RFLP

DNA SEQUENCING TECHNIQUES

1. SANGER'S TECHNIQUE → Dideoxy nucleotide method is used
2. MAXAM & GILLBERT TECHNIQUE → Chemical clevage method
3. NEXT GENERATION SEQUENCING

EPIGENETICS AND GENOMIC IMPRINTING

BASICS

→ "Epi" genetics means "above" genetics

→ In other words, this is change in DNA but not change in DNA code

→ Chemical modification of DNA e.g. DNA methylation

→ Transferred to next generation

→ Unlike mutations, these changes are reversible

→ Can lead to gene activation or gene inhibition

GENOMIC IMPRINTING

→ an Epigenetic phenomenon

Imprinting means inhibited; Genomic means related to genes

→ In most genes, both alleles are expressed

→ < 1% of genes, only one allele is expressed & other is imprinted

Maternal Imprinting

- allele from mother is imprinted/inhibited
- Allele from father is working

Paternal Imprinting

- allele from father is imprinted/inhibited
- Allele from mother is working

MECHANISMS OF GENOMIC IMPRINTING

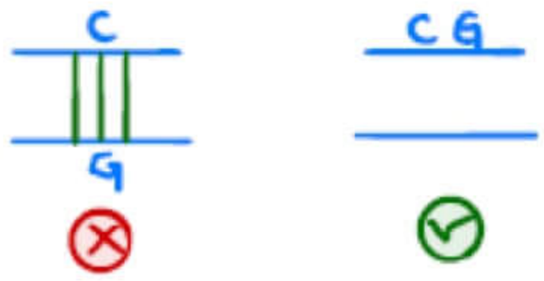
1. DNA METHYLATION

→ most common mechanism

→ occurs at CG site / CG Island / CpG dinucleotide

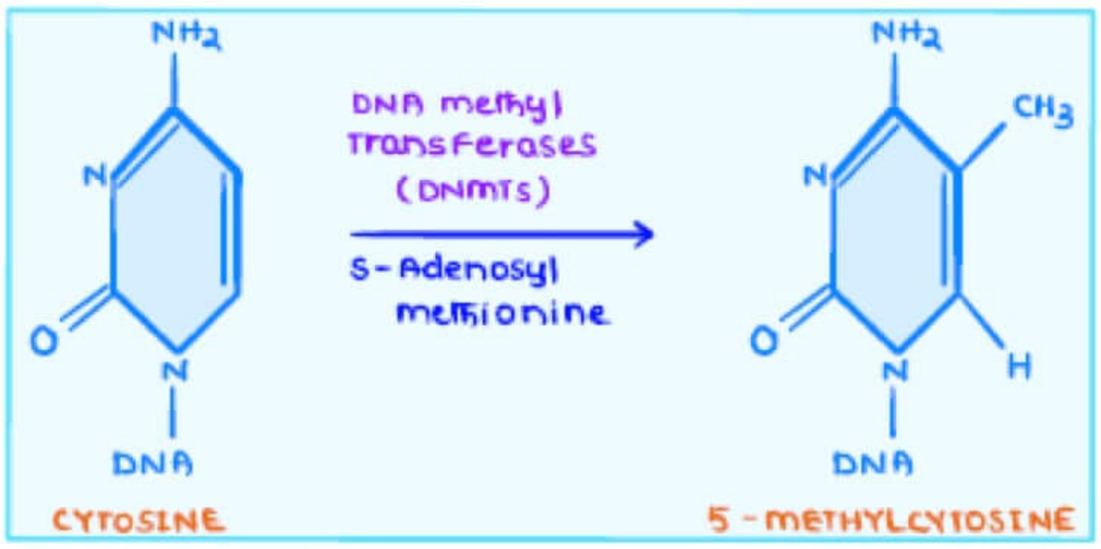
CG ISLANDS / CG SITES

→ C & G together present on same strands



→ cytosine is usually methylated in CG site

- causes inactivation of the gene



2. Histone deacetylation/methylation

→ less common mechanism

→ Post Translational Modification (PTM)

Methods to detect genomic imprinting

1. **Sodium Bisulfite method**

→ required for detection of DNA methylation

2. **Chromatin Immuno Precipitation (CHIP)**

→ CH → chromatin; I → Immuno; P → precipitation

→ required for detection of PTMs

Disorders caused due to genomic imprinting

1. **PRADER WILLI SYNDROME [PWS]**

Related to a particular allele on chromosome 15

For this allele:

Under normal situation

Maternal allele is imprinted/inhibited and only paternal allele is working

In PWS

→ Loss of paternal copy of allele on chromosome 15 by deletion

→ Maternal uniparental disomy

- 2 maternal copies are present
- But both of them are imprinted

2. **ANGEL MAN SYNDROME**

Related to a other gene on chromosome 15

For this allele:

Under normal situation

Paternal allele is imprinted/inhibited and only maternal allele is working

In Angelman syndrome

→ Loss of maternal copy of allele on chromosome 15 by deletion

→ Paternal uniparental disomy

- 2 paternal copies are present
- But both of them are imprinted

Other diseases involving genomically imprinted alleles are

3. Beckwith-Wiedemann syndrome
4. Russel-silver Syndrome

CRISPR

CRISPR – CAS 9 System

→ causes double strand break.

→ CRISP – clustered regularly interspersed Short palindrome Repeats

→ Cas-9 → CRISPER associated endonculease

→ This endonuclease is from cas-9 CRISPER gene

→ It is an immune system in bacteria against bacteriophages

- (memory transfered to future generation also)

→ Transmitted to progeny

→ So many generations protected from viruses

USES

→ Can be adopted to be used in eukaryotes for purposes like:-

- Gene deletion
- Exogenous gene insertion
- Multigene editing possible
- Altering gene transcription and regulation

Advantages over older technique of DNA breaks

- Cheap
- More accessible
- Simple
- Highly efficient
- Can target specific gene
- Rapid technique

MISCELLANEOUS

RESPIRATORY QUOTIENT

$$RQ = \frac{\qquad}{\qquad}$$

RQ of Glucose (Carbohydrates)

$C_6H_{12}O_6 + 6O_2 \longrightarrow 6\,CO_2 + 6\,H_2O$

RQ → 6/6 → 1

RQ VALUES

1. Carbohydrates → 1
2. Proteins → 0.8
3. Fats → 0.7
4. Mixed diet → 0.85
5. Brain → 0.97 – 0.99 [Principal fuel is carbohydrate]

DIET

1. Exclusive carbohydrate diet → 1
2. Carbohydrate Rich diet → > 1 (1.2)
   - → Extra carbohydrates convert to fats
   - → Macro molecule with more O_2 [Carbohydrates] ⟶ Macromolecule with less O_2 [Fats]

     - Amount of O_2 used is decreased
     - RQ increased

RQ TELLS US

1. Type of macromolecule used in the body
2. Conversion of one macromolecule to another

FASTING / STARVATION → RQ decreases [Fats are used]

RQ in diabetics → RQ decreases [Fats are used]

→ On giving insulin → RQ Increases [Carbohydrates are used]

ACIDOSIS

→ RQ increases because CO_2 output increases

→ CO_2 output is greater than O_2 consumption

ALKALOSIS

→ RQ decreases because respiration is depressed

→ CO_2 retained in the body

→ So less CO_2 is produced

FEVER

→ RQ increases

→ ↑ Breathing

→ Wash out CO_2

→ So CO_2 production increases

EXERCISE	RECOVERY FROM EXERCISE
→ ↑ Lactic Acid	→ Less CO_2 produces
→ ↑ RQ	→ So RQ decreases

ALCOHOL METABOLISM

→ Occurs in LIVER (organ)

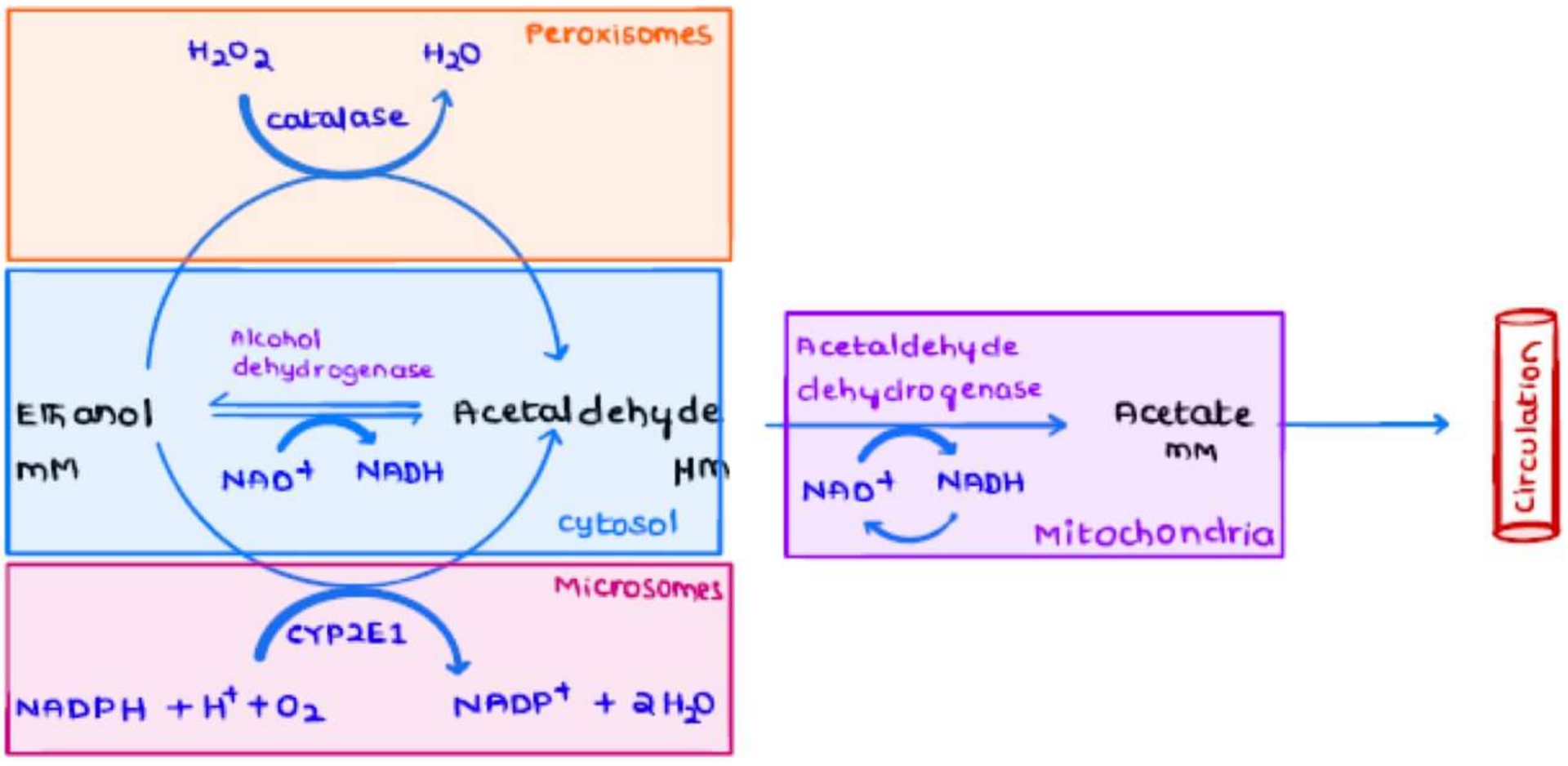
→ Occurs in cytoplasm & mitochondria [organelle]

→ Energy produced → 7 kcal/gm

→ Site of absorption → Mainly small Intestine

→ Follows zero order kinetics

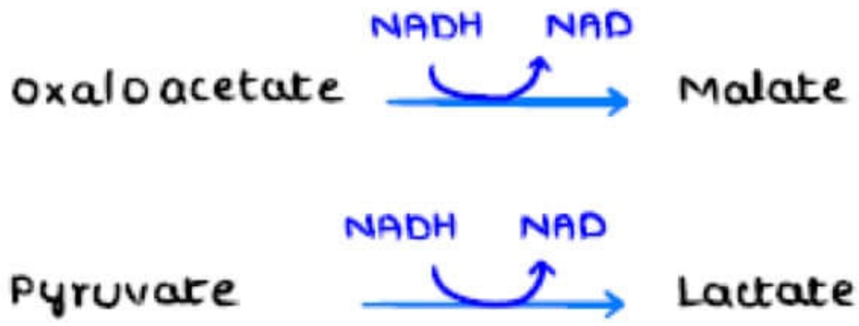
→Pleasurable effect is due to increased dopamine



→ Microsomal metabolism is activated when high amount of alcohol is ingested

INCREASED NADH

→ ↑ NADH in Liver occurs d/t Alcohol metabolism & causes



→ ↑ NADH causes ↓ oxaloacetate &↓ pyruvate & leads to

1. Lactic Acidosis [Pyruvate to Lactate]

2. Hyperuricemia

   → Alcohol increases the breakdown of purine nucleotides also causes Hyper uricemia

   → Both over production & under excretion occurs

3. Decreased Gluconeogenesis [no pyruvate & OAA]

4. Hypoglycemia (also d/t poor glycogen reserves)

5. TCA inhibited (no NAD & OAA)

6. β Oxidation inhibited

7. TG synthesis increased

Fatty Liver is not because of FA derived from adipose tissues, but this is due to endogenous synthesis of TG in Liver

8. Alcoholic Ketosis

9. Fatty Liver

   → d/t ↑ TG

   Impaired formation or release of VLDL

   β - Oxidation inhibited

→ **NO NEGATIVE FEEDBACK CONTROL FOR ALCOHOL METABOLISM**

↓

So Alcohol oxidation is preferred over other macromolecules

→ Alcohol also affect the absorption of

- Vitamin B3
- Vitamin B1 [usually found]
- Vitamin B6
- Vitamin B9
- Vitamin A

ALCOHOL DEHYDROGENASE

- ADH
- Many isoenzymes
- Most Abundant – ADH 1A
- Present in Liver and Adrenal Glands
- Has NAD containing domain known as ROSSMAN FOLD

ALDEHYDE DEHYDROGENASE [ALDH]

→ **2 Iso enzymes**

- ALDH-1 → Present in cytoplasm (minor role)
- ALDH – 2 → present in Mitochondria (major role)

MICROSOMAL ETHANOL OXIDISING SYSTEM [MEOS]

→ Occurs in Endoplasmic Reticulum

→ Inducible

→ Induced after ingestion of lots of alcohol

→CYP - 2E1 → High Km [requires lots of substrate]

→ NADPH involved

- protective in chronic Alcoholics

→ But too much use will produce ROS [Reactive oxygen species] which will damage DNA, proteins & Lipids

ACCESSORY PATHWAY IN PEROXISOMES

→ Produces H_2O_2

→Catalase needs to detoxify this

WERNICKE – KORSAKOFF SYNDROME

→ d/t Thiamine deficiency

→ **THIAMINE DEFICIENCY IN ALCOHOLICS IS DUE TO**

1. Intake of improper amount in diet
2. Alcohol interferes with the absorption of B1

→ Wernicke peripheral neuropathy

→ Korsakoff psychosis

→ SIGNS & SYMPTOMS

- Ataxia
- Memory toss
- Confabulations
- Ophthalmoplegia, Nystagmus
- Cerebral haemorrhage

→ TREATMENT

→ Thiamine supplementation before giving glucose for hypoglycemia

- Thiamine supplementation prevents extra Lactic Acidosis

→ But there is incomplete recovery of memory

ALDEHYDE DEHYDROGENASE

→ Inhibited by Antabuse → DISULFIRAM

→ Genetic variations seen (particularly in ASIAN POPULATION)

→ ASIAN FLUSH SYNDROME

→ d/t ↑ Acetaldehyde (d/t Aldehyde Dehydrogenase)

→ Nausea, Tachycardia, Vomiting, Hyperventilation, Flushing

Sweating.

→ DISULFIRAM must be given under medical supervision

HOW ACETALDEHYDE IS TOXIC

→ Forms adducts with proteins & AA

→ Binds glutathione

→Damage mitochondria

→ Inhibit microtubules

METHANOL POISONING

→ Methanol forms Formaldehyde [TOXIC]

- Enzyme involved Alcohol dehydrogenase

→Rx

1. ETHANOL

→Ethanol competes with methanol for ALDH and forms Acetate [safer]

2. FOMEPIZOLE (costly)

ETHYLENE GLYCOL

(Ethylene glycol)
↓
(Glyco Aldehyde)
↓
(Glycolic Acid)

→ used as ANTI FREEZE compound in western countries.

FETAL ALCOHOL SYNDROME

→ If mother taking alcohol during pregnancy can cause it.

- no defined amount of alcohol

→ C/I in pregnancy

→ ADH inhibited

(Retinol)
↓ADH
(Retinaldehyde)
↓
(Retinoic Acid)

→ important for cellular signaling during growth & development

→ Both alcohol & acetaldehyde crosses placenta

→ Low IQ, low birth weight

VITAMINS GENERAL

→ Vitamins are organic compounds which are essential in diet.
- Required in minute quantities

Atypical vitamins – can be synthesized in our body

Synthesized by Human enzymes
1. Vitamin D ← Synthesized from cholesterol (skin)
2. Niacin (B_3) ← Synthesized from amino acid-Tryptophan

Vitamins synthesized by bacterial flora
- Vit K
- Vit B_2
  B_5
  B_7

→ Vitamin B complex which are helping in energy release B_2, B_5, B_7, B_1, B

Classification of Vitamins

Fat Soluble	Water Soluble
- A, D, E, K	- B complex, C
- All made from common Isoprene structure (5c)	- No common structure
- They do not have common function	- Common function ↓ Co enzyme

Fat soluble Vitamin which act as co-enzyme Vit K

Q Water Soluble form available for which fat soluble vitamin?

Ans Vit K ⟶ Synthetic form – K_3 /menadione water soluble

General features of Fat soluble vitamin

1 Common Isoprene unit

2 All are absorbed from intestine and are assembled into chylomicrons. Along with dietary lipids, Pancreatic enzyme and bile salts have a role.

3 Non-polar ⟶ cannot be excreted via kidneys

So tend to accumulate

Stored in ⟶ LIVER ⟶ If Excess ⟶ Toxicity of fat Soluble Vitamin can occur

→ Water Soluble vitamins are not stored in body they are excreted out

Except ⟶ Vitamin B_{12}

Out of all fat-soluble vitamin → Vitamin E has least toxic effect

Fat soluble vitamin deficiency - Rare as they are stored in our body

Causes

1. Steatorrhea & Malabsorption

   Ex: - Cystic Fibrosis, Celiac diseases

   Vitamin K is the first fat soluble vitamin to be excreted in acute malabsorption.

2. Mineral oil intake Ex: - Paraffin Oil

Q Richest source of vitamin A –

Halibut fish liver oil » Cod fish liver oil » Shark fish liver oil

Q Which fruit has vitamin A – Ripe Mango

Q Which vegetable has vitamin A – Carrot

Q Richest source of vitamin D – Halibut fish liver oil

Q Which fruit has vitamin D – None

Q Which vegetable has vitamin D – None

(No plant source for two vitamins – Vitamin D & vitamin B_{12})

Q Strict vegetarians will be deficient in which Vitamin – Vitamin B_{12} » Vitamin D

(Vitamin D can also be obtained from sunlight)

Richest source of Essential Fatty acid

Safflower oil> Sunflower oil> Corn oil, Soyabean oil

Other sources → Olive oil, Ground nut oil, Coconut oil, Flaxseed oil, Fish oil

- Richest source of Linoleic acid → Safflower oil
- Richest source of Arachidonic acid → Safflower oil
- Richest source of Linolenic acid → Flaxseed oil > Soyabean oil
- Richest source of Eicosa Pentaenoic acid or Timnodenic 20C → Fish oil
- Richest source of PUFA → Safflower oil
- Richest source of Saturated Fatty acids → Coconut oil
- Richest source of Mono unsaturated fatty acids → Olive oil

Limiting Amino Acids

Maize lacks → Tryptophan + Lysine

Wheat lacks → Threonine + Lysine

Pulses lacks → Methionine + Cysteine

VITAMIN – A

→ 3 Active forms

1. Retinol (OH)
   - Role in reproduction mainly

2. Retinal (Aldehyde groyp)
   - Role in vision

3. Retinoic acid (COOH)
   - Role in cell differentiation & growth

→ Main form of Retinol – can be converted to other forms also.

→ These Retinoid compounds source in animal origin

Ex: - Eggs, fish, liver, milk, cheese

→ From Plant source the form of Vit A is ⟶ β carotene

↓

Structure having (2 retinal)

→ Richest plant source of Vitamin A ⟶ Carrot

→ Vit A is absorbed in Intestine in the form of

- Retinol

↓

Liver

Stored as Retinol esters in Peri-sinusoidal cells or Ito cells

↓

Circulation

Retinol – RBP (LMW protein synthesized in liver)

Retinol binding Protein

↓

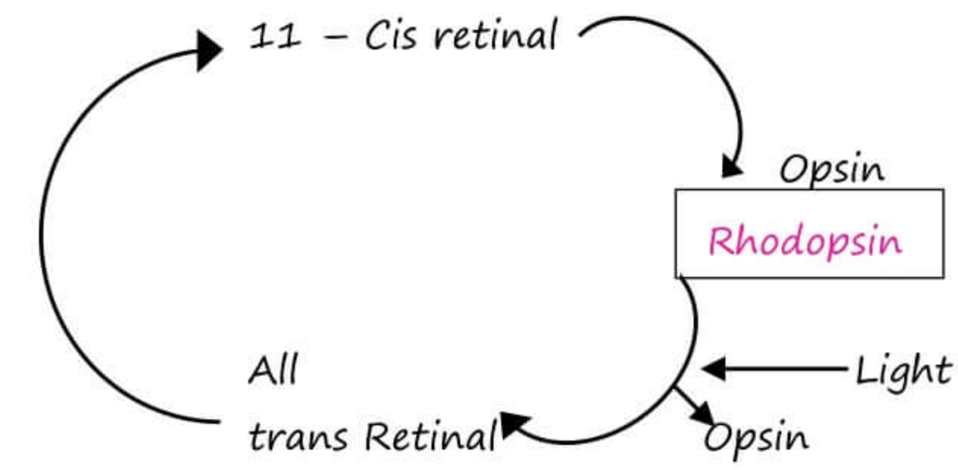
In Circulation for the transport of Retinol

Retinol – RBP – Transthyretin

TERNARY COMPLEX

Trans	Thy	retin
Transport	↓	↓
Protein	Thyroxin	RBP

Role of vitamin A in Vision



Protein Responsible for NIGHT VISION

This cycle called – WALD's VISUAL CYCLE

Rhodopsin – In bright light (Bleached + non functional)

Dark Light (Rhodopsin resynthesized)

Time ⟶ DARK Adaptation time

Increased in Vit A deficiency

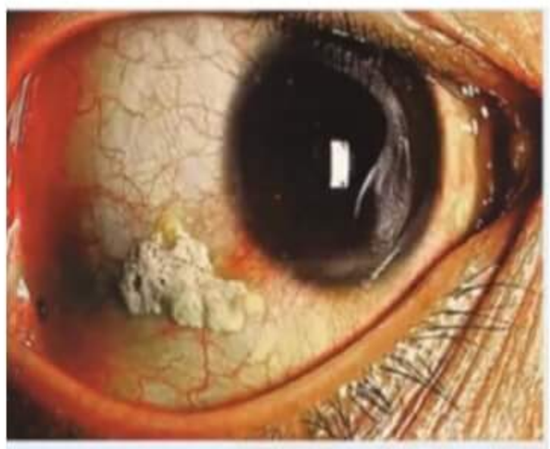
Other uses

- Anti Oxidant
- Photo protective
- 13-Cis Retinoic acid ⟶ Severe cystic acne
  ↘ (Teratogenic)

Deficiency of Vit A

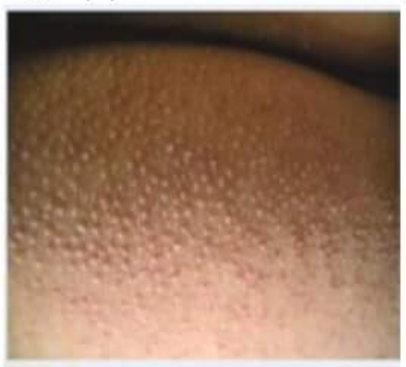
→ Earliest sign → Loss Sensitivity to green light
→ Earliest symptom → Night blindness
→ Earliest manifestation → Night blindness
→ Most specific manifestation → Bitot's spots

→



Bitot's spots

- Foamy appearance in conjunctiva due to superficial deposition of keratin in conjunctiva



Follicular hyperkeratosis

Follicular Hyperkeratosis

→ Dry scaly skin
→ Skin ulceration

→ Corneal ulceration
→ Urinary & respiratory tract epithelium affected leading to infection
→ Vitamin A deficiency is most common cause of preventable blindness

Vitamin A toxicity

→ Organelle affected ⟶ Lysosomes
→ As vitamin A is stored in Liver
It occurs in people who consume bear's liver
→ It resembles ⟶ Brain Tumour
Known as Pseudo tumour Cerebri
→ Also patients have
- Hepatomegaly
- Hyper Lipidemia
- Blurred Vision

Note

Carotenemia

→ Occurs due to excess intake of carotenoids.
→ Yellow staining of skin (Not Sclera)

→ In Hyperbilirubinemia Jaundice – Yellow staining of skin + sclera.

VITAMIN D / SUN SHINE VITAMIN

→ Synthesized in body from cholesterol
→ Active form → CALCITRIOL 1,25 dihydroxy cholecalciferol
→ CALCITONIN → Synthesized from Thyroid gland
→ ↓ blood calcium

2. FORMS

D_3 → Cholecalciferol
D_2 → Ergo calciferol (obtained from plants, fungi, yeast)

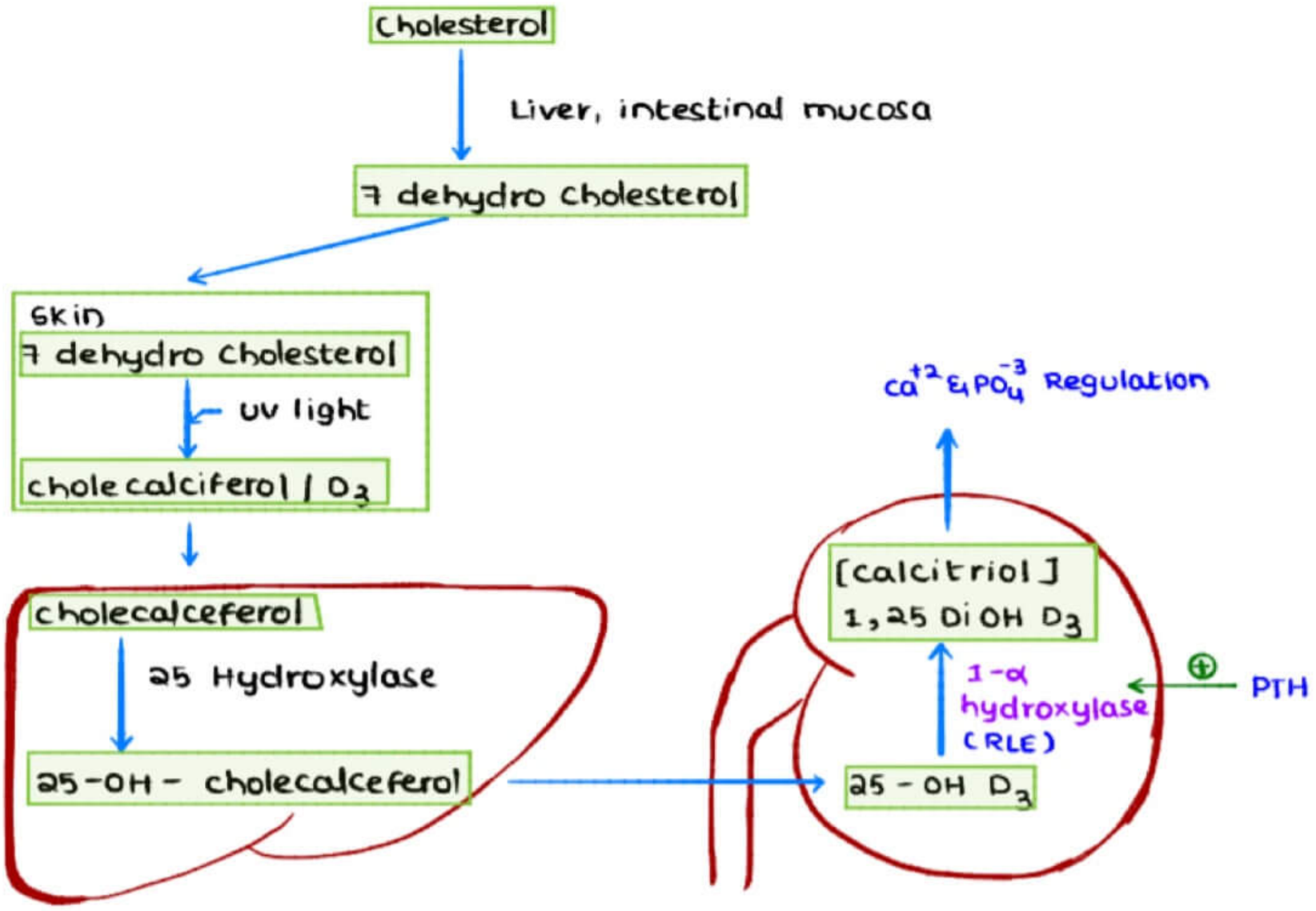
Both forms are converted to 25-OH D_3 in Liver

3. CALCITRIOL – CONSIDERED AS HORMONE

1. Synthesized in body (kidney)
2. Released in circulation
3. Has distant site of action (intestine, kidney, Bones)
4. Bind to nuclear receptors like steroid hormones
5. Subjected to feed back regulation like hormones

NOT A PROPER HORMONE – Because it is not produced by some gland

4. SYNTHESIS OF VITAMIN – D



5. Ca Homeostasis = Calcitriol & PTH

Vit D	PTH	CALCITONIN
↑ Ca^{+2} Blood	↑ Ca^{+2} in Blood	↓ Ca^{+2} in
↑ PO_4^{-3} in Blood	↓ PO_4^{-3} in Blood	Blood

6. ROLE OF VIT D

1. ↑ Ca & P absorption from intestine
2. Cause Reabsorption of Ca^{2+} from kidney
3. Bone → ↑ Bone mineralisation during bone growth and development. But if there is Ca ↓ level in Blood. Then Vitamin D will activate osteoclasts of bone and will ↑ Blood Ca^{+2}.

7. SOURCES

1. Adequate sun light
2. Fish Liver Oil
3. Egg yolk
4. Liver

$$\text{Production of Vitamin D} \propto \text{Exposure to sunlight} \propto \frac{1}{\text{Pigmentation of Skin}}$$

→ Highest levels of Vit D are synthesized at the end of Summers

→ Lowest levels of Vit D are synthesized at the end of Winters

8. CAUSES OF DEFICIENCY

1. Inadequate Sunlight

→ Common in womens (Pardah I Burkha system)

Hospitalised bed ridden patients

Climate Where Sunlight is not enough

2. Chronic kidney disease
3. Premature Infants, pregnancy + Lactation.

→ Deficiency of Vit D → ↑ Alkaline of Phosphatase

DEFICIENCY IN CHILDREN – RICKETS

1. Bow legs, Knock Knees } Bending Of long bones
2. Beaded appearance of RIBs
   - Pigeon breast appearance → Rachitic Rosery

DEFICIENCY IN ADULTS – OSTEOMALACIA

→ Bone pain

→ Muscle weakness

→ Soft bones

- Pelvic bones affected → Waddling gait

→ ↑ Serum ALP

→ ↓ Serum Ca^{+2} & Po_4^{3-}

RENAL RICKETS

→ When the deficiency of Vitamin D is d/t defective formation of Calcitriol in Kidney

HYPERVITAMINOSES D

→ Calcification of Soft tissues occur

→ Kidney stones

VITAMIN E / α – TOCOPHEROL

→ ANTI STERILITY VITAMIN (earlier name)
→ Most abundant & potent → α – Tocopherol

SOURCE

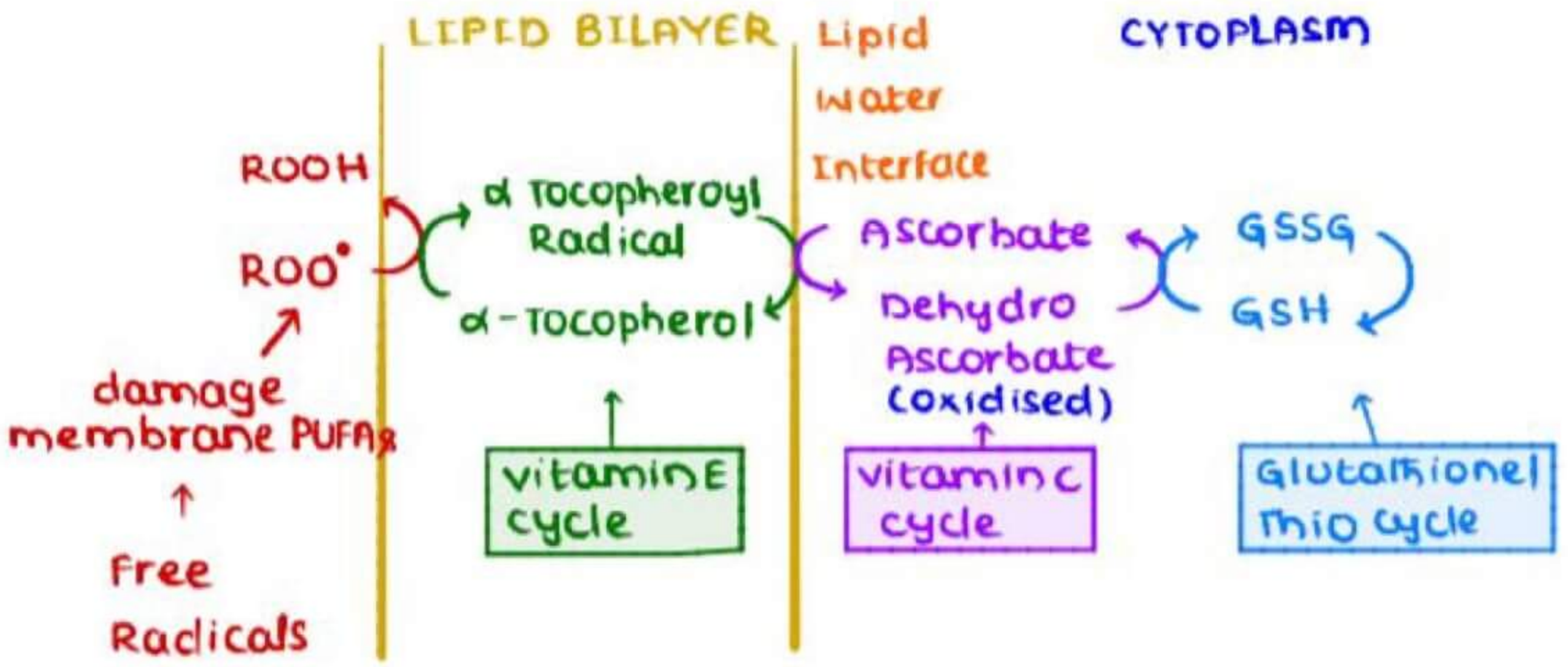
1. Vegetable oils – wheat germ oil, cotton seed oil, Sunflower oil
2. Nuts

EXCRETION → Mainly in faeces via Hepato biliary root

FUNCTIONS

1. **Antioxidant**
   → Most potent lipid phase antioxidant chain breaking antioxidant
   → Protects RBC & other membranes from free radical damage
   - Aka CELL SCAVENGER

   Anti Atherogenic - Converts oxidized LDL to normal LDL
   → Boost Immune response
   → Protect RBCS from Hemolysis
   → Keeps structural & functional integrity of all cells
   → Slow ageing process
   → Protects nervous system from degenerative action from over production of glutamate.



INTER RELATIONSHIP OF VITAMIN E WITH SELENIUM

→ Se decreases the symptoms of Vitamin E deficiency

- It is required for enzyme Glutathione peroxidase

GSH ⟶ GSSG

(red) **(ox)**

→ Vit E also decreases the requirement of Se

BOTH ACT SYNERGISTICALLY

DEFICIENCY SYMPTOMS

→ Hemolysis (d/t lack of protection of RBCs)

→ Hypersegmented Neutrophils Opthalmoplegia

→ Neurological Presentation

- Peripheral Neuropathy ≡ to Vit B deficiency
  - No megaloblastic anaemia

VITAMIN K → Koagulation /Coagulation

→ Only fat soluble Vitamin with co enzyme function

3 FORMS

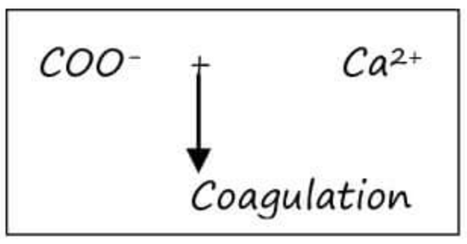
(K1)	Phyloquinone	Plant source
(K2)	Menaquinone	Animal source synthesized by bacterial flora intestine stored in Liver
(K3)	Synthetic form Menadione	Water soluble Can be converted to K2 in Liver

ROLE

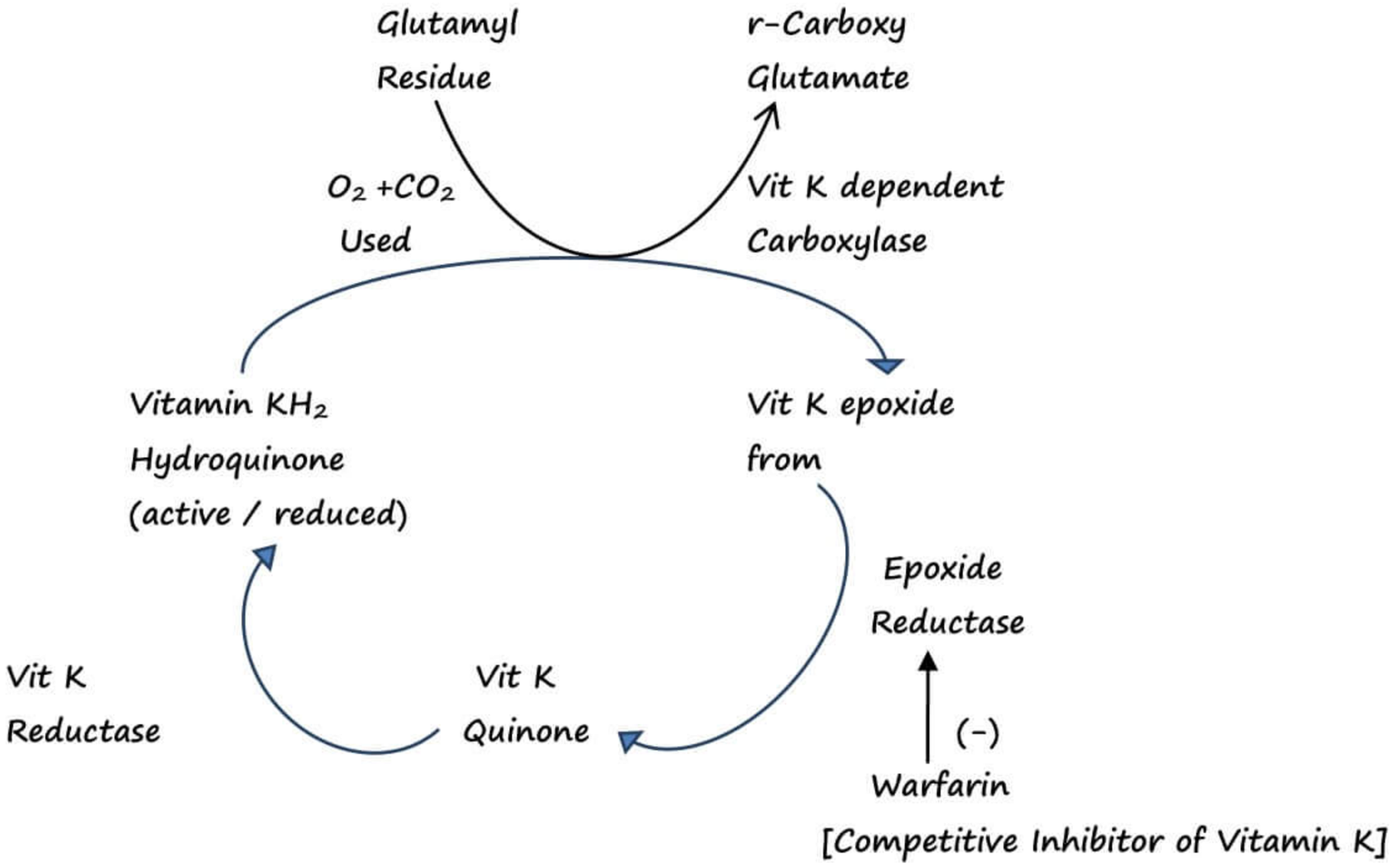
Helps in coagulation

→ Acts as a co enzyme in carboxylation of Glutamyl residues present in some proteins.

Ex: Clotting factor 2 (Prothrombin)
Clotting factor 7,9,10
Osteocalcin
Nephrocalcin
Protein C & S



VITAMIN K CYCLE



→ Vitamin K can be used as antidote for warfarin.

→ Vitamin K cycle occurs in microsomes of Liver cells.

SOURCES

1. Green leafy vegetable (cabbage, cauliflower, spinach)
2. Cereals

DEFICIENCY SYMPTOMS

→ Bleeding

- Easy bruising
- Ecchymotic patches
- Mucous membrane Haemorrhage
- ↑ Prothrombin time
- Post traumatic bleeding
- Fetal Haemorrhagic disease of new born
- Internal bleeding

HAEMORRHAGIC DISEASE OF NEW BORN

→ Fatal

→ Occurs quite often

→ Reasons

1. Poor placental transfer
2. Hepatic Immaturity → Inadequate synthesis of coagulation proteins
3. Low Vit K content in early breast milk (colostrum)
4. Vitamin K regeneration cycle is not fully developed
5. Intestine of new borns is sterile
6. Prothrombin levels are only 25% of adult levels

→ Prophylactic administration of Vit K for all new borns advised

B COMPLEX VITAMINS

→ All water soluble vitamins have coenzyme role

→ B soluble have role in the energy metabolism

→ Vit B_1 (Thiamine) → Carbohydrate metabolism

→ The Vit for which RDA is based on carb intake → B_1

The Vit for which RDA is based on protein Intake → B_6

B_1 → Thiamine

Active form – TPP (Thiamine PyroPhosphate)

Thymine → Pyrimidine

Source → Richest source → Rice Polishing

Grains Outer layer → Aleurone layer which contain Vit B_1

Role

→ Oxidative Decarboxylation

Transketolase is marker for Vit B_1 deficiency

Deficiency of Vit B_1 → Beri-Beri

a DRY beri beri
→Affect CNS
b Wet beri beri
→affect CVS
→edema

→ Usually mixed Beri-Beri occurs
→ In case of alcoholics severe Vitamin B_1 deficiency occurs this is known as Wernicke Korsakoff Psychosis
→ Vit B_1 deficiency Lactic Acidosis occurs

Pyruvate $\xrightarrow{\frac{B1}{PDH\ complex}}$ Acetyl Co A

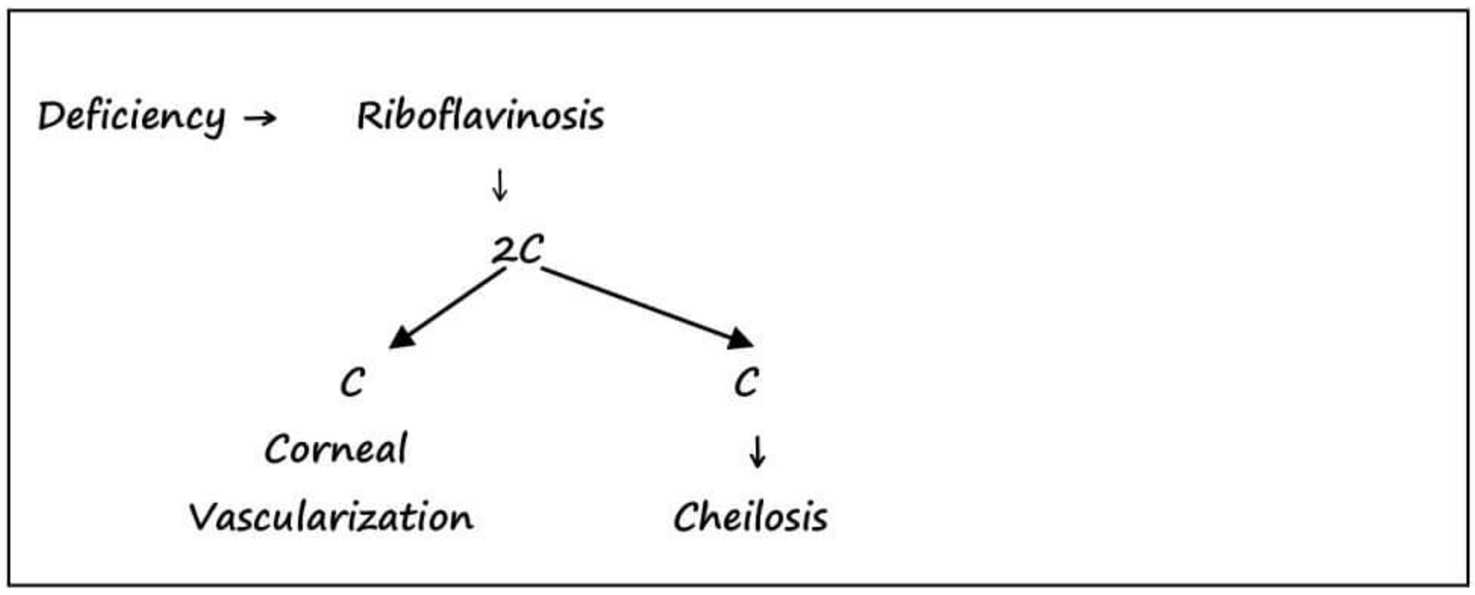
- So in thiamine deficiency Pyruvate excess can cause lactic acidosis

B2 → Riboflavin

Active form – FMN & FAD

Role – Oxidative – reductive reaction
→ Enzyme which require FAD
- Succinate Dehydrogenase
- Branched chain Amino acid DH PDH
- α - Ketoglutarate DH
- Complex II ETC

FMN → Complex I ETC
Marker enzyme → RBC Glutathione Reductase Activity



This patient has Glossitis / Magenta tongue / Geographical tongue

→ Also have angular Stomatitis

B3 → Niacin

→ Synthesize from tryptophan
→ 60mg of tryptophan is used to form 1mg Niacin

→ Active → NAD, NADP
Role → Oxidative → Reduction reaction

Deficiency of B_3

Causes → Staple diet like maize
→ Maize protein zein lacks tryptophan
→ Anti TB drug – ISONIAZID
→ Hartnup's disease
→ Carcinoid syndrome → Serotonin make by Tryptophan

Deficiency of B_3 know as → Pellagra (3D)

D → Dermatitis (Photosensitive)
Casal's necklace appearance
D → Diarrhoea
D → Dementia
4th D → Death
5th D → Delirium
6th D → Depression

→ Niacin use in Hyperlipidaemia
→ Niacin - decreases TG, LDL and increases HDL because Its Inhibits hormone sensitive lipase

B5 → Pantothenic acid

Active → Coenzyme A
Ex: - Acyl Co A, Acetyl Co A
Deficiency → Burning foot syndrome

B6 → Pyridoxine

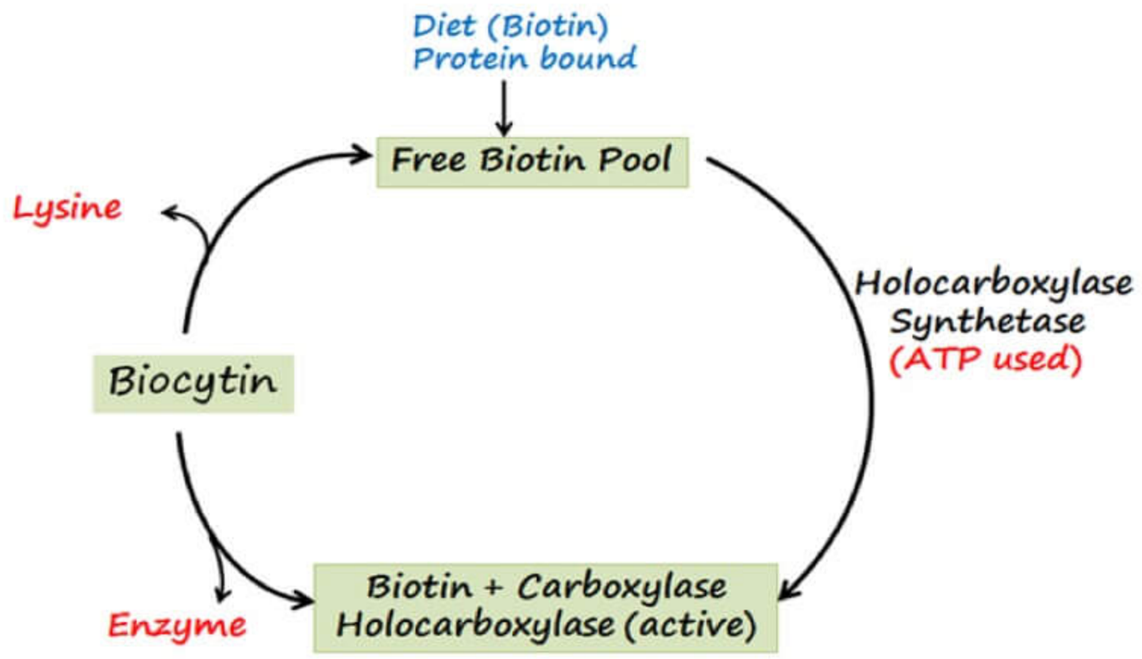
Active → PLP (Pyridoxal Phosphate)
Role → Transamination

Trans sulfuration
Deamination
Simple decarboxylation
Haem synthesis
Cysteine syndrome
Glycogen Phosphorylase

Deficiency of Vit B_6 → Anaemia with Neurological symptoms

Q Seizures in Infants can be caused by which Vitamin deficiency
ANS B_6

Multiple Carboxylase Deficiency



Multiple Carboxylase Synthetase → Deficiency of Holocarboxylase Synthetase + Biotinidase.

Deficiency of Holocarboxylase Synthetase → Early onset (Infantile)

Deficiency of Biotinidase → Late onset (Juvenile)

Multiple Carboxylase Disease

- Autosomal recessive
- Odour → Tom Cat urine

C/F

An organic acidemia

- Metabolic acidosis with ↑ anion gap
- NH_3 normal or ↑
- Ketosis

CNS → Encephalopathy, Seizures, Developmental delay.
Hair → Alopecia
Skin → Eczema
Diagnosis → Enzyme Assay – Lymphocytes
Treatment→ Biotin

B7 → Biotin

→ Role in carboxylation
→ Few reactions where CO_2 added but Biotin not used

1. CPS I & II
2. Malic enzymes
   Pyruvate(3C) ⟶ Malate (4C)
3. Carbon number 6 in Purines
4. Gamma carboxylation of clotting factor done by Vit K

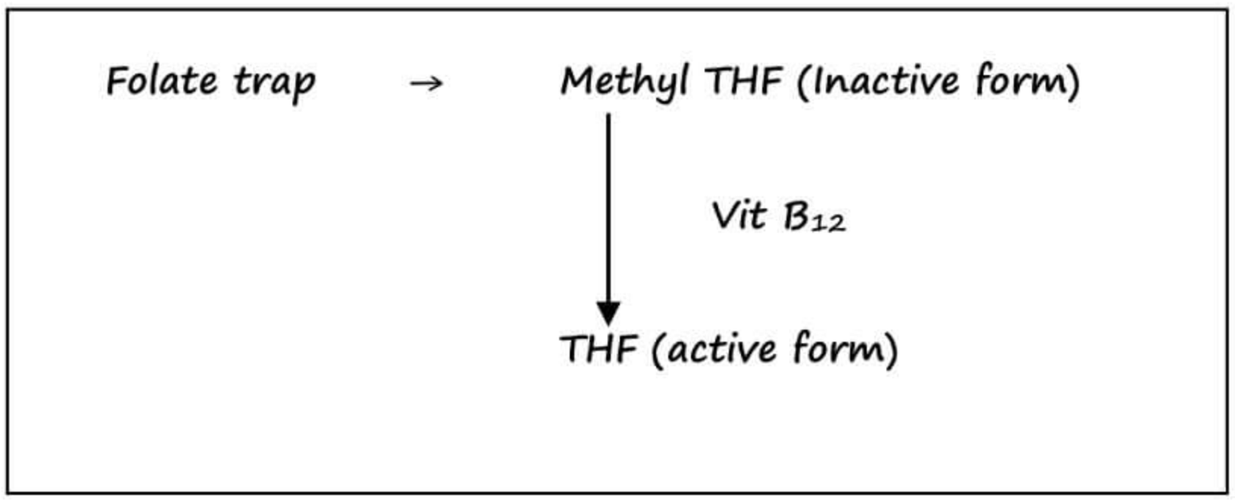
Biotin → Also known as anti egg white injury factor
Egg white contains protein avidin (raw egg)
↓
Deficiency of biotin ← Bind with biotin

1st enzyme of Gluconeogenesis Pyruvate Carboxylase requires B_7
↓
So deficiency of B_7 lead to Hypoglycaemia

B_9 (Folate)

Active – Tetra Hydro Folate

Deficiency of Folate → Megaloblastic Anaemia + Neural tube defects
→ FIGLU and Homocysteine present in Urine
Def of B_{12} → Megaloblastic anaemia + Neurological symptoms
→ L-Methyl malonic acid & Homocysteine in urine



If Vit B_{12} is deficient in body active form of folate THF is not formed & Folate trapped in methyl THF.

Its called functional deficiency of Folate.

B12-Cobalamine

Role

1. Homo cysteine to Methionine
2. Ribonucleotide Reductase → DNA synthase
3. Methyl malonyl CoA Mutase

Intrinsic factor → Glycoprotein required for absorption of Vit B_{12}

Pernicious Anaemia → Auto Immune disease

VITAMIN C

→ **Ascorbic Acid**

Active moiety – Ascorbic Acid

Non primates can synthesize Vitamin C due to the presence of L-Gulono lactone oxidase.

Source – Fresh fruits

→ Destroyed on heating

Richest source – AMLA

Role

1. Hydroxylation reaction
   → Bile acid (7α Hydroxylase)
   → Tryptophan Hydroxylase

→ Tyrosine catabolism

→ Dopamine → NE (Dopamine Hydroxylase)

2. Required for wound healing
3. Anti oxidant
4. Vit C helps in absorption of Iron
5. Meth Hb $\xrightarrow{\text{Vit C}}$ Hb

   so it prevents hemolysis

Deficiency → SCURVY

→ Collagen formation Defective

C/F

→ Bleeding gums

→ Bruises

→ Petechiae

→ Poor wound Healing

→ Anaemia (Microcytic Anaemia)

Toxicity

→ Oxalate stone formation

→ Iron overload

Richest source – Amla (Indian Gooseberry) > Guava > Cabbage >other citrus fruits

Q Which is the non-citrus fruit having vitamin C – Guava

Q Vegetable which is rich in vitamin C – Cabbage

Iron

Richest source of iron – Dried pumpkin seeds > nuts & oil seeds eg: – pistachio nuts, > Cashews

Golden rice

Genetically modified crop which is rich in two nutrients – Vitamin A & Iron.

Poor sources

Egg → Poor in carbohydrates & vitamin C

Milk → Poor in Fe & Vitamin C

Meat → Poor in Ca^{2+}

Fish → Poor in carbohydrates & Iodine specially fresh water fishes.

MINERALS

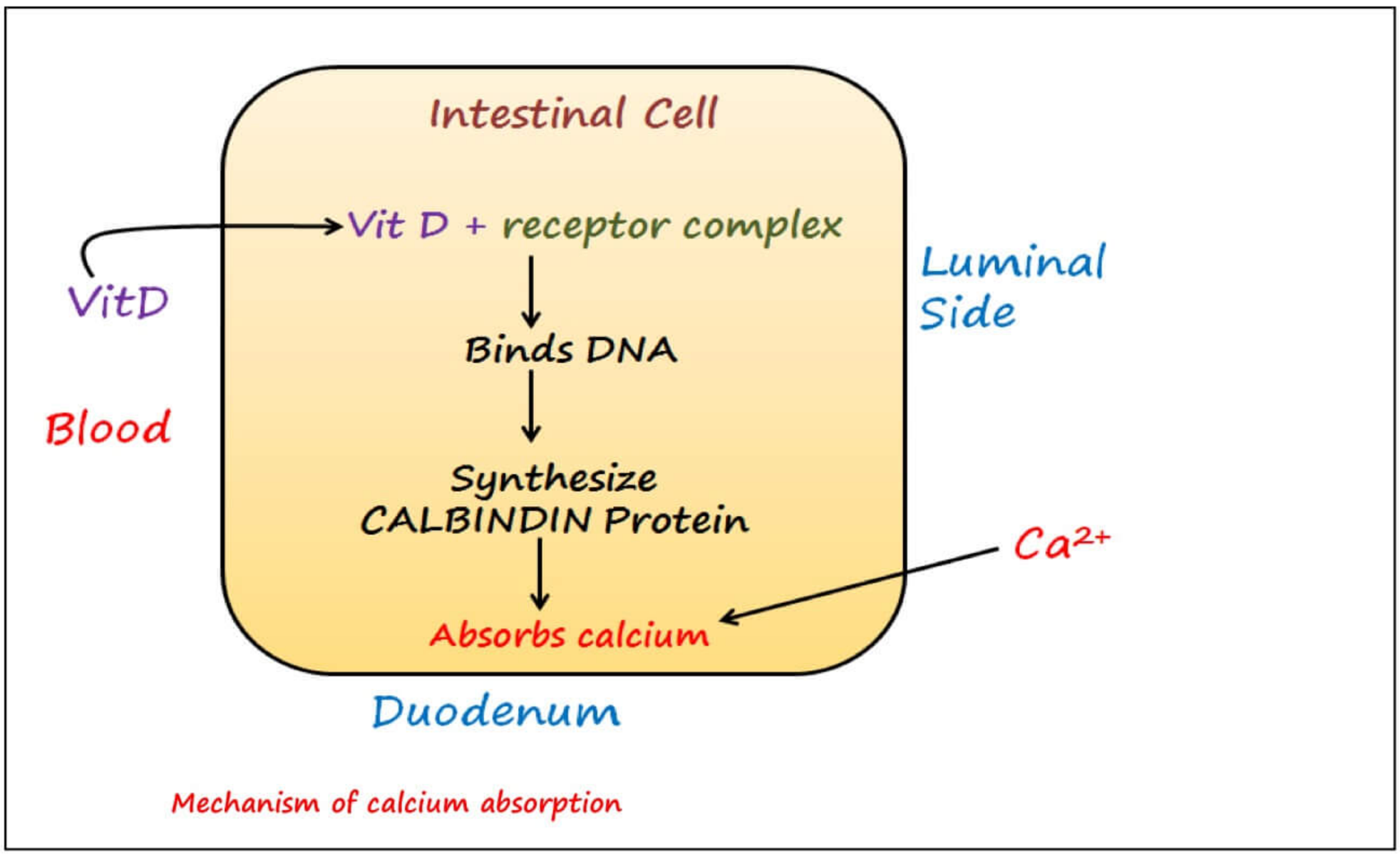
Classification of Minerals

Major elements or Macro – Minerals	Trace elements or Micro-Minerals
→ Requirement > 100 mg/day	< 100 mg/day
e.g.: → Ca, P → Na, K, Cl → Mg, S	e.g.: → Fe, Fl → Cu, Co, Cr → Mn, Mo → Zn, I, Se

CALCIUM

Major source – Milk,

- Also egg, fish, meat etc.



Mechanism of calcium absorption

→ Factors that favor Ca absorption

- Vit D, PTH, Acidity, Lysine, Arginine

→ Factors that inhibit Ca absorption

- Phytate, Oxalates, phosphates, Malabsorption syndrome

TRACE ELEMENTS

1. Iron

Sources

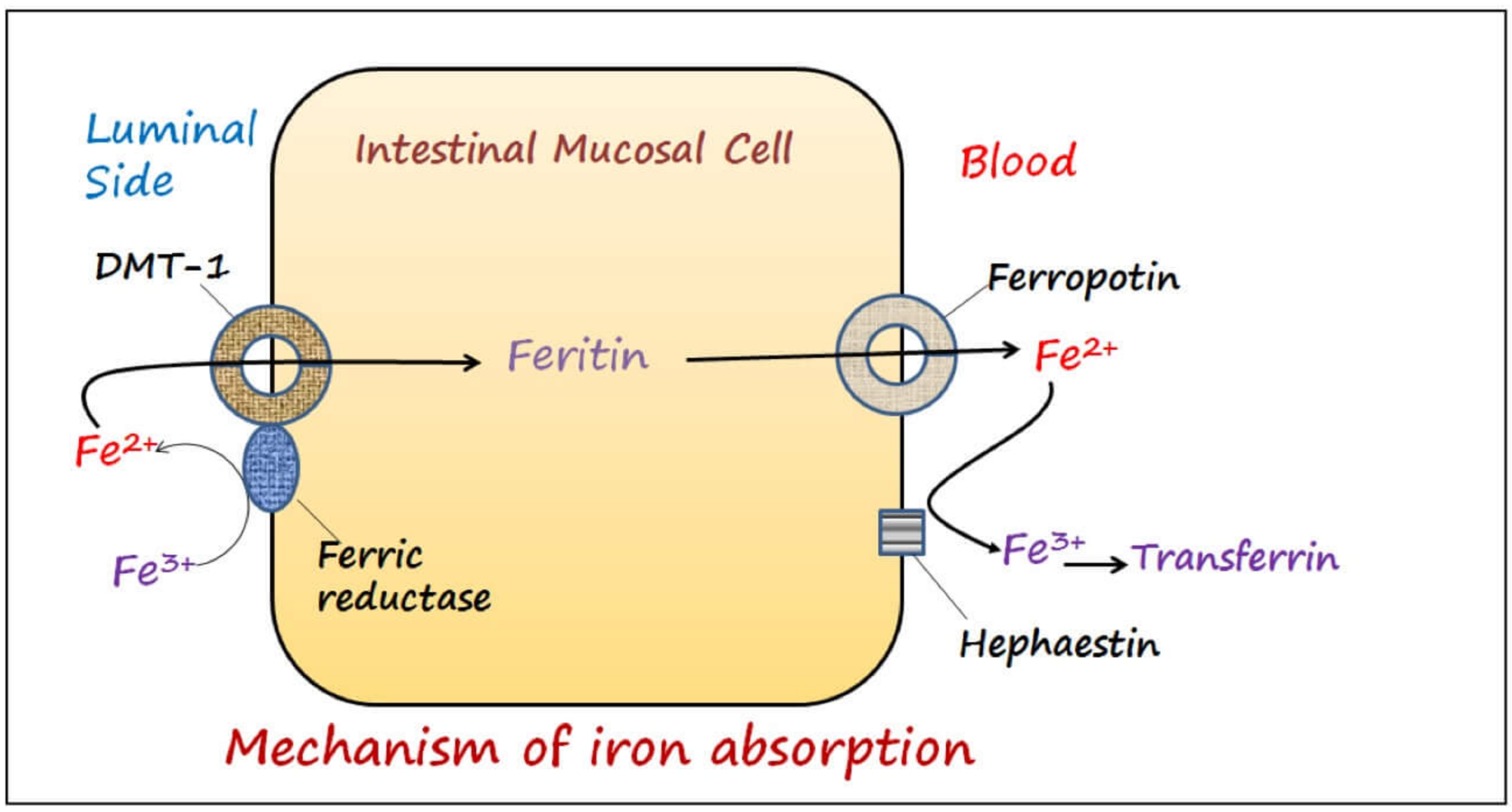
- green leafy veg, jaggery, pulses
- Milk is a poor source of Fe & Cu
- Food has Fe^{+3}, absorption occurs in Fe^{+2} form

→ Factors that ↓ abs.

- Phytates, oxalates, Phosphates, tanntes

→ Factors that ↑ abs

- Vit C, Cysteine, HCL



Mechanism of iron absorption

DMT – 1 (Divalent metal Transporter)

→ Transports Fe^{+2}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+}

→ Coupled with H^{+}

→ Expression regulated by body iron stores

Hephaestin

→ Homology with ceruloplasmin

→ A transmembrane protein, has ferroxidase activity

HEPCIDIN

→ Acute phase protein synthasized by liver

→ Inhibits ferroportin and ↓ iron absorption

→ responsible for Anemia and chronic inflammation

Mucosal block theory

→ Iron homeostasis regulated at the level of absorption, not excretion

→ Iron is a one way element

Transferrin

→ to transport iron

→ It is measured as TIBC (Total Iron Binding capacity)

Iron storage proteins

1. Ferritin	2. Hemosiderin
→ Readily mobilized form of stored iron	→ Aggregates of several ferritin → Higher iron content → Release iron more slowly

Fe def. Anemia

→ Most common nutritional problem in India

→ Hb ↓, Fe ↓, TIBC ↑

→ Microcytic hypochromic

Rx →

Iron + Vit C + Vit E

Iron overload

Excess iron → Ferritin → Hemosiderin

leads to ↓

Hemochromatosis ← excess Leads to ← Hemosiderosis

C/F ↓

1. Diabetes
2. Skin pigmentation
3. Liver cirrhosis

} Together known as **Bronze Diabetes**

Copper

Sources:

→ Nuts, cereals, green leafy veg

→ Meat, liver

Functions

1. Oxidases
2. Ceruloplasmin required for iron metabolism

→ So Cu def. anemia is also microcytic hypochromic

→ Ceruloplasmin (CP) → Acute phase protein

→ Synthesis by liver in the form of Apo – ceruloplasmin

(Apo Cp + CU → CP)

Plasma Cu transport

80% Ceruloplasmin

10% transcuperin

} tightly bound

10% Albumin → loosely bound Cu → so better Cu Transporter

Disorders related to Cu

1. Menke's kinky hair syndrome

→ XR

→ Cu deficiency, ↓ Cu in blood and urine, ↓ cp

→ Defective ATP 7A protein

→ Cu stays in intestinal cell, unable to enter blood

→ Mental retardation, hypotonia, premature birth

→ Presents in infancy, death usually by 3 years

→ As tyrosinase is affected which forms melanin so patient has grey depigmented hair

→ As lysyl oxidase affected, so defective collagen leads to brittle kinky hair

2. Wilson's hepatolenticular degeneration

→ Cu excess in body

→ ↑ Cu in blood and urine

→ Defective ATP 7B protein

→ AR

→ ↓ cp

→ Cu excess in liver hepatosplenomegaly, cirrhosis

→ Eyes

- KF rings (Kayser-Fleisher rings)
- sunflower shaped cataract

→ Hemolytic anemia, renal damage

Rx

→ penicillamine → chelates Cu

Cu toxicity → excess use of brass utensils

C/F

- Blue green stools and saliva
- hemolysis and renal damage

Selenium

→Toxicity known as selenosis

→ Accidental ingestion of metal polishing, antirust chemicals

C/F

- hair loss,

- weight loss
- falling of nails
- garlic breath odour
- dirrhoea

Se Deficiency

→ An endemic cardimyopathy → Known as Keshan's disease
  - Due to low Se content in soil
  - Usually affects women of child bearing age and children

C/F

→ weakness, eczema
→ hypertension
→ increased risk of stroke and even cancer

Fluorine

→ Strengthens bones and teeth
→ Double edged sword as deficiency and excess both are common
→ Only source is drinking water
  - If levels are < 0.5 ppm → dental caries
  - If levels are > 5 ppm → dental fluorosis
  - If levels are > 20 ppm → skeletal fluorosis

Zinc

Uses:

→ Cofactor for enzymes
→ Prevents diarrhoea
→ Stabilize Insulin
  - So deficiency leads to impaired glucose metabolism

Zn deficiency

- ↓immunity
- poor wound healing
- diarrhoea
- hypogonadism

Acrodermatitis and Enteropathica

- AR, rare
- Due to Zn deficiency

- Simultaneous diarrhea + dementia
- Inflammation around nose, mouth, anus, cheeks, elbow etc.

Chromium

→ GTF (glucose tolerance factor)
- This factor is synthesized in vivo from dietary chromium and enhances the action of insulin

→ Chromium deficiency leads to glucose intolerance.

FREE RADICALS

→ Any molecule / molecular fragments having 1 or more than 1 unpaired electrons in it's outer orbit. It has an independent existence

→ Short lived

- Gains e- from surrounding compounds & produce more dangerous free radicals

→ Can start chain of reaction

FORMATION

$$O_2 \xrightarrow[+\,4e^-]{\text{Reduced}} H_2O$$

Complete

INCOMPLETE REDUCTION

Incomplete Reduction

$$O_2 \xrightarrow{e^-} O_2^{\bullet} \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} OH^{\bullet} \xrightarrow{e^-} H_2O$$

$O_2^{\bullet}$: Super Oxide Radical (precursor)

H_2O_2: Reactive Oxygen Species (ROS)

$OH^{\bullet}$: Hydroxy Radical [most powerful / most dangerous]

Oxygen free radicals

1. $O_2^{-\circ}$ – Superoxide radical
2. OH° – Hydroxyl radical
3. HO_2° – Hydroperoxyl radical

Other free radicals

NO – Nitric oxide

- EDRF (Endothelium derived relaxation factor)

NO ⟶ Peroxy nitrite ⟶ $OH^\bullet$

How oxygen free radicals are generated

1. ETC
2. Oxidation – reduction reactions
3. Exogenous agents
    - Carbon tetra chloride
    - Ionizing radiation
    - Cigarette smoke
4. Transition metals
    - Fe, Cu
    - Cuprous & Ferrous are more reactive than cupric & Ferric
    - Fenton reaction

$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + OH^\bullet$

Uses

1. Phagocytes ⟶ OFR Resp. burst ⟶ Kill bacteria
2. Enzymes ⟶ at active site, OFR helps in catalysis

Damage – Macromolecules

1. Most susceptible – PUFA & (lipid peroxidation)

PUFAs (Lipid Peroxidation)

- ALEs – Advanced Lipid Peroxidation End Products are formed
- Chain of reactions started

2. Nucleic acids – DNA
    - Chain breaks
    - Mutations
    - Cell death
    - Cancer
3. Hb ⟶ met Hb
4. Proteins ⟶ conformational change occurs SH group oxidized
5. Carbohydrates ⟶ AGEs (Advanced Glycation End Products)

Diseases

1. Parkinsonism
2. Alzheimer's disease
3. Cancer
4. Rheumatoid arthritis
5. Ageing
6. Infertility
7. Autoimmune Diseases
8. DM
9. Atherosclerosis

Anti-oxidants

Enzymatics	Non Enzymatic
1. SOD	
2. Glutathione Peroxidase	
3. Catalase	

SOD (SUPER OXIDE DISMUTASE)

→ Carries Dismutation reactions

$$2O_2^{\bullet} + 2H^+ \xrightarrow{SOD} H_2O_2$$

Super Oxide Radical (Substrate)

H_2O_2 (ROS) – Relatively harmless

→ 3 Forms

1. Cytoplasmic – Requires Cu
2. Mitochondrial – Requires Manganese
3. Extra cellular – Requires Cu + Zn

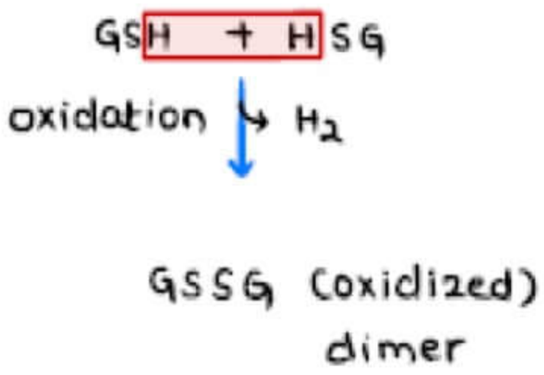
GLUTATHIONE PEROXIDASE

→ Tripeptide, reducing agent

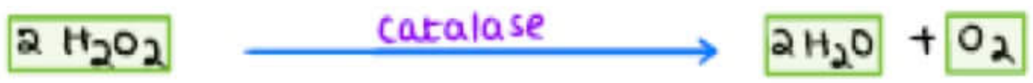
→ has 3 AA, 2 peptide bonds

- 3 AA
  - Glutamate
  - Cysteine (responsible reducing property)
  - Glycine

→ GSH



CATALASE



Non Enzymatic Anti-oxidant

1. **Vitamins:** E, A, D, C

   E – Tocopherol (most important)

2. **Thiol Antioxidants** e.g. Glutathione, Thioredoxin, Lipoic acid

1. Flavinoids
2. Melatonin
3. Selenium

4. Transferrin, ceruloplasmin
5. Co enzyme Q/Ubiquinone
6. Uric Acid

2 Classes of Anti-oxidants

1. Chain breaking anti-oxidants

- Interfere with chain propagation
- Ex.

1. Alpha- Tocopherol (E)
2. Beta carotene (A)
3. Vit C
4. SOD
5. Uric Acid

2. **Preventive anti-oxidant**

- Reduce the rate of chain initiation

1. Glutathione peroxidase
2. Catalase

Artificial Anti-oxidants

1. Propyl gallate
2. Butylated hydroxyl toluene (BHT)
3. Butylated hydroxyl anisole (BHA)

Measurement of Free Radicals

1. FOX assay (Ferrous oxidation in Xylenol)
2. Estimation of Dialdehydes

MDA – Malon Dialdehyde – Marker of Lipid Peroxidation

3. Pentane & Methane measurement

XENOBIOTICS

→ Foreign substances to which human body exposed & metabolized in body & excreted out of body safely

→ **Detoxification**

- Making the substance inactive & more soluble so as to excrete out of the body safely
- Mainly those substances thrown out of the body by kidneys

→ Major organ for Xenobiotic metabolism & for detoxification is Liver

2 PHASE REACTIONS

PHASE I	PHASE II
- Makes the compound Hydrophilic / Polar	- Makes the compound Soluble & throws out of the body via Kidneys

Phase 1 reactions

1. Hydroxylation (mc)
2. Hydrolysis
3. Oxidation
4. Reduction

Cyt P_{450} enzymes are used for hydroxylation

Cyt P_{3A4} – most common & most versatile biocatalyst

- Uses NADPH & O2
- Haem containing enzyme
- Most important enzyme of Xenobiotic metabolism
- Catalyze hydroxylation / Mono oxygenation reactions [O].
- Membrane bound enzyme present in microsomes & IMM
- Absorb light at 450 nm
- Highly inducible by their own substrates (by ↑ the rate of transcription of genes)
- Highly efficient
- Rapid development of tolerance against the drugs occurs

Ex: Epileptic drug – Phenobarbital

Dose should be increased to 3-4 time within the 1st week of starting of drug.

ISOENZYMES

- Comes from different genes (closely related)

- Broadly divided into
    - Lipid metabolizing enzymes
    - Drug metabolizing enzymes

Lipid metabolizing enzymes

- Has tight substrate specificity
- Responsible for
    - ω- oxidation of FA
    - Denaturation of FA
    - Synthesis of Steroids

Drug Metabolizing enzymes

- Has broad specificity
- Variety of drugs can be metabolized
- Responsible for metabolizing the drugs

Phase II reaction

Conjugation

- MC of all Xenobiotic reactions
- **Conjugating agents**

1. **Glutathione**
2. **Methylation** (SAM – S- Adenosyl Methionine)
3. **PAPS**
    - Phospho Adenosyl Phospho Sulphate
    - Responsible for Sulfation reactions
4. **Glycine** – responsible for conjugating
    - **Bile acids**
    - **Benzoic acids – Hippuric acid**
5. Glucuronic acid – For conjugation of Bilirubin
6. Acetylation – Require Acetyl CoA

MUSCLE ENERGY SYSTEM

In Sequence

Muscle energy systems	Power (Rate of ATP production)	Capacity (Total ATP produced)	Fuel uses
Phosphagen system	Very high	Very low	Creatine-Phosphate or Phospho-creatine
Anaerobic system/ Lactate system	High	Low	Muscle Glycogen
Aerobic system/ Mito-chondrial respiration	Low	Very High	Muscle glycogen, blood glucose, adipose tissue and intramuscular fat

Phosphagen system (First 3-10 seconds)

- Quickest source of energy
- Does not uses O2
- Does not produce lactate
- Most direct form of energy production
- But phosphocreatine is of limited supply in muscles, so depleted quickly.

Anaerobic Glycolysis or Lactate system (1-3 minutes)

- Produce ATPs using Glucose derived from Muscle Glycogen
- Lactate is formed

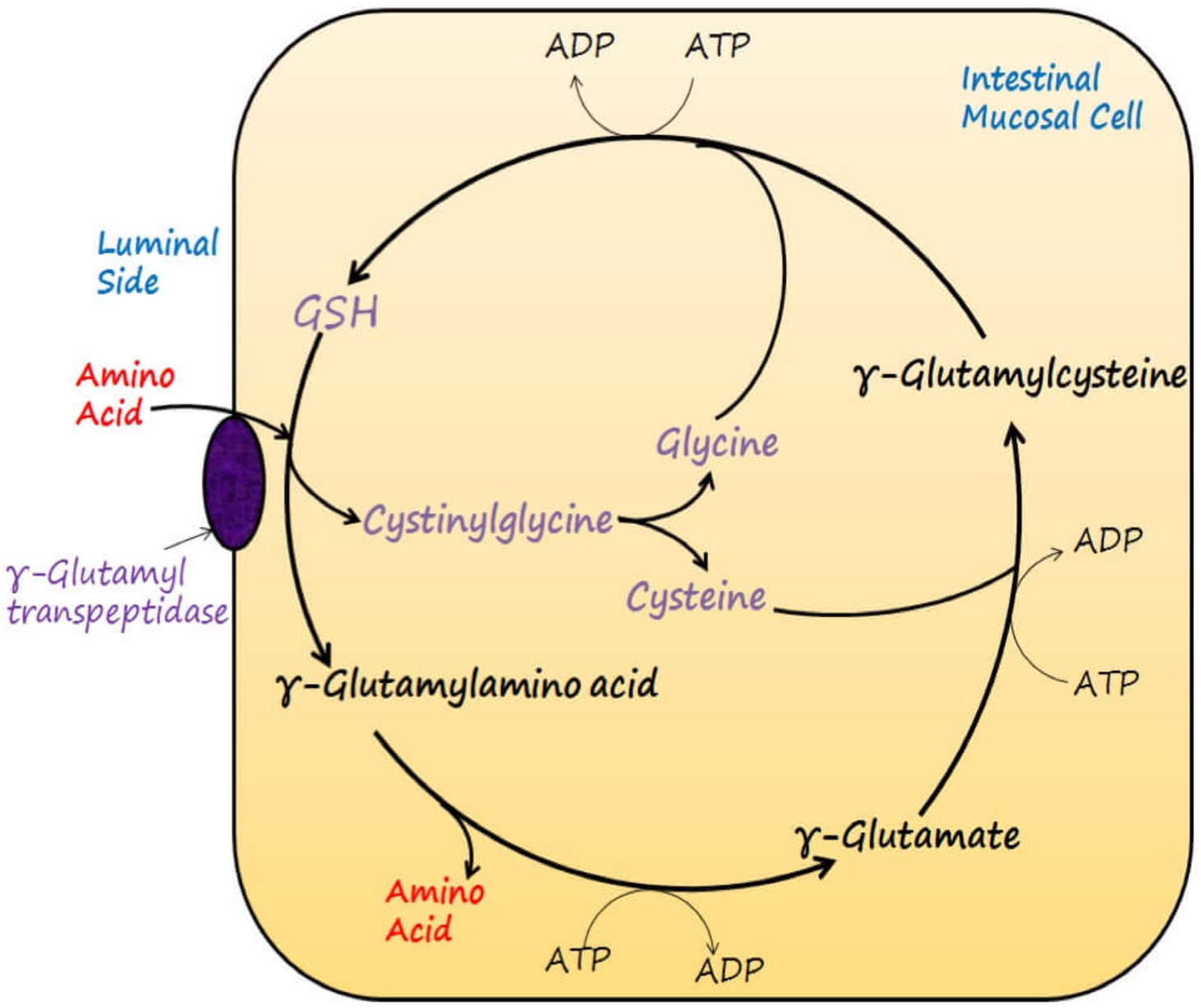
Aerobic System or Mitochondrial Respiration (After 3 minutes)

- uses carbohydrates, Fats, or Proteins to produce energy
- Slowest but most efficient in providing energy
- Uses TCA & ETC mainly
- Provides more amount of energy (32 ATP from 1 glucose).

MEISTER CYCLE (γ-GLUTAMYL CYCLE)

Glutathione is used in this cycle for:

- For entry of amino acids in to the cells
- Occurs in Intestine, Kidney and Liver



Meister Cycle

Limitations:

- Not present in all the cells
- Cannot be used for transfer of proline and OH-proline
- Cost of transfer is high (3 ATPs)