# FED - FAST CYCLE

## Fed fast cycle 00:06:36

- Well fed state :
  - 1 – 4 hrs after food

- Fasting – Stages
  - Early fasting : 4 – 16 hrs after food
  - Fasting : 16 – 48 hrs after food
  - Prolonged fasting ( Starvation ) : 2 – 5 days after food .
  - Prolonged starvation : > 5 days after food .

Well fed state

- Blood glucose level ↑ ↑ .

## Fasting 00:10:55

- Early fasting ( 4 – 16 hrs ) :
  - Blood Glucose ↓ .
  - Glycogenolysis .

- Fasting : ( 16 – 48 hrs ) :
  - 16 – 18 hrs – liver glycogen depleted .
  - Gluconeogenesis .
  - $\beta$ Oxidation of Fatty acids .

- Prolonged fasting ( 2 – 5 days ) :
  - ↓ Gluconeogenesis .
  - Hydrolysis of triacylglycerol in adipose tissue .
  - Ketone body synthesis .

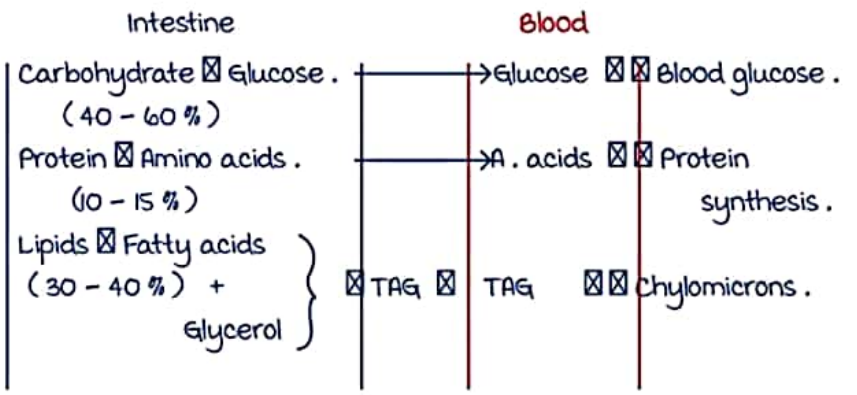
- Prolonged starvation ( > 5 days ) :
  - ↓ Fatty acid oxidation .
  - ↓ Acetyl coA .
  - ↓ Ketone body synthesis .
  - ↑ muscle proteolysis → Cachexia .

Active space

# BIOCHEMISTRY OF FED STATE

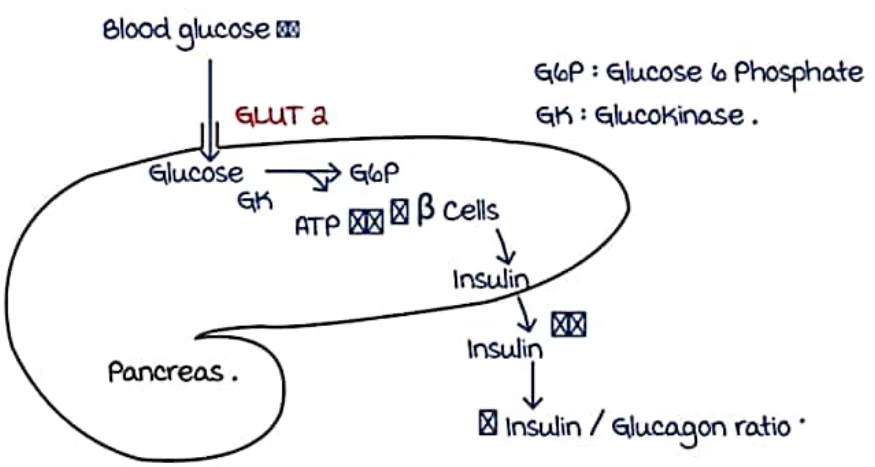
## Fed state

00:00:36




## Blood glucose regulation in fed state

00:05:40




## Action of insulin in fed state

00:18:09

Early response : ☒ Blood glucose ☒☒ Insulin

☒

☒ GLUT 4

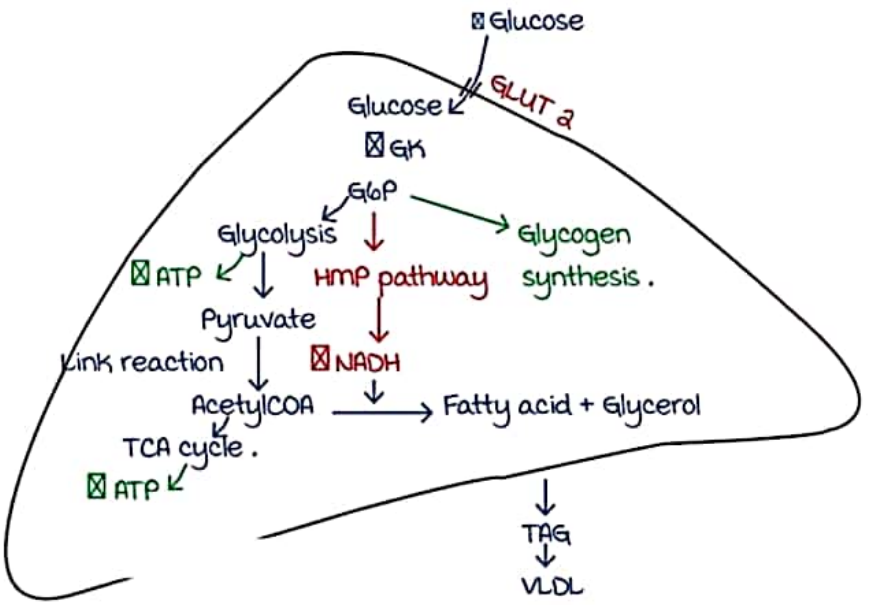
☒

☒ Glucose uptake by :
- Skeletal muscle .
- Adipose tissue .
- Heart .

Active space

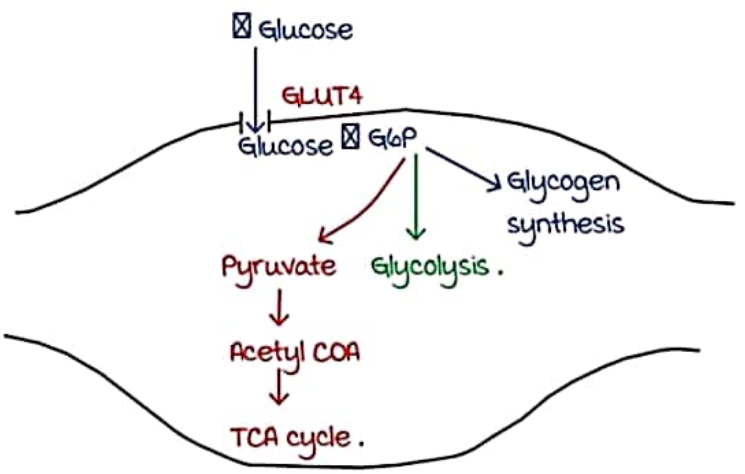
## Liver in fed state

00:11:31



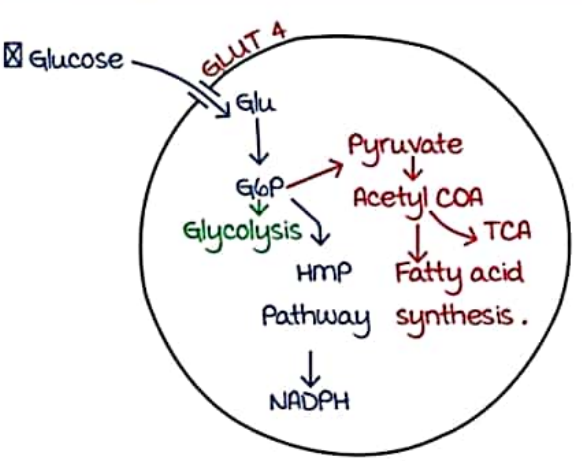

## Skeletal muscle in fed state

00:17:04




## Adipocytes in fed state

00:19:07




Active space

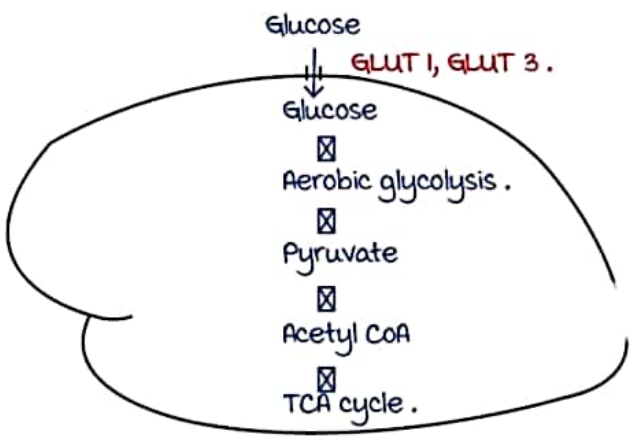
## RBC in fed state

00:22:26

- There is obligatory requirement for glucose in RBC.
- Anaerobic glycolysis takes place because of absence of mitochondria.
- Rapaport Leubering cycle.
- ☒ HMP pathway.
- GLUT 1 is present in RBC.

## Brain in fed state

00:24:13



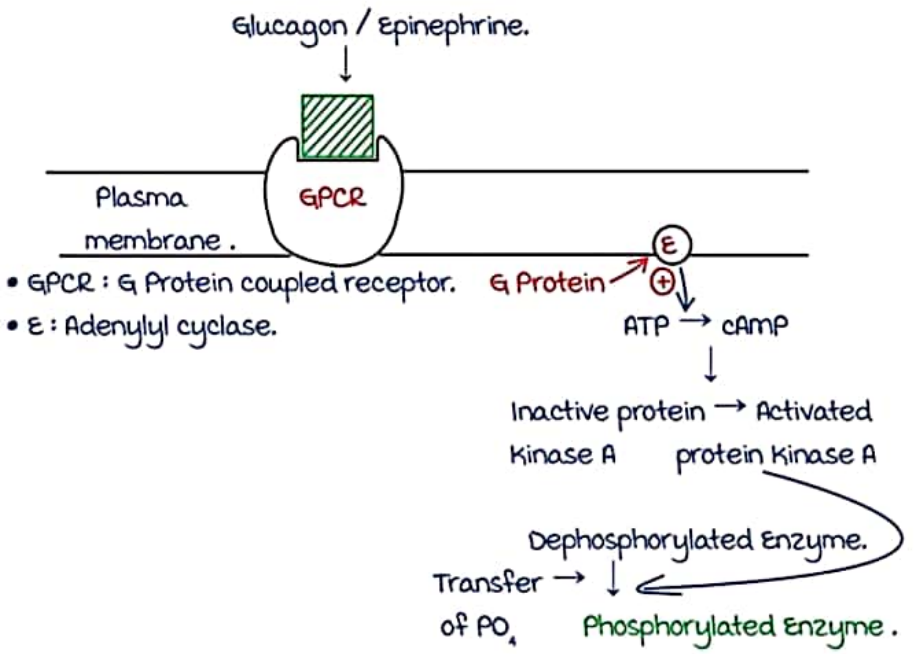

- During starvation brain depends on Glucose > Ketone bodies.

Active space

# MECHANISM OF ACTION OF GLUCAGON & INSULIN

## Mechanism of action of Glucagon / Epinephrine 00:00:21

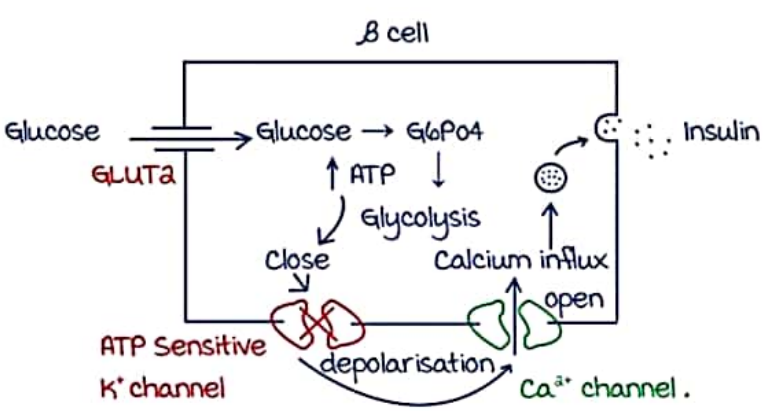
- Fasting state : <50 mg / dl .
  Glucagon released by ∝ cells.




- GPCR : G Protein coupled receptor.
- E : Adenylyl cyclase.

## Insulin- secretion 00:12:09

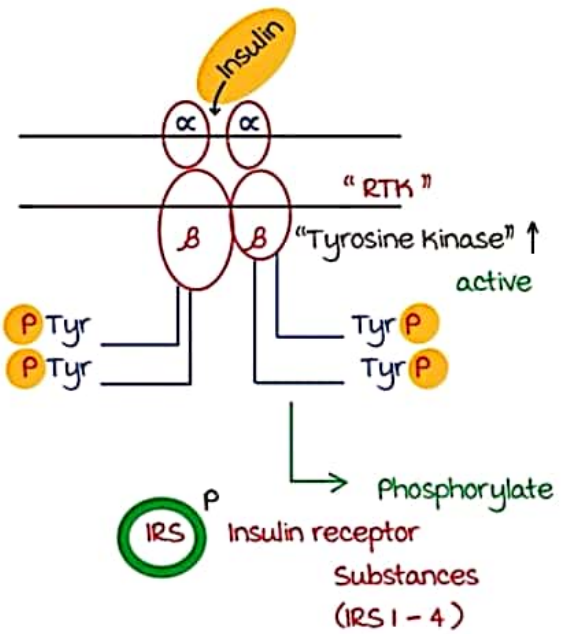
- Insulin secretion starts when blood glucose > 80 mg / dl.
- Secreted by the β cells of pancreas .




Active space

## Mechanism of action of Insulin

00:17:33




- Insulin dephosphorylates the enzymes.
- ATP —Glucagon++→ cAMP ⟶ 5' AMP → ↓ ↓ cAMP
  - Phosphodiesterase
  - Insulin ⊕
  - ↓ cAMP dependent protein Kinase A.
- Insulin favours phosphatase.
  - ↓
  - Phosphorylated enzyme. → Dephosphorylated enzyme.

Active space

# CONCEPT OF HORMONAL REGULATION

## Hormonal regulation 00:00:31

- Well fed state → Insulin.
- Fasting state → Glucagon.
- Regulation of enzyme activity → Covalent modification
  - ↓
  - Phosphorylation.
  - Dephosphorylation.

## Insulin - Well fed state

Insulin is the hormone in well fed state

Insulin
↓
Generally dephosphorylate regulatory enzymes of metabolic pathway
↓
Enzymes active under the influence of insulin active in Dephosphorylated state.

## Glucagon - Fasting state 00:04:18

Glucagon is the hormone in fasting state.

Glucagon
↓
Generally phosphorylate regulatory enzymes.
↓
Enzymes active under glucagon is active in Phosphorylated state.

## Metabolic pathways & Their active state 00:06:06

- 3 factors:
  - Well fed / Fasting.
  - Insulin / Glucagon.
  - Dephosphorylated / Phosphorylated.
- Glycolysis → Well fed → Insulin → Dephosphorylated state.

Active space

- Glycogen synthesis → Well fed → Insulin → Dephosphorylated State.
- Gluconeogenesis → Fasting → Glucagon → Phosphorylated State.
- Glycogenolysis → Fasting → Glucagon → Phosphorylated State.
- Link reaction PDH → Well fed → Insulin → Dephosphorylated State.
- Fatty acid synthesis → Well fed → Insulin → Dephosphorylated State.
- Cholesterol synthesis → Well fed → Insulin → Dephosphorylated State.

Active space

# METABOLIC FUELS & METABOLIC PATHWAYS IN DIABETES MELLITUS

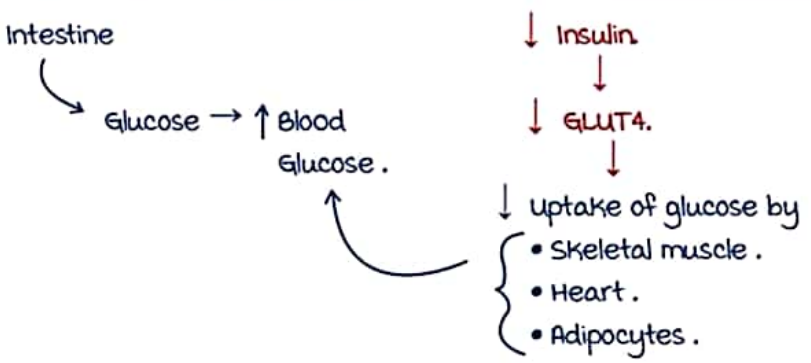
## Metabolic fuels

00:00:44

| Organs. | Fed state | Early fasting / fasting | Prolonged fasting, starvation . |
|---|---|---|---|
| • Liver . | Glucose > FFA | FFA / Glucose. | Amino acids / FFA (never use KB) |
| • Heart . | FFA > Glucose | FFA / Glucose | Ketone bodies. |
| • Brain . | Glucose | Glucose | • Glucose (80%)<br>• Ketone bodies (20%). |
| • Skeletal muscle . | Glucose > FFA. | FFA > Glucose . | • FFA<br>• Ketone bodies.<br>( Slow twitch muscle ). |
| • RBC | Glucose | Glucose | Glucose. |
| • Adipo-cytes . | Glucose > FFA | FFA > Glucose | • FFA<br>• Ketone bodies . |

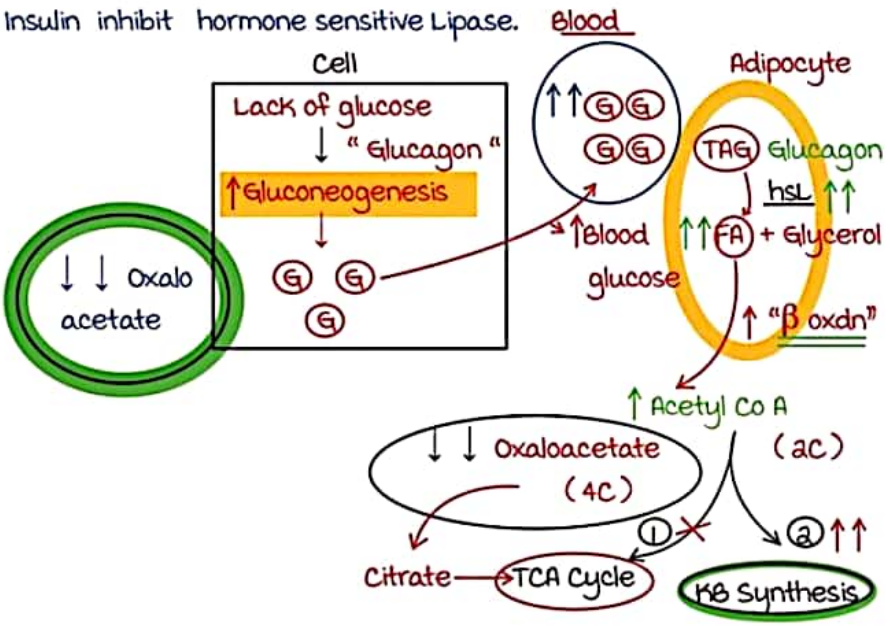
## Metabolic pathways in DM

00:16:05




Active space

• Insulin inhibit hormone sensitive Lipase.




HSL-hormone sensitive lipase
TAG-triacylglycerols

Active space

# CLASSIFICATION OF ENZYMES

International Union of Biochemistry and Molecular Biology (IUBMB) → 7 classes of enzymes (6 classes + 1 newly added)

Enzyme classes :

I – Oxidoreductase
II – Transferase
III – Hydrolase
IV – Lyase
V – Isomerase
VI – Ligase
VII – Translocase

## Class I - Oxidoreductases 00:04:24

Catalyse oxidation and reduction reactions .

Subclasses :

1. Dehydrogenase
2. Oxidase
3. Oxygenase
4. Peroxidase eg : Glutathione peroxidase .
5. Catalase
   - Present in peroxisomes
   - Scavenge $H_2O_2$
6. Reductase
   - require NADPH

## Subclasses of oxidoreductase 00:08:04

Dehydrogenase

Remove hydrogen from one substrate and donate to another substrate

↓

acceptor substrate usually is : B -complex vitamin

eg : 1. $NAD^+ \longrightarrow NADH$ (m.c)

2. $FAD^+ \longrightarrow FADH_2$

in : Succinate dehydrogenase

Acyl CoA dehydrogenase

Active space

3. $NADP^+ \longrightarrow NADPH$
   - 1st two reactions of HMP pathway.
   - Cytoplasmic Isocitrate dehydrogenase
   - Malic enzymes

Oxygenase

Directly add oxygen to the substrate

| 1 atom added | Both atoms of $O_2$ added |
|---|---|
| Monooxygenase | Dioxygenase |
| (Mixed function oxidase) | eg: |
| • includes most hydroxylases and cytochromes | 1. Tryptophan dioxygenase (Pyrrolase) |
| eg: Tyrosine hydroxylase | 2. Homogentisate dioxygenase |
| Phenylalanine hydroxylase | |
| Tryptophan hydroxylase | |
| 7-α hydroxylase | |
| Cytochromes | |

Oxidases:

Remove hydrogen from substrate and donate to oxygen.

eg: Cytochrome C oxidase (Complex IV)
Monoamine oxidase

## Class II - Transferase

00:15:49

Transfer the functional group from one substrate to another

A. Transaminase
Transaldolase
Transketolase
Transmethylase
Any enzyme with 'Trans' in the name

B. Kinases.
- Transfer of $PO_4$ from organic $PO_4$ molecule (ATP)

eg: 1. Glucose $\xrightarrow{\text{Glucokinase}}$ Glucose-6-$PO_4$

1 ATP → 1 ADP

(Require $Mg^{2+}$)

reaction catalysed by glucokinase

↓

type of hexokinase (add $PO_4$ to 6th position of a hexose sugar)

Active space

a. Fructose – 6 – $PO_4$ —(Phosphofructokinase; 1 ATP → 1 ADP)→ Fructose 1, 6 Bis $PO_4$

c. Phosphorylase
- transfer inorganic $PO_4$ group

eg : Glycogen —(Glycogen phosphorylase; Pi)→ Glucose – 1 – $PO_4$

## Class III - Hydrolase

00:22:36

Breaks covalent bonds ( C-C, C-N ,C-O) by adding water .

↓

Covalent bonds present in : Primary structure of macromolecule .

| Macromolecule | Covalent bonds | Hydrolase |
|---|---|---|
| Carbohydrate | Glycosidic bond | Amylase, Maltase, Sucrase |
| Protein | Peptide bond | Peptidases<br>Proteases<br>(Trypsin, Chymotrypsin, Elastase) |
| Nucleic acid | Phosphodiester bond | Nucleases<br>( Endonucleases and Exonucleases ) |
| Lipids | Ester bond | Esterase / Lipases |

Other hydrolases :
- Arginase
- Phosphatases

Glucose -6-$PO_4$ —(Glucose – 6 – Phosphatase; $H_2O$ → Pi)→ Glucose

Active space

## Class IV - Lyase

00:28:47

1. Break covalent bond without adding water .

eg : A. Fructose - 1, 6 - bis $PO_4$ ⟶ Glyceraldehyde- 3 - $PO_4$ + DHAP

(6C) (3C) (3C)

Enzyme → Aldolase

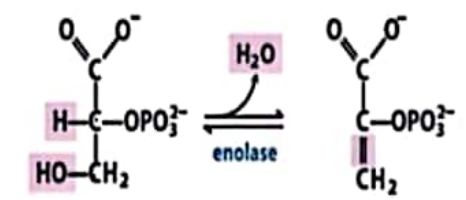
B. Any enzyme with Lyase in the name
- HMG CoA lyase
- Argininosuccinate lyase
- ATP Citrate lyase .

2. Break / make double bond by atom elimination .

eg : Enolase

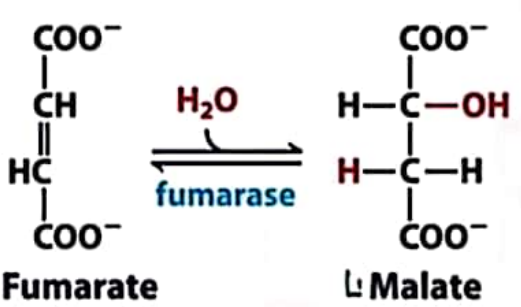
2 - Phosphoglycerate ⇌ Phosphoenolpyruvate + $H_2O$

$Mg^{2+}$ dependant .




$\Delta G'^{\circ} = 7.5$ kJ/mol

- Fumarase : Fumarate ⟶ L - Malate




Active space

- Aconitase
- Decarboxylase

Simple

- Histidine ⟶ Histamine
- Glutamate ⟶ GABA
- Tryptophan ⟶ Tryptamine

These reactions :
- Liberate $CO_2$
- Co-enzyme ⟶ PLP

Oxidative decarboxylation

eg :
- Pyruvate dehydrogenase
- $\alpha$ - Ketoglutarate dehydrogenase
- Branched chain Ketoacid dehydrogenase

In these reactions :
$NAD^+ \longrightarrow NADH$
∴ belong to class I oxidoreductases .

## Class V - Isomerase 00:36:22

Catalyse isomerisation reactions

Glucose - 6 - $PO_4$ $\overset{\text{Phosphohexose isomerase}}{\rightleftharpoons}$ Fructose - 6 - $PO_4$

Glyceraldehyde - 3 - $PO_4$ $\overset{\text{Phosphotriose isomerase}}{\rightleftharpoons}$ DHAP

D Glucose $\overset{\text{Racemase}}{\rightleftharpoons}$ L Glucose

Glucose - 6 - $PO_4$ $\overset{\text{Phosphoglucomutase}}{\rightleftharpoons}$ Glucose - 1 - $PO_4$

In this reaction :
Intramolecular transfer of $PO_4$ occurs

## Class VI - Ligase 00:39:25

- Join two substrates
- Makes a covalent bond
- ATP is required

eg : 1. Carboxylase
2. Synthetase

Active space

1. Carboxylases

In all reactions :

- $CO_2$ added
- ATP required
- Coenzyme : Biotin
- Catalysed by ligases

eg :

Pyruvate (3C) —Pyruvate carboxylase→ Oxaloacetate (4C)

Propionyl CoA (3C) —Propionyl CoA carboxylase→ methylmalonyl CoA (4C)

Acetyl CoA (2C) —Acetyl CoA carboxylase→ Malonyl CoA (3C)

Biotin independant carboxylation reaction :

- Carbamoyl Phosphate Synthetase I and II
- Gamma carboxylation
- Addition of 6$^{th}$ carbon of purine ring (AIR Carboxylase)
- Malic enzymes

2. Synthetase

- Require ATP

eg : Glutamine synthetase
Carbamoyl $PO_4$ Synthetase

NOTE:-Synthases : do not require ATP
Belongs to any class other than class VI
(generally class IV)

## Class VII - Translocase 00:45:21

- Transfer molecules and ions across the membrane
- Earlier called ATPases
- Belonged to class III previously

Active space

# CHEMISTRY OF CARBOHYDRATES

## Definition of carbohydrates 00:02:03

- Hydrates of carbon
- General formula → $C_n(H_2O)_n$
  n → No. of carbon atoms
- These are aldehyde or keto derivatives of polyhydroxy alcohol
- Simplest carbohydrate → Glyceraldehyde & Dihydroxyacetone

## Classification of carbohydrates 00:10:07

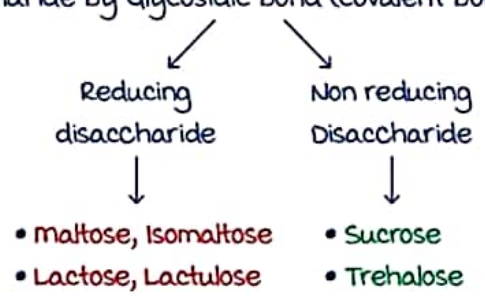
- Monosaccharide : Single sugar unit
- Disaccharide : Two potential sugar units
- Oligosaccharide : 3 – 10 sugar unit
- Polysaccharide : > 10 sugar unit

## Monosaccharide 00:13:21

| No. of carbon atoms | Aldoses | Ketoses |
|---|---|---|
| • 3 → Trioses | Glyceraldehyde | Dihydroxyacetone |
| • 4 → Tetroses | Erythrose | Erythrulose |
| • 5 → Pentoses | Ribose<br>Xylose<br>Arabinose | Ribulose<br>Xylulose |
| • 6 → Hexoses | Glucose<br>Galactose<br>Mannose | Fructose |

## Disaccharides 00:18:08

- Joining a monosaccharide by Glycosidic bond (covalent bond)




Active space

Reducing disaccharides:

- Maltose: Glucose + Glucose
  α 1→ 4 glycosidic linkage
- Isomaltose: Glucose+Glucose
  α 1 → 6 glycosidic linkage
- Lactose : Galactose+Glucose
  β 1 → 4 glycosidic linkage

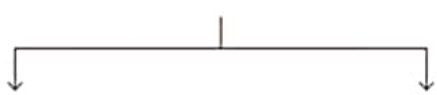
Lactulose : Galactose + Fructose
Synthetic disaccharide
Osmotic Laxative

Non reducing disaccharide:

- Sucrose : Glucose+Fructose
  α 1 β 2 linkage
  Cane sugar

Trehalose : Glucose + Glucose
α 1 → 1 linkage

## Polysaccharides 00:33:48



| Homopolysaccharide | Heteropolysaccharide |
|---|---|
| Homoglycan | Heteroglycan |
| One type of monomer | more than 1 type of monomer |
| Glycogen | GAG |
| Starch | Agarose |
| Chitin | Pectin |
| Cellulose | |
| Inulin | |

## Biochemistry significant carbohydrates: glycogen 00:36:18

Glycogen :

- made of α D Glucose
- Branched polymer

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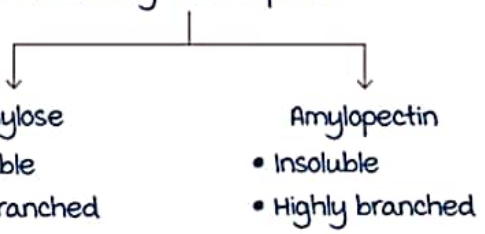
- Storage form of carbohydrate in animals
- Animal starch
- Straight chain → α 1, 4 linkage
- At Branches → α 1, 6 linkage
- In liver & muscle it occurs as β particle
- 1β particle → 60,000 glucose residues

## Starch 00:40:11

- Monomer of β D Glucose
- Storage form of carbohydrate in plants




## Cellulose 00:41:37

- Major dietary fibre
- β D Glucose
- β 1 → 4 linkage
- Human lacks the enzyme cellulase to hydrolyse the β 1-4 linkage

## Chitin 00:42:37

- Exoskeleton of Insects
- Homopolysaccharide
- N - Acetyl glucosamine(monomer)

## Inulin 00:43:24

- Homopolysaccharide found in Dahlia,chicory roots and garlic
- Polymer of β D Fructose
- Fructosan
- Used to assess GFR → Inulin clearance test

Active space

## Dextran vs Dextrin 00:44:37

| Dextran | Dextrin |
|---|---|
| • Homopolysaccharide of glucose | • Hydrolytic product of starch |
| • Plasma volume expander | • Oligosaccharide |

## Pectin 00:47:03

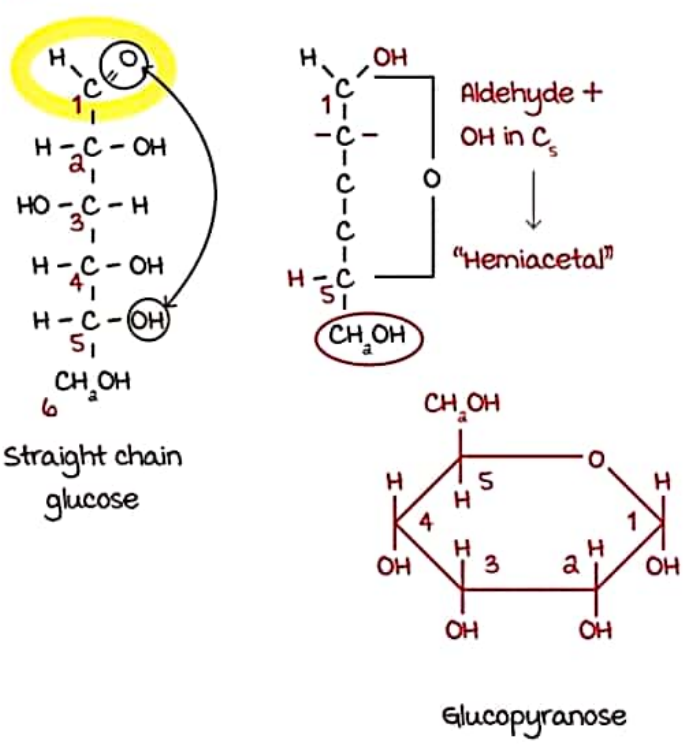
- Heteropolysaccharide.
- Galacturonic acid with arabinose,galactose.
- Dietary fibre

- Lectin: polypeptide
  Agglutinin- binds to specific glycosyl residues.

## Ring structure of monosaccharides 00:49:38

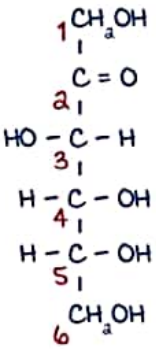
- Ring structure of glucose:

Ring structure of monosaccharides

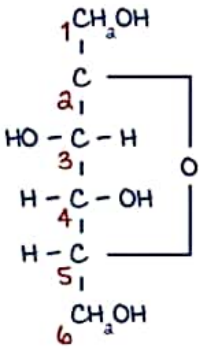



Active space

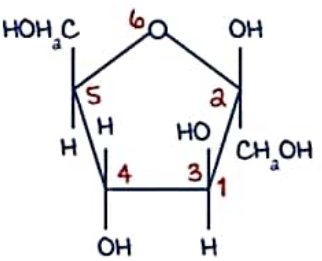
- Ring structure of fructose




Fructose




Ketogroup + OH
= "Hemiketal"




Furanose

## Oxidation of sugars 01:02:50

- Sugars → Aldehyde / Keto → Oxidised → Sugar acid
  - Reduced
    - Sugar alcohol

Glucose oxidase method

- Glucose —($C_1$ oxidised)→ Gluconic acid
- enzymatic method for estimation of blood glucose.
- Glucose —($C_6$ oxidised)→ Glucuronic acid → conjugation of Bilirubin, synthesis of GAG and proteoglycan
- Glucose —($C_1$ & $C_6$ oxidised)→ Saccharic acid → Glucosaccharic acid
- Galactose —($C_1$ & $C_6$ oxidised)→ Galactosaccharic acid (mucic acid)

## Reduction of sugars 01:10:09

- Glucose —(Aldose reducase)→ Sorbitol
  - → Diabetic cataract.
    - Sorbitol pathway/polyol/ pathway

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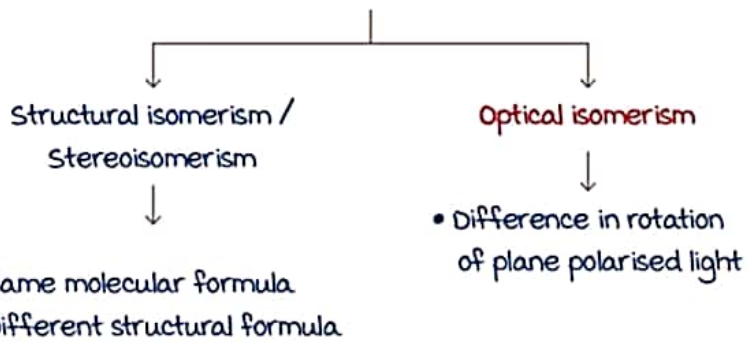
- Galactose → Galactitol/Dulcitol
- Fructose → Sorbitol and Mannitol
- Mannose → Mannitol
  ↳ ↓ICT

Active space

# ISOMERISM IN CARBOHYDRATES

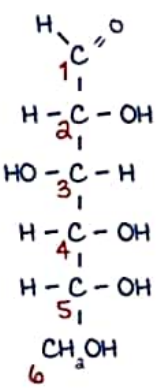
## Isomerism: Types

0:00:40

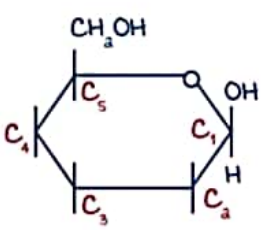



## Asymmetric carbon atom

- 4 valencies of carbon atom occupied by 4 different groups




Glucose




Glucose

- In straight chain 4 asymmetric carbon atoms → $C_2$, $C_3$, $C_4$, $C_5$
- In ring form 5 asymmetric carbon atoms → $C_1$, $C_2$, $C_3$, $C_4$, $C_5$
- In fructose:
  - Straight chain asymmetric carbon atoms → $C_3$, $C_4$, $C_5$
  - Ring form → $C_2$, $C_3$, $C_4$, $C_5$
- Le Bel- Van't Hoff rule:
  - Number of structural isomers → $2^n$
  - n → Number of asymmetric carbon atoms

Active space

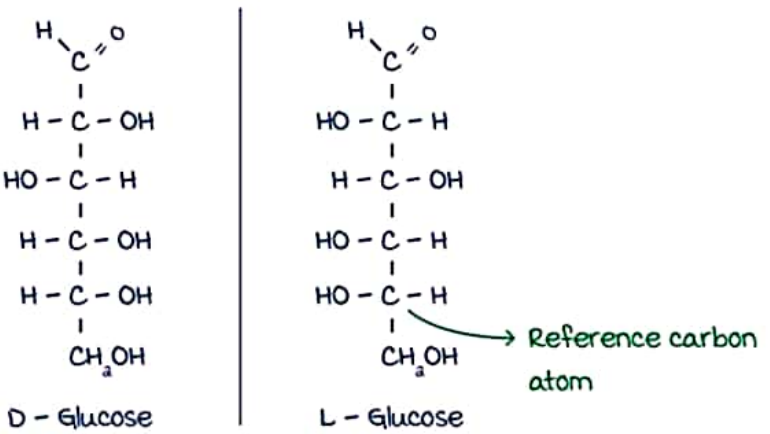
## Structural isomers of monosaccharides 00:13:14

- D and L isomerism
- Anomerism
- Epimers

## D and L Isomerism / Enantiomers 00:13:57

- Difference in orientation of H and OH groups. In the penultimate carbon atom / Reference carbon atom

Mirror images



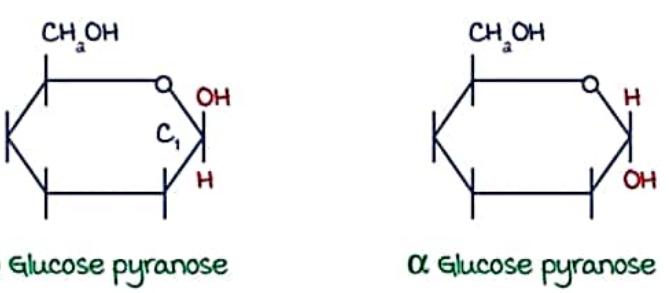

## Anomerism 0:22:57

- Isomerism at Functional carbon atom
- In aldoses at → $C_1$
- In Ketoses at → $C_2$

Examples of Anomerism

Eg :- α Fructose & β Fructose

α Glucose & β Glucose



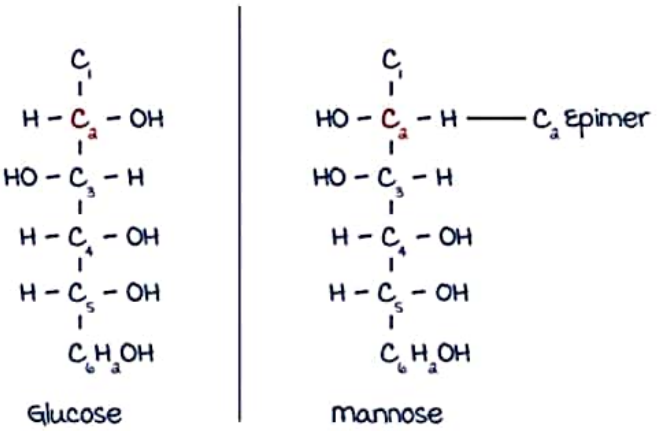

Active space

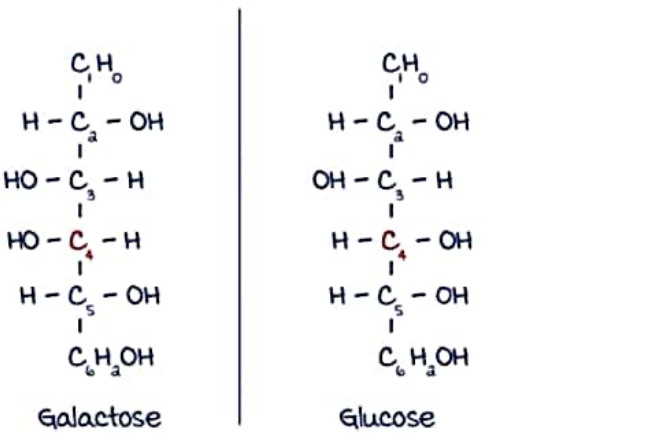
## Mutarotation 00:26:53

- Change in the rotation of plane polarised light with time
- α Glucose → + 112°
- β Glucose → + 19°
- α Glucose $\underset{\text{Equilibrium}}{\rightleftharpoons}$ β Glucose ⟶ + 52°
- Racemic mixture: • Mixture of α and β anomers
  • So that net rotation is Zero

## Epimerism 0:29:34

- Isomerism at a single carbon atom other than functional and penultimate carbon atom
- Examples:
  - At $C_2$ → Glucose & Mannose
  - At $C_3$ → Glucose & Allose
  - At $C_4$ → Glucose & Galactose




Active space

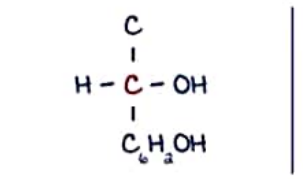
## Diastereoisomers 00:34:12

- Differ in the orientation of H & OH groups > 1 carbon atom other than the penultimate & the anomeric carbon atom
- Not mirror images
- Eg: D. Mannose & D. Galactose

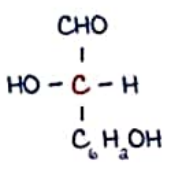
## Optical isomerism 00:37:41

- Ability to rotate the plane polarised light
- Rightward / Clockwise rotation → Dextrorotation ('D' or '+')
- Leftward /Anticlockwise rotation → Levorotation ('L' or '–')

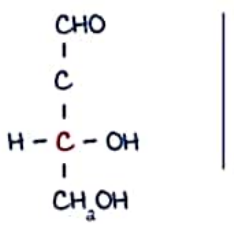
D and L isomerism → Examples.



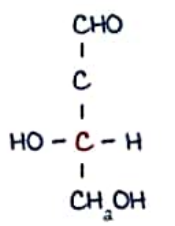

D – Glyceraldehyde




L – Glyceraldehyde



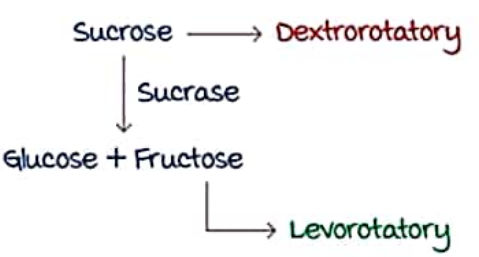

D – Erythrose




L – Erythrose

## Invert sugar & invertase 00:40:37

- Sucrose is an invert sugar
- Sucrase → Invertase




Active space

## One liners of isomerism

00:44:01

- Monosaccharide with single asymmetric carbon atom → Glyceraldehyde
- Ketoses have 1 asymmetric carbon less than corresponding aldoses
- Carbohydrate with no asymmetric carbon atom → DHA
- Amino acid with no asymmetric carbon atom → Glycine
- Most predominant form of glucose → β D glucopyranose
- Most carbohydrate exist in → D form
- Most Aminoacids exist in → L form

Active space

# GLYCOSAMINOGLYCANS

## Glycosaminoglycans (GAG) 00:00:12

- Also called as Mucopolysaccharides
  ↓
  seen in mucin
- These are long unbranched heteropolysaccharide with repeating disaccharide units
- Repeating disaccharide unit:

Amino sugar + Acidic sugar

Amino sugar → Glucosamine, Galactosamine

Acidic sugar → Glucuronic acid, Iduronic acid

## Properties of Glycosaminoglycans 00:03:31

- Negatively charged (polyanions)
- Repel each other → mucus secretions are slippery
- Absorb water (due to negative charge) → Abundant in ECM
- Resilient → synovial fluid, vitreous humor

## Chondroitin sulphate 00:10:07

- Most abundant GAG
- Most heterogeneous GAG
- Mainly present in cartilage, bones, CNS
- Responsible for compressibility of cartilage and weight bearing bones

## Keratan sulphate 00:13:02

- N – Acetyl Glucosamine + Galactose
- Keratan sulphate I → 1st isolated from cornea
- Keratan sulphate II → Cartilage, Loose connective tissue
- Responsible for corneal transparency
- GAG with no uronic acid

Active space

## Dermatan sulphate & Heparan sulphate 00:15:08

Dermatan sulphate :

- Widely located GAG
- Most abundant GAG in Skin

Heparan sulphate :

- Glucosamine + Glucuronic acid
- Seen in plasma membrane receptors
- Lipoprotein Lipase anchored by Heparan sulphate
- Present in Basement membrane of renal glomeruli
  - → Charge selectiveness of renal glomeruli
- Present in synaptic & other vessels

## Hyaluronic acid & Heparin 00:18:29

Hyaluronic acid :

- N – Acetyl Glucosamine + Glucuronic acid
- No sulphate group
- Not covalently attached to protein
- Helps in cell migration:
  - Tumor cell metastasis
  - morphogenesis
  - Wound repair
- Also found in Bacteria

Heparin :

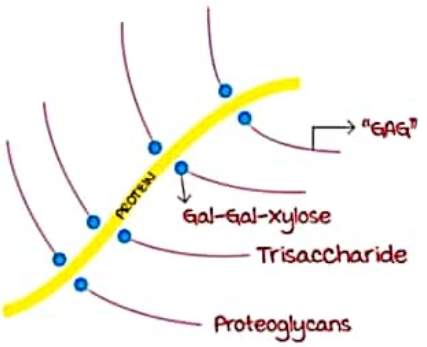
- Glucosamine + Iduronic acid
- Anticoagulant
- Only intracellular GAG
- Present in mast cells, Lung

## Proteoglycans & glycoprotein 00:21:45

- Proteoglycans: protein (5%) + GAG (95%)
- Glycoproteins: carbohydrate (5%) + protein (95%)
- Structure: Bottle brush shape

Active space

- Structure: Bottle brush shape




## Synthesis and degradation of Glycosaminoglycans 00:25:08

- Synthesis :
  - Occurs in Endoplasmic reticulum & Golgi apparatus
- Degradation :
  - Occurs in Lysosomes
  - Defect in degradation leads to Mucopolysaccharidoses (MPS)
  - MPS belongs to group of Lysosomal storage disorders

Active space

# MUCOPOLYSACCHARIDOSIS

## Mucopolysaccharidosis 00:00:08

- Defect in degradation of GAG(Glycosaminoglycans) in Lysosomes
- Lysosomes → Lack of enzyme (Hydrolases)

↓

Intralysosomal accumulation of GAG / MP (mucopolysaccharidosis)

- It belongs to group of Lysosomal storage disorder

## Hurler's disease (MPS 1H) 00:04:54

- Biochemical defect : α – L – Iduronidase deficiency
- Accumulation of Heparan sulphate & Dermatan sulphate
- Gene defective: 'IDA' gene
- Rapid progression
- Clinical features:
  - Abdominal protrusion
  - Umbilical hernia
  - Short stature
  - Coarse facial features
  - Depressed nasal bridge
  - Claw hand
  - Frontal bossing
  - Steamy cornea
  - Bullet shaped middle phalanx
  - Reilly body inclusions in the Leucocytes

## General clinical features of MPS 00:09:14

- Coarse facial features
  - Frontal bossing
  - Corneal clouding*
  - Depressed nasal bridge
  - Gingival Hypertrophy
  - Large tongue

Noisy breathing

Ear infection → Hearing loss

URTI → copious nasal discharge

Active space

- Skeletal System:
  - Skeletal dysplasia
  - Dysostosis multiplex
  - Short stature*
  - Bullet shaped middle phalanx*
  - Clawing of hand*
- Visceral manifestation
  - Visceromegaly*: • Protuberant abdomen
    • Umbilical Hernia
  - Inguinal hernia*
- Heart:
  - Valvular Heart disease
  - Mitral and Aortic reauraitation.
- Leukocytes*
  - Reilly body inclusions
- Intellectual disability*

* → Not commonly seen in all MPS

( Impaired degradation of Heparan Sulfate → Mental retardation )

## Scheie's disease (MPS 1S) 00:16:39

- Biochemical defect: Partial deficiency of α - L - Iduronidase
- 'IDA' gene defect
- Clinical features similar to Hurlers except there is no Intellectual disability
- normal intelligence
- Accumulation of Dermatan Sulphate

( Impaired degradation of Dermatan Sulfate→ Mesenchymal abnormality )

## Hunters disease (MPS II) 00:20:50

- Only males are affected
- Slow progression
- X linked recessive disorder
- Clear vision, no corneal clouding
- Biochemical defect: L- Iduronate sulfatase
- Gene defect: 'IDS'
- Accumulation of Heparan sulphate & Dermatan sulphate

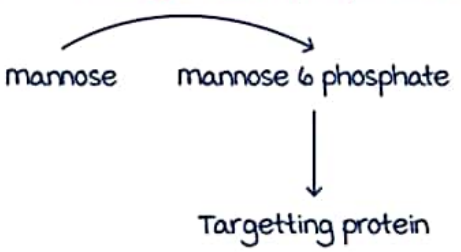
Active space

## General characteristics of other MPS 00:26:15

- All MPS are autosomal recessive except Hunters (XLR)
- Mc type of MPS: Sanfilippo > Hunter & Hurler
- MPS with no Intellectual disability:
  - Scheie disease (MPS IS)
  - Morquio disease (MPS IV)
  - Maroteaux Lamy disease (MPS VI)
- MPS with no corneal clouding: Hunters & Sanfilippo
- MPS with no visceromegaly: Morquio disease
- Skeletal deformity associated with all MPS: Dysostosis Multiplex
- MPS with no leukocyte inclusion: Morquio disease

## I (Inclusion )- cell disease 00:28:24

- Protein targetting disorder to lysosomes
- Resembles MPS but severe form
- Enzyme defect → N Acetyl Glucose phosphotransferase




- This leads to deficiency of Mannose 6 phosphate (Targetting protein) & accumulation of mucopolysaccharides in the Lysosomes

## Enzyme replacement therapy 00:36:40

- MPS I (Hurlers disease ) → Aldurazyme
- MPS II (Hunter) → Elaprase
- MPS VI (Maroteaux Lamy disease) → Naglazyme

## Natowicz syndrome 00:37:51

- Genetic defect in hyaluronidase
- Hyaluronic acid accumulation in joints
- Also called as MPS IX

# GLUCOSE TRANSPORT

Types of glucose transporters

- Sodium dependent Glucose transporters: SGLT
- Sodium independent Glucose transporter: GLUT

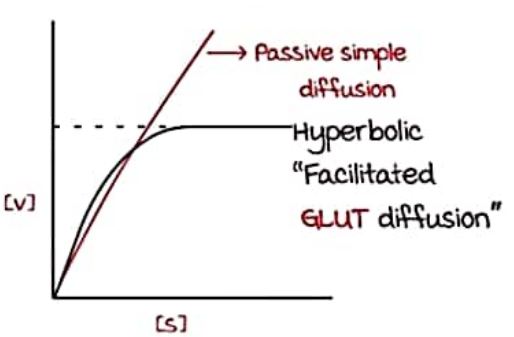
## SGLT

00:01:36

- Sodium dependent
- Secondary active transport
- Unidirectional
- Transport of glucose against concentration gradient
- Types:
  - SGLT 1 :
    - Intestine (Luminal side)
    - Renal tubules
  - SGLT 2 :
    - Renal tubules
    - Defect in SGLT 2 → Renal glycosuria

## GLUT

- Sodium independent
- Facilitated carrier mediated → Passive process
- Moves along concentration gradient
- Ping pong mechanism
- Bidirectional transport
- Hyperbolic curve on velocity V/s substrate concentration graph




Active space

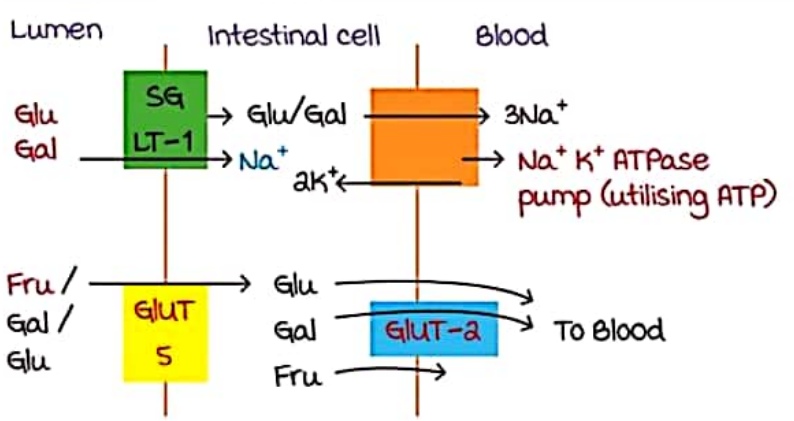
## Concept of distribution of GLUT

00:09:02

| GLUT | Distribution | Concept |
|---|---|---|
| • GLUT 1 | • Widely distributed<br>• Brain, placenta, kidney, RBC | • Insulin independent<br>• Basal glucose uptake |
| • GLUT 2 | • β cells of pancreas | • Insulin independent<br>• insulin secretion |
| | • Sinusoidal liver cells | • ↓ Blood glucose |
| | • Basolateral side of intestine | • Transport glucose to blood |
| | • PCT | • Glucose reabsorption |
| • GLUT 3 | • Neurons | • High affinity to glucose |
| • GLUT 4 | • Heart, Adipocytes, skeletal muscle | • Insulin dependent<br>• ↓ Blood glucose in post-prandial State |
| • GLUT 5 | • Testis, sperm, Intestine (luminal side) | • Fructose transport |
| • GLUT 6 | • Spleen,Leucocyte | • Pseudogene<br>• No transporter functions |
| • GLUT 7 | • Liver endoplasmic reticulum | |

## Absorption of glucose in intestine

00:23:50




Active space

## One liners

00:30:26

- Insulin responsive glucose transporters → GLUT 4, GLUT 8, GLUT 12
- Fructose transporters → GLUT 5 & GLUT 11
- Most widely distributed GLUT → GLUT 1
- Most abundant glucose transporter of RBC → GLUT 1
- GLUT present in Blastocyst → GLUT 8
- Neuronal glucose transporter → GLUT 3

Active space

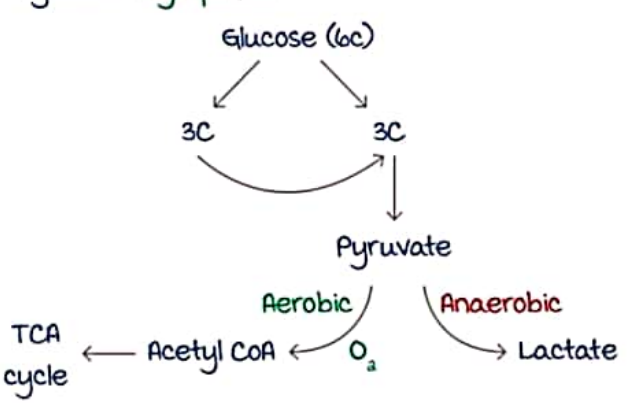
# GLYCOLYSIS & RAPOPORT – LUEBERING CYCLE

## Glycolysis 00:01:55

- Well fed state → Insulin
- All cells & tissues
- Aerobically as well as anaerobically
- In erythrocytes:
  - Only Anaerobic glycolysis
  - Depend wholly on glucose
  - Defect in glycolytic enzymes → Hemolysis
- Heart → Low glycolytic capacity → Cannot survive ischaemia
- Skeletal muscle:
  - Enormous capacity for glycolysis
  - Ischemia → Survive
  - Defect in glycolytic enzymes → Fatigue

## Overview of glycolysis

- Site: All organs
- Organelle: Cytoplasm




## Steps of glycolysis 00:11:15

- Preparatory phase → • Stage of phosphorylation
  • Stage of splitting

ATP consumed in preparatory phase

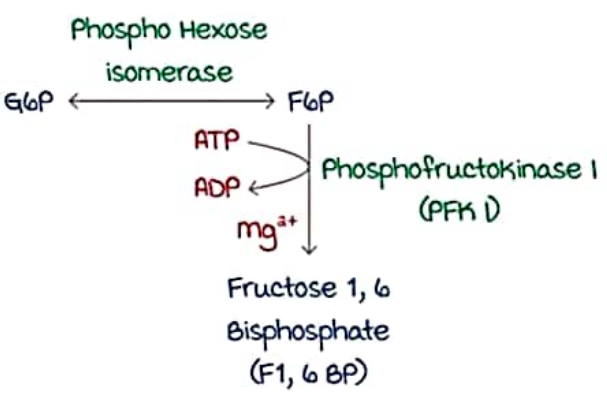
- Pay off phase → ATP generated
  (oxidation / Reduction)

Active space

## Preparatory phase: Stage of phosphorylation 00:13:20


- Significance of the step:
  - Trap glucose for cellular metabolism
  - 1 ATP is utilized
  - 1st irreversible step- Flux generation steps
  - Regulatory step

| Hexokinase ( HK) | Glucokinase (HK IV) |
|---|---|
| • House keeping enzyme | • Inducible |
| • Not induced by Insulin | • Induced by Insulin |
| • Inhibited by G6P | • Not Inhibited by G6P |
| • High affinity | • Low affinity |
| • Low Km | • High Km |
| • No postprandial regulation of blood glucose | • Postprandial regulation of blood glucose ⊕ |
| | • In pancreas, Liver |

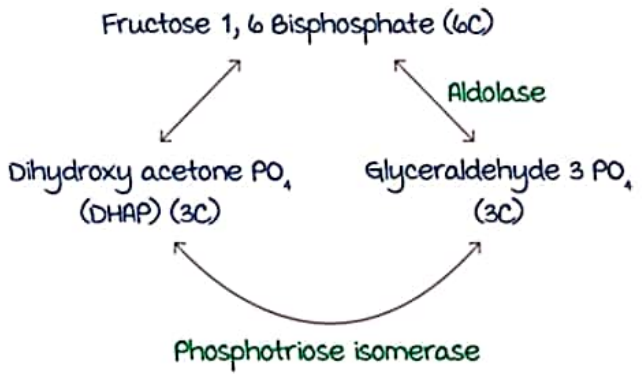



⇒ Significance
- 2nd irreversible step
- Rate limiting enzyme
- Committed step

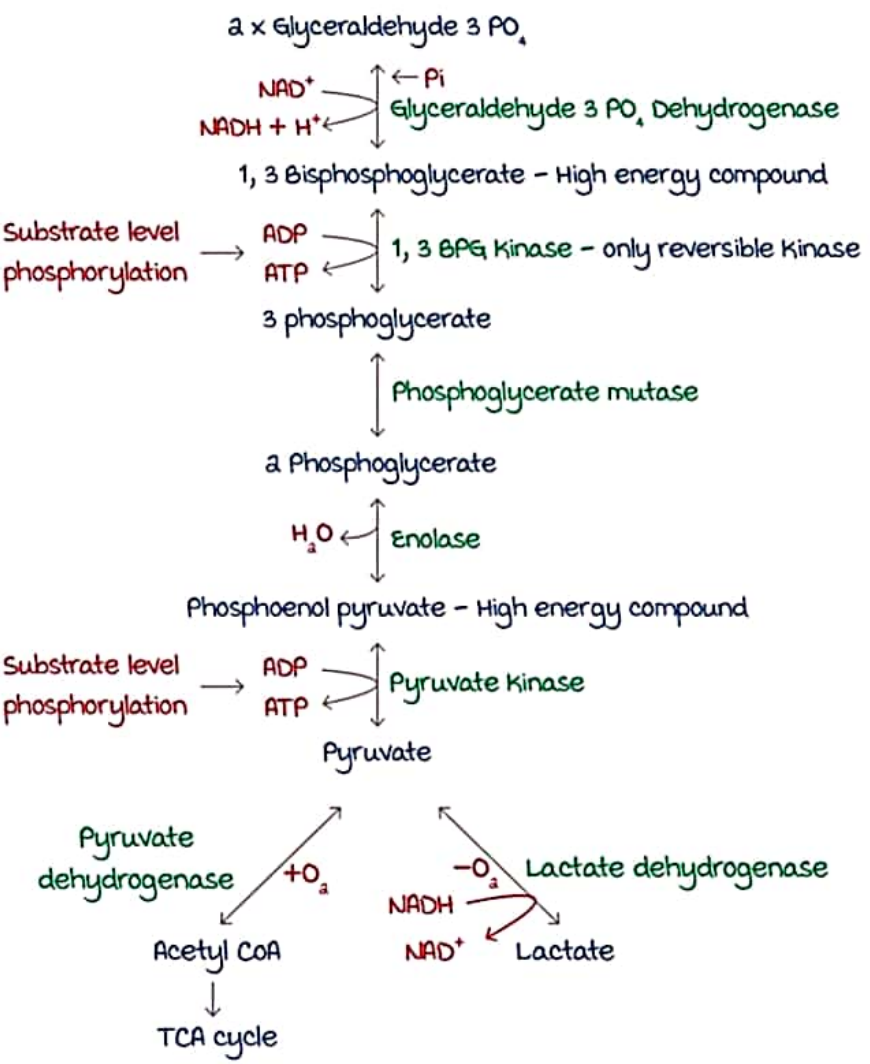
Bottle neck of the pathway

Active space

## Preparatory phase: Stage of splitting 00:28:18

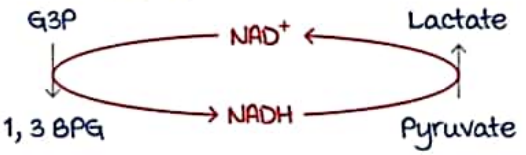



## Payoff phase 00:32:49



## Glycolysis in RBC 00:49:25

- No mitochondria – No ETC and NADH is not converted to $NAD^+$
- Only anaerobic glycolysis




- No net generation of 'NADH' in anaerobic glycolysis

## Energetics of glycolysis 00:54:32

- Aerobic glycolysis:
  - ATP generated = 4ATP
  - NADH generated = 2 NADH = 2 × 2.5 ATP = 5 ATP
  (1 NADH = 2.5 ATP)
  - ATP consumed = 2 ATP
  - Total ATP = 4 + 5 − 2
  = 7 ATP's generated
- Anaerobic glycolysis:
  - ATP generated = 4 ATP
  - ATP consumed = 2 ATP
  - Net ATP = 2 ATP

## Regulation of glycolysis 1:03:43

- Well fed state
- Insulin
- Active in dephosphorylated state
- Allosteric regulation:
  Substrate favours forward reaction
  Product inhibit forward reaction

Active space

| Enzyme | Activator | Inhibitor |
|---|---|---|
| Hexokinase | | Glucose 6 phosphate |
| PFK – 1 | 5' AMP<br>Fructose 6 phosphate<br>Fructose 2, 6 bisphosphate | ATP<br>Citrate<br>Low pH |
| Pyruvate Kinase | | ATP |

## Clinical correlation of glycolysis 01:12:22

- Pyruvate Kinase → 2nd mc enzyme defect in Humans
  - → Hemolysis
- PFK-1 in muscle →
  - Glycogen storage disorder
  - Muscle fatigue

## Inhibitors of glycolysis 1:14:15

- Iodoacetate inhibit Glyceraldehyde 3 phosphate dehydrogenase
- Fluoride inhibit Enolase
- Arsenate inhibit 1, 3 BPG Kinase

## Rapaport leubering cycle / 2,3 BPG shunt 1:18:15

- It occurs in RBC only
- 10% glucose
- Significance: generates 2, 3 Bisphosphoglycerate

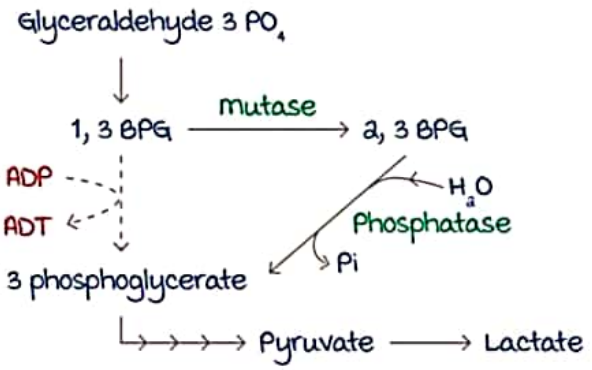
↓

Shift ODC to right

↓

$O_2$ unloading

Active space

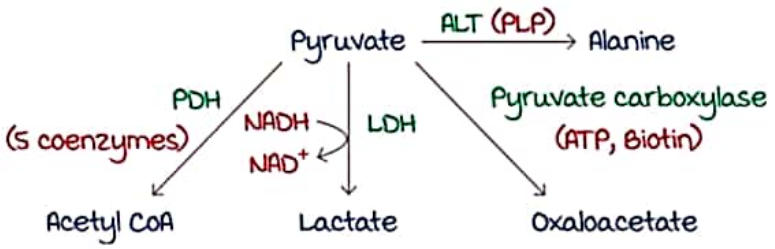



- Energetics:
  - Total ATP generated = 2 ATP
  - Total ATP consumed = 2 ATP
  - Net ATP generated = 0

Active space

# PYRUVATE DEHYDROGENASE

## Fates of pyruvate




PLP-Pyridoxal phosphate(Coenzyme)

PDH-Pyruvate dehydrogenase

LDH-Lactate dehydrogenase

ALT-Alanine aminotransaminase

## Pyruvate dehydrogenase

00:04:15

- Site: Mitochondria
- PDH is a multienzyme complex

| Enzymes | Coenzymes |
|---|---|
| Pyruvate dehydrogenase | Thiamine(active form-TPP/ TDP) |
| Dihyrolipoamide transacetylase | Lipomide |
| Dihydrolipoamide dehydrogenase | FAD, $NAD^+$, CoA |

TPP-Thiamine pyrophosphate

TDP-Thiamine diphosphate

- Pyruvate $\xrightarrow{PDH}$ Acetyl CoA ($NAD^+$ → NADH → ETC → 2.5 ATPs)

## Significance of Pyruvate dehydrogenase

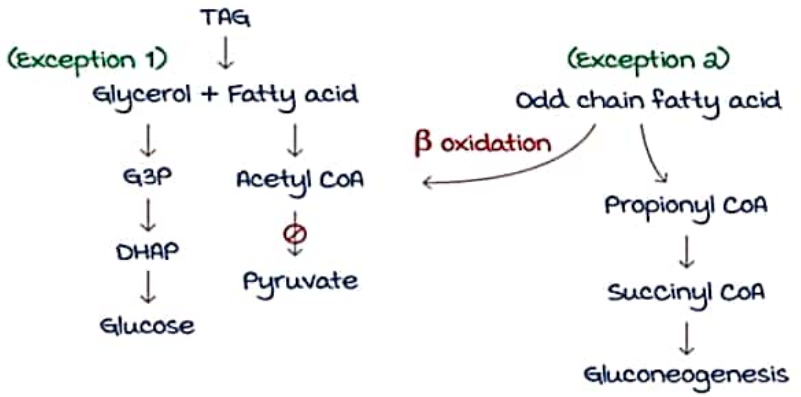
00:10:17

- Pyruvate $\xrightarrow{\text{Irreversible}}$ Acetyl CoA
- Acetyl CoA cannot be used as a substrate for gluconeogenesis
- Acetyl CoA is an allosteric activator of pyruvate carboxylase

Active space

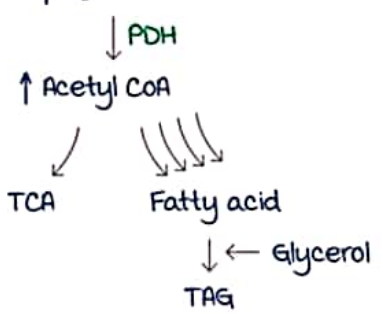
- Pyruvate carboxylase is an enzyme of gluconeogenesis.
- Fat cannot be convered to glucose because the product is

acetyl CoA

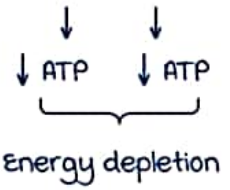



G3P-Glyceraldehyde 3 phosphate
DHAP-Dihydroxyacetone phosphate

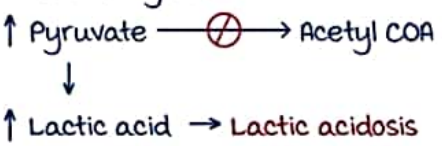
- ↑carbohydrate diet → ↑ Glucose → ↑ Pyruvate




- Irreversible link between glycolysis & TCA - PDH
- In chronic alcoholics → Energy depletion
  - Coenzymes of PDH & α KGDH-CoA(pantothenic acid)
    Thiamine
    FAD($B_2$)
    $NAD^+$($B_3$)
    Lipoamide
  - In chronic alcoholic thiamine deficiency → ↓ PDH & ↓ α KGDH




- In deficiency of PDH




Active space

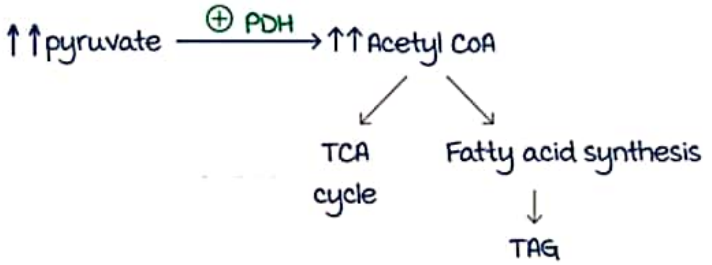
## Regulation of pyruvate dehydrogenase 00:27:46

- Covalent modification/ Covalent regulation

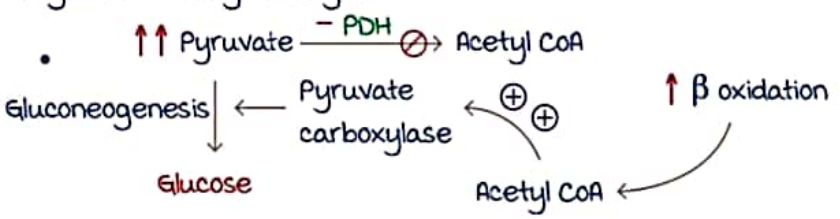
  PDH active in dephosphorylated state

- Allosteric regulation/ End point regulation
  - Increase in the products inhibit PDH.
  - ∴↑ NADH, ↑ ATP, ↑ Acetyl COA $\xrightarrow{\ominus}$ PDH

- Regulation during fed state:




- Regulation during fasting state:




Active space

# GLUCONEOGENESIS

## Gluconeogenesis concept

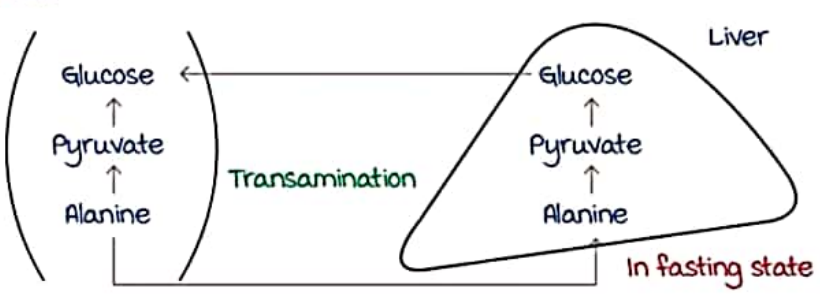
- Synthesis of glucose from non carbohydrate substrate
- Early Fasting (4–16 hrs) → Glucogenolysis
  ↓
  Fasting (16–48 hrs) → Gluconeogenesis
  ↳ β oxidation → ATP requiring ⊕ ⊕
  ↳ Acetyl CoA
- In diabetes mellitus,
  ↳ Fasting → ↑↑ Gluconeogenesis → Hyperglycemia
- Site:
  - Liver, Kidney
- Organelle:
  - Both cytoplasm & Mitochondria

## Non carbohydrate substrates 00:08:22

- Glucogenic amino acid (Principal = Alanine)
- Lactate
- Glycerol
- Propionyl CoA

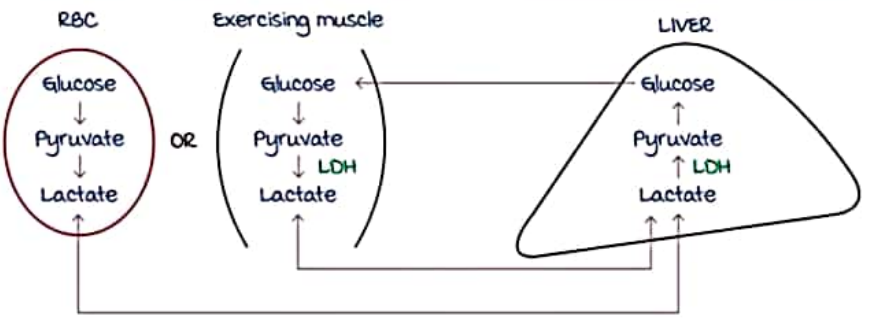
## Glucose-alanine cycle / Cahill cycle 00:10:38

Muscle:




Active space

## Glucose - lactate cycle / Cori's cycle 00:12:21




Lactate accumulation in muscle → fatigue → prevented by gluconeogenesis

## Key enzymes of gluconeogenesis 00:15:21

- Reversible steps in glycolysis is common to gluconeogenesis
- Irreversible steps of glycolysis is bypassed / Reversed / Circumvented by Key enzymes

- Irreversible steps of glycolysis
- Glucose $\xrightarrow{\text{Hexokinase / Glucokinase}}$ G6P
- Fructose 6PO$_4$ $\xrightarrow{\text{PFK-1}}$ F1, 6BP
- Phosphoenolpyruvate
  ↓ PK
  pyruvate

- Key enzymes of gluconeogenesis
- G6P $\xrightarrow[H_2O\ \ Pi]{\text{Glucose 6 phosphatase}}$ Glucose
- F1, 6BP $\xrightarrow[H_2O\ \ Pi]{\text{Fructose 1, 6 Biphosphatase}}$ F6P
- Pyruvate (3C)
  ATP, ADP, CO$_2$ ← Pyruvate carboxylase + Biotin
  Oxaloacetate (4C)
- This reaction takes place →
  mitochondria
  Oxaloacetate (mitochondria)
  ↓ malate aspartate shuttle
  Oxaloacetate (cytoplasm)

Active space

- PEP → Pyruvate

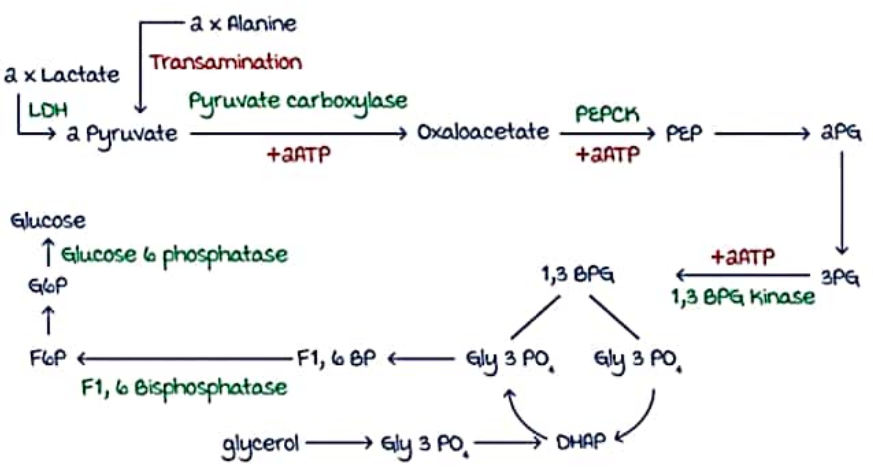
- Oxaloacetate

$CO_2$ ← , GTP → GDP ← : PEP carboxykinase (PEPCK)

Phosphoenol pyruvate (PEP) (3C)

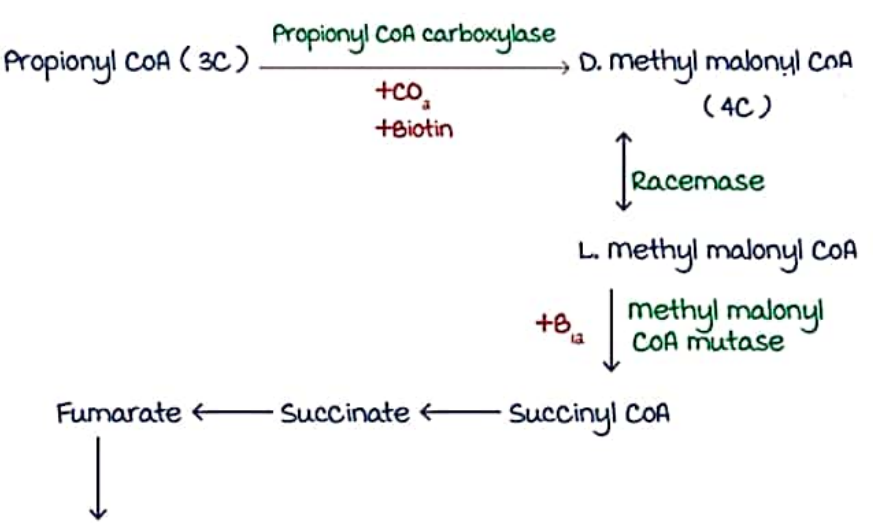
- decarboxylation
- Phosphorylation

## Steps of gluconeogenesis 00:29:24




- ∴ 2 Lactate → 1 Glucose
- 6 ATP's are utilized for conversion of 2xLactate → 1 Glucose

## Entry of propionyl CoA to gluconeogenesis 00:40:38



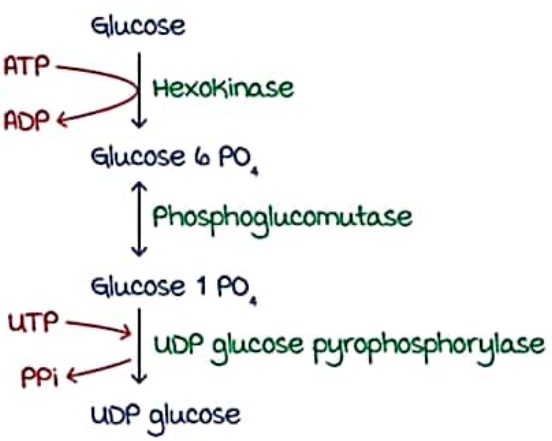

Active space

# GLYCOGEN METABOLISM

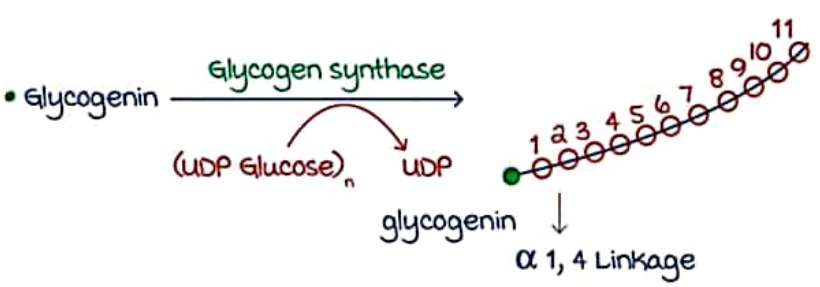
## Glycogenesis / glycogen synthesis 00:02:26

- Glycogen : storage form of carbohydrate
- Organs: Liver & muscle
- Organelle: Cytoplasm
- Rate Limiting Enzyme : Glycogen synthase
- Steps:
  - Synthesis of UDP glucose
  - Glycogen synthase reactions
  - Branching
- Glycogen synthesized on a primer → Glycogenin(polypeptide)

## Synthesis of UDP glucose 00:10:54




## Action of glycogen synthase 00:14:57

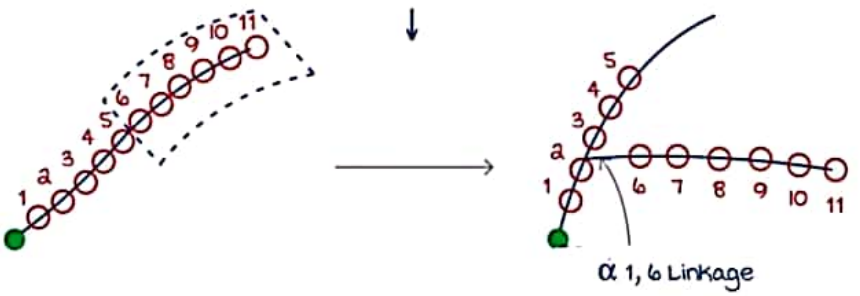



- Glycogen synthase : rate limiting enzyme.

Active space

## Branching 00:18:07

- Enzyme: α 1, 4 – 1, 6 glucan transferase

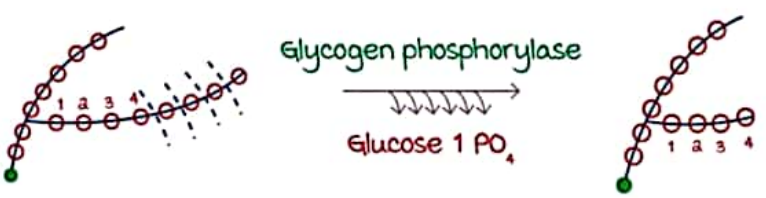



- In the muscle & liver glycogen is stored as → β particle
- one β particle-60,000 glycogen residues
- In liver : arranged in rosette pattern

## Glycogenolysis 00:22:50

- Site:
  - Organ: Liver & muscle
  - Organelle: Cytoplasm, Lysosomes (1-2%),SER
- Rate Limiting Enzyme : Glycogen phosphorylase. (Coenzyme → Pyridoxal phosphate)
- Steps:
  - Action of glycogen phosphorylase
  - Removal of branches
  - Conversion of glucose 1 $PO_4$ → glucose

## Action of Glycogen phosphorylase 00:28:34

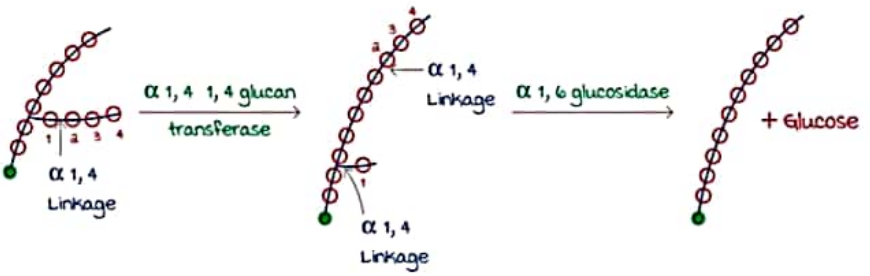



## Removal of branches 00:31:46

- Debranching enzyme → Bifunctional enzyme

| | |
|---|---|
| α 1, 4 1, 4 glucan transferase | α 1, 6 Glucosidase / Amylo 1, 6 glucosidase |

Active space




## Conversion of Glucose 1 $PO_4$ to glucose

- In Liver:

Glucose 1 $PO_4$
↓ Phosphoglucomutase
Glucose 6 $PO_4$
$H_2O$ → ↓ Glucose 6 phosphatase
Pi ←
Glucose

- In muscle:

Glucose 1 $PO_4$
↓ phosphoglucomutase
Glucose 6 $PO_4$
↓ Absence of glucose 6 phosphatase in muscle
Anaerobic glycolysis
↓
Pyruvate → Lactate

- ∴ In muscle during exercise,

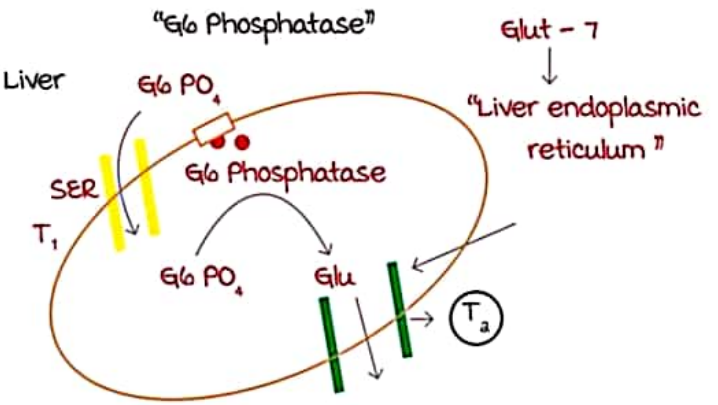
Number of ATP produced from glucose 6 $PO_4$ → 3ATP

## Glucose 6 phosphatase

00:49:55

- It is present in liver
- Absent in muscle
- This enzyme is present in cytoplasmic side of SER
- It is common to gluconeogenesis & glycogenolysis

Active space




Active space

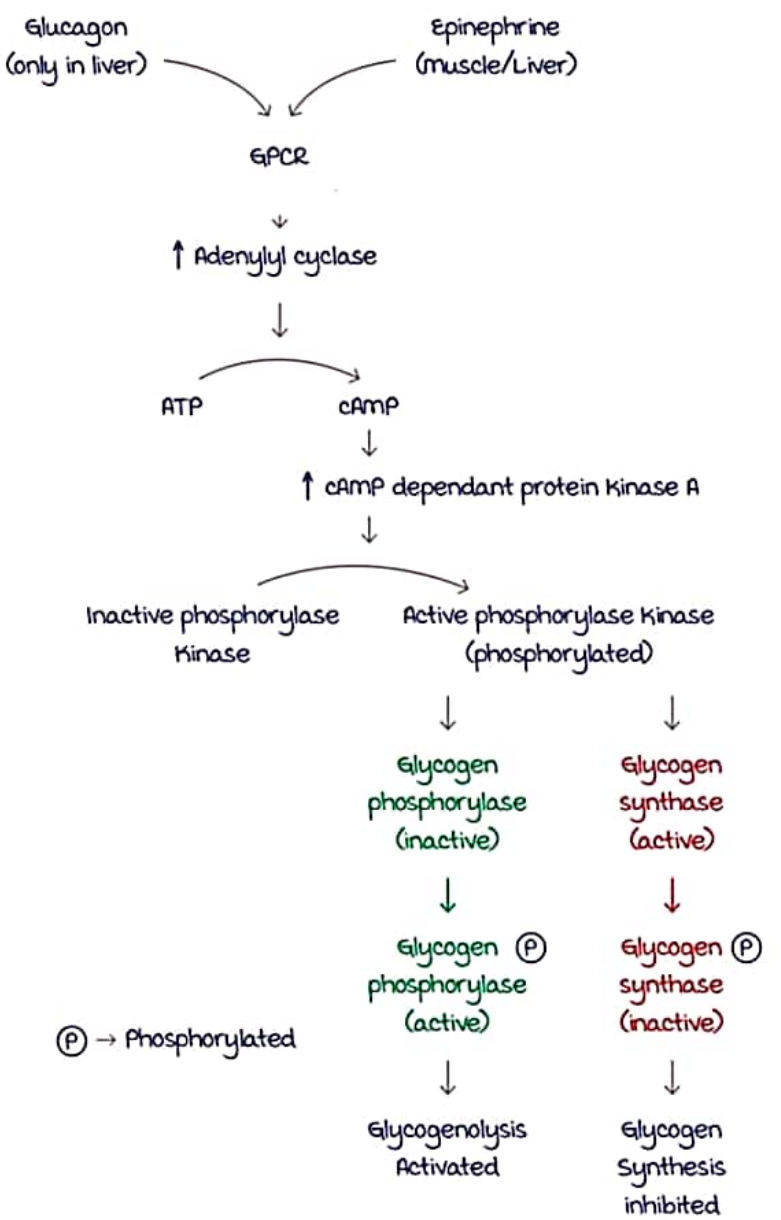
# REGULATION OF GLYCOGEN METABOLISM

Types of regulation

- Hormonal regulation
- Allosteric regulation

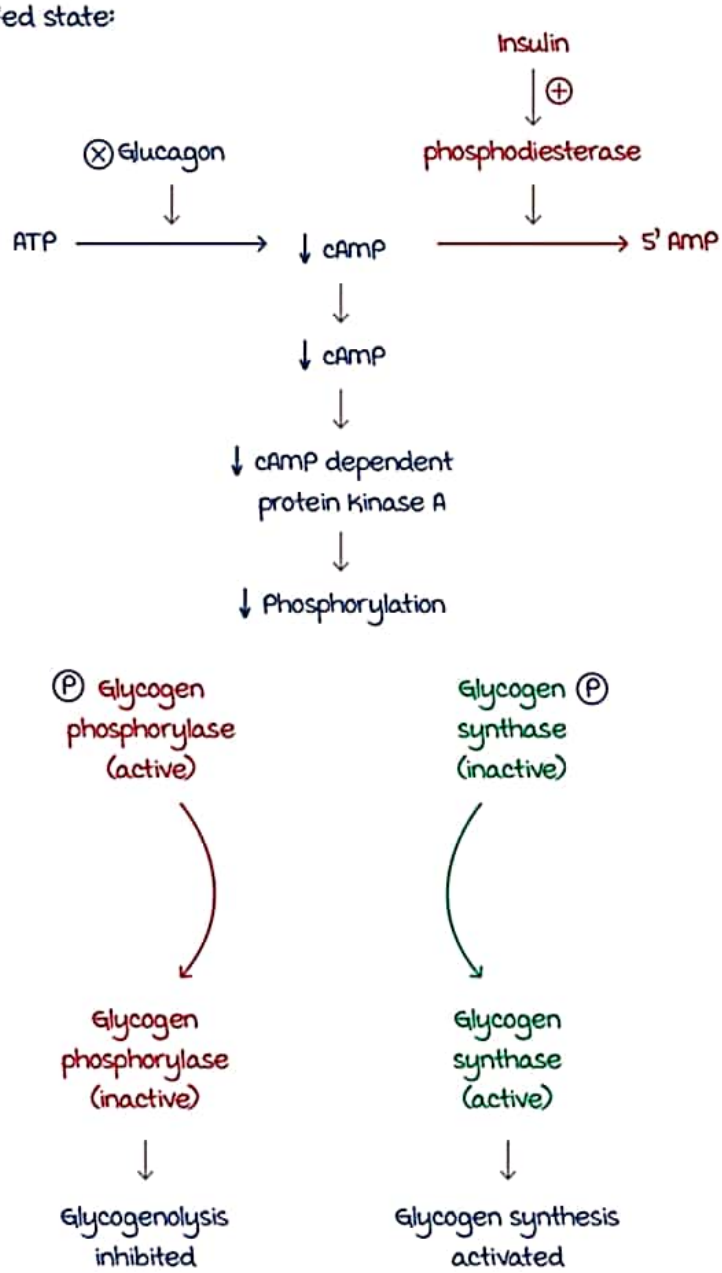
## Hormonal regulation 00:00:58

- Depends on the dietary status.
- During Fasting: Early fasting (4–16 hrs)



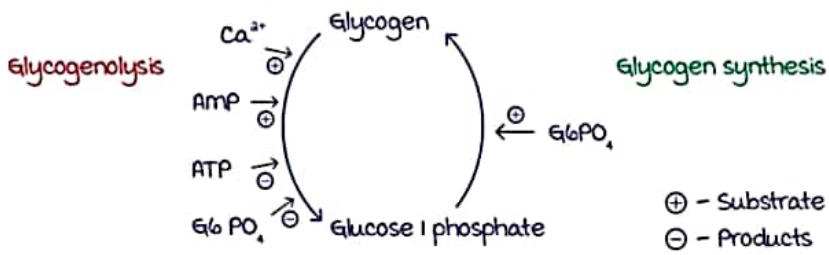

Active space

- Well-fed state:



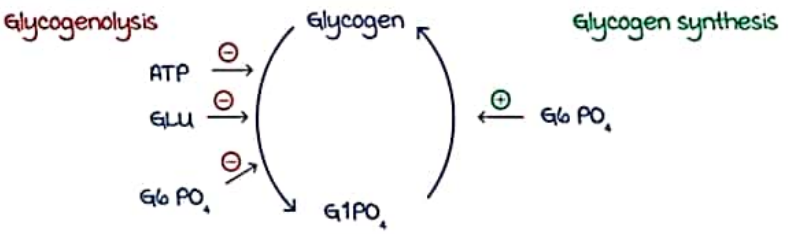

## Allosteric regulation in muscles

00:11:18



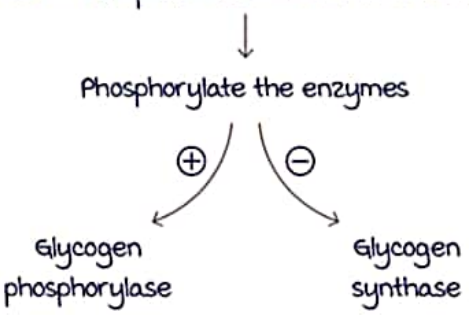

Active space

## Allosteric regulation in liver 00:14:06

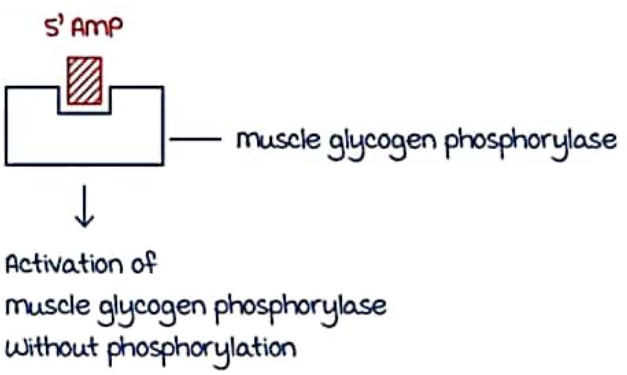



## Special mechanism of regulation in muscles 00:16:20

- cAMP independent calcium calmodulin dependent Kinase.




- In extreme anoxia:




Active space

# GLYCOGEN STORAGE DISORDERS

## Glycogen Storage Disorder ( GSD ) 00:01:14

- Liver GSD
  - Hypoglycemia
  - Hepatomegaly
  - No exercise intolerance
- Muscle GSD
  - Normoglycemia
  - Exercise intolerance

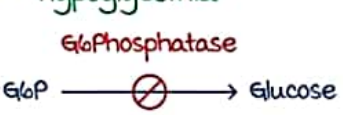
## Von gierke's disease (type Ia GSD) 00:03:49

- Mc GSD
- Biochemical defect: Glucose 6 phosphatase
- ↑↑ Glucose 6 phosphate
- ↑ Glycogen in organs 'Liver'
- Clinical features:
  - Presents at 3-4 months of age
  - Doll like facies with thin extremities
  - Massive hepatomegaly
  - No splenomegaly
  - Renomegaly
  - Milky white plasma → Triglyceridemia

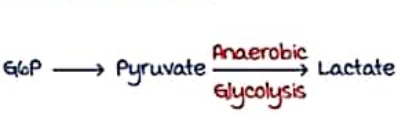
## Biochemical hallmarks of Von gierke's disease

- Hypoglycemia
- Lactic acidosis
- Hyperlipidemia
- Ketosis
- Hyperuricemia

- Hypoglycemia:

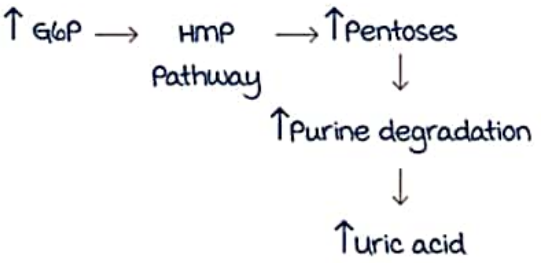



- Lactic acidosis:

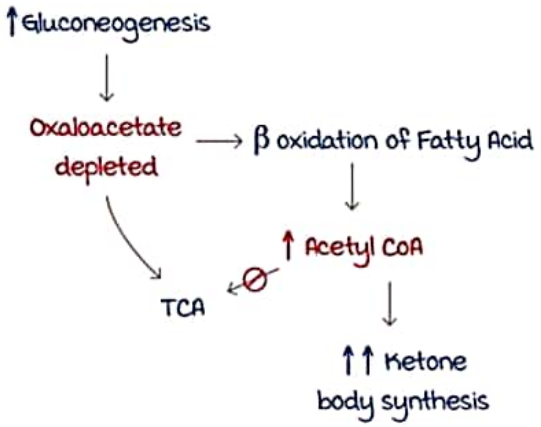



Active space

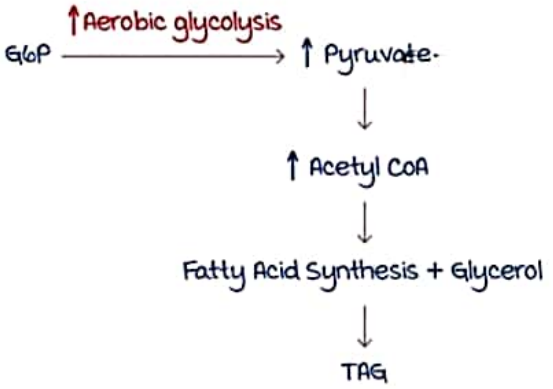
- Hyperuricemia:




- Ketosis:




- Hyperlipidemia:




## Type I b GSD

00:18:23

- Biochemical defect: $G6PO_4$ transporter in liver endoplasmic reticulum
- Clinical features:
  - Similar to Von gierke's disease
  - Neutropenia & Recurrent bacterial infections

Active space

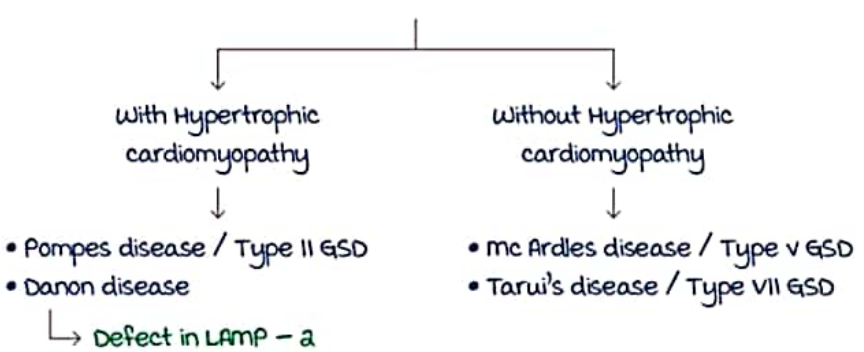
## Type III GSD / Cori's disease / Forbes disease / Limit dextrinoses 00:20:05

- Biochemical defect: Debranching enzyme
- Abnormal glycogen ( Limit dextrin with few outer branches)
- Clinical features:
  - Hypoglycemia
  - Hepatomegaly
  - Splenomegaly
  - No renomegaly
  - Progressive liver cirrhosis → Death
  - I.V Glucagon → • Response in well fed state
    • No Response in overnight fasting

## Type IV GSD / Anderson disease / Amylopectinoses 00:28:45

- Biochemical defect: Branching enzyme
- Abnormal glycogen → Insoluble in water (Amylopectin like)
- Clinical features:
  - Hypoglycemia
  - Hepatomegaly
  - Splenomegaly
  - No renomegaly
  - Progressive cirrhosis → Portal HTN → Death in 5 yrs

## Muscle glycogen storage disorder 00:33:43




Active space

## Pompe's disease (type II GSD) 00:36:17

- Muscle GSD
- Lysosomal storage disorder
- Enzyme defect: Acid maltase / Acid α 1, 4 glucosidase
- Clinical features:
  - Early onset
  - Hypotonia
  - Floppy infant
  - Macroglossia
  - Cardiomegaly
  - Failure to thrive
  - Cardiac failure → Progressive → Death in 2 yrs
- Diagnosis:
  - ↑ Serum CK ⊕⊕
  - ↑ Serum LDH
  - ↑ Acid phosphatase
- Enzyme replacement therapy:
  - Myozyme / Alglucosidase α / Recombinant acid α glucosidase

## Mc Ardles disease / Type V GSD 00:43:05

- Enzyme: Muscle (glycogen phosphorylase)
- Clinical features:
  - Normoglycemia
  - Excercise intolerance
  - Rhabdomyolysis → myoglobinuria → Burgundy coloured urine
  - Second wind phenomenon
    - → 1st pain —Rest→ Resume exercise with more ease
- Mc GSD in adults & adolescent age

Active space

## Tarui's disease / Type VII GSD 00:46:37

- Enzyme defect: Muscle & Erythrocyte PFK
- Clinical features:
  - Exercise intolerance
  - No second wind phenomenon
  - Hemolysis

## GSD types overview 00:49:40

| Type of GSD | Name (One) | Enzyme |
|---|---|---|
| I | Von Gierke | G6Pase |
| II | Pompe's (M) | Acid Maltase |
| III | Cori's (L) | Debranching enzyme |
| IV | Anderson (L) | Branching enzyme |
| V | McArdle's (M) | Muscle Phosphorylase |
| VI | Her's disease | Hepatic Phosphorylase |
| VII | Tarui's | Muscle / Erythrocyte PFK-1 |

- GSD with brain involvement (Anterior horn cells) → Type II GSD
- Recently added GSD (GLUT – 2) → Fanconi Bickel syndrome
- Type 0 glycogen storage disorder → Glycogen synthase defect
- GSD – type I GSD
- Mc GSD in adolescents and adults – type 5 Mc ardle's
- GSD with hyperglycemia and hepatomegaly – liver GSD
- GSD with liver cirrhosis – type 3, type 4
- GSD with renal dysfunction – Von gierke's
- Liver GSD with myopathy – type 3 and type 4

Active space

# REGULATION OF GLUCONEOGENESIS

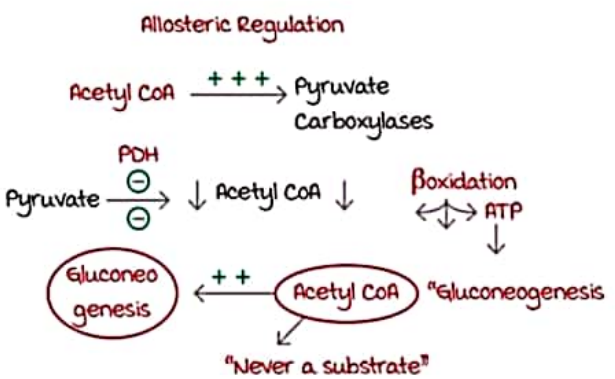
## Types of regulation 00:00:28

- Covalent modification
- Allosteric regulation
- Reciprocal regulation of glycolysis and gluconeogenesis

## Covalent modification 00:01:10

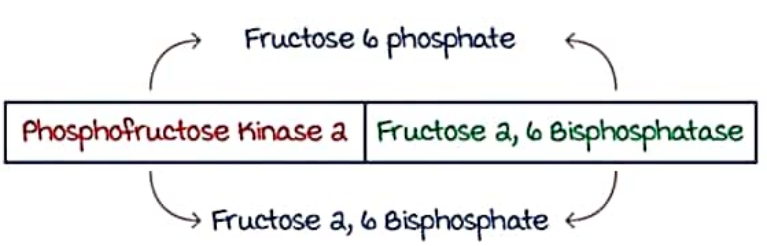
- Fasting state (16–48 hrs)
- Glucagon
- Regulatory enzyme active in phosphorylated state
  - The enzymes are: • Pyruvate carboxylase
    • Fructose 1, 6 Bisphosphatase

## Allosteric modification 00:03:03



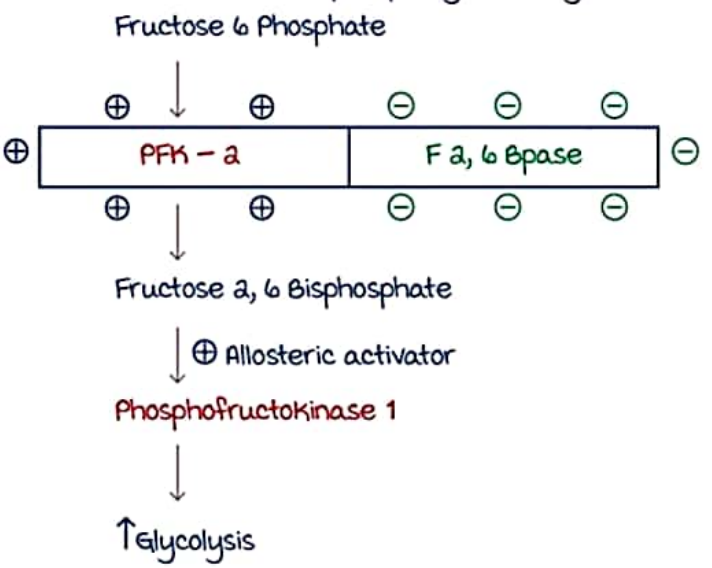

## Reciprocal regulation 00:06:00

- Reciprocal regulation with the use of Tandem enzyme / Bifunctional enzyme
- Single polypeptide with a enzyme activity

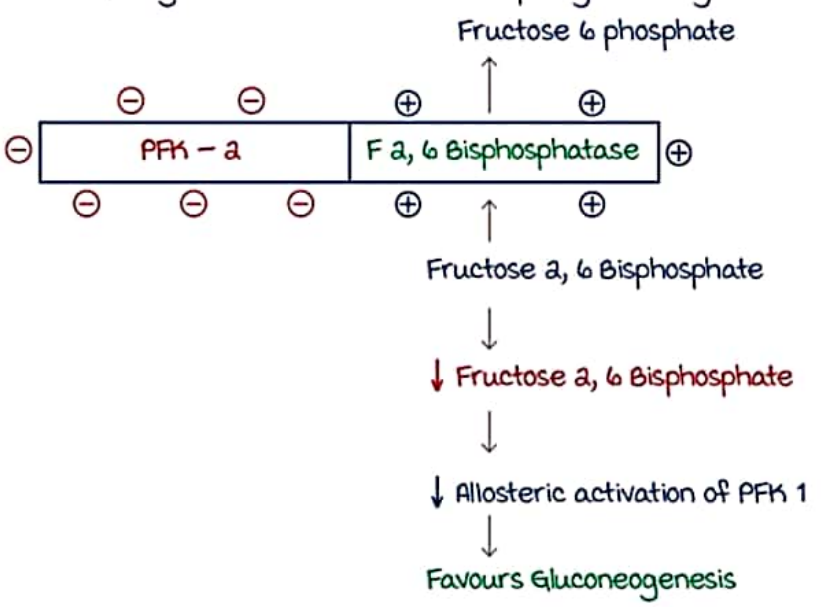



Active space

- In well fed state:
  - Insulin is the hormone → Dephosphorylate enzymes




- In fasting state:
  - Glucagon is the hormone → Phosphorylate enzymes




Active space

# HMP PATHWAY

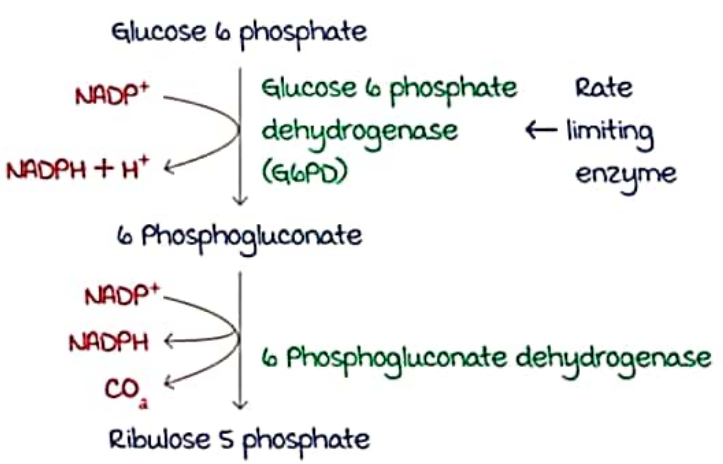
## HMP pathway 00:01:09

- Hexose monophosphate pathway
- This pathway is also called as
  - Pentose phosphate pathway
  - Dickens-Horecker pathway
- Site: Cytosol
- Significance:- Major source of NADPH
  - contributor of pentoses

## Phases of HMP pathway 00:05:02

- Oxidative phase
  - Irreversible
  - NADPH production
- Non oxidative phase:
  - Reversible
  - Pentose production

## Oxidative phase 00:06:01




## Function of NADPH 00:09:11

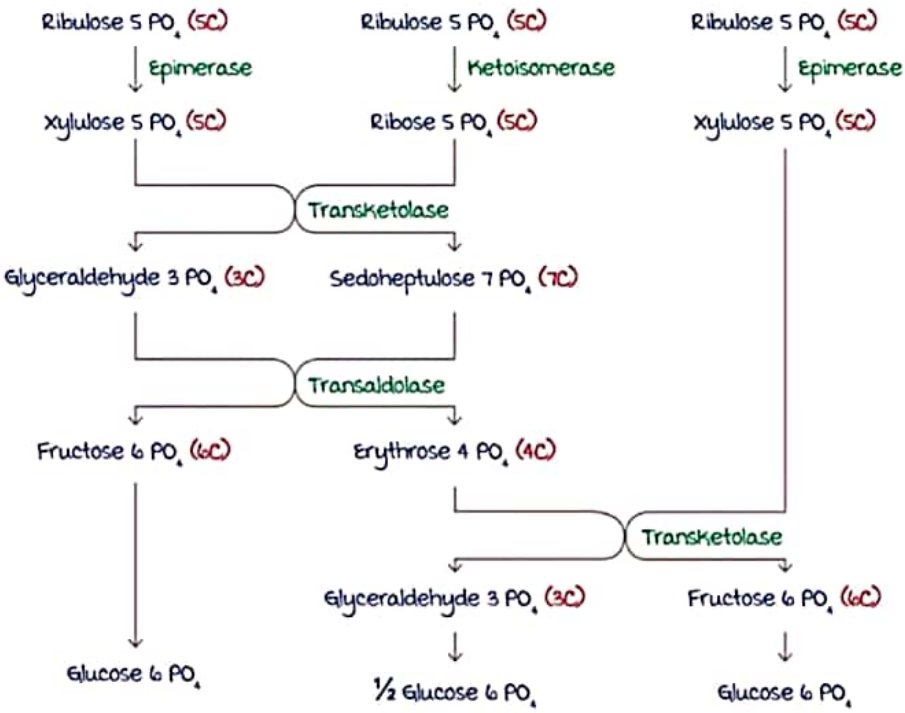
- Reductive biosynthesis of fatty acids, steroids & cholesterol
- Free radical scavenging
  - RBC → To maintain membrane integrity
  - Lens → To keep transparency of lens
  - Keeps iron in the ferrous state in hemoglobin

Active space

## Non oxidative phase

00:15:33

- Reversible
- Synthesis of pentoses
- Occurs in all organs, specifically in
  - -Skin
  - -Intestinal mucosa } Rapid cell turn over
  - -Bone marrow




- ATP – not generated
- $CO_2$ – liberated

## Clinical correlation of HMP pathway

00:27:21

- mc enzyme deficiency in humans → G6PD

↓G6PD

↓

↓NADPH

↓

↓ Free radical scavenging

↓

  - RBC → Hemolysis → anemia, jaundice
  - Hb → methemoglobinemia
  - Heinz bodies in RBC
  - consumption of Sulfadrugs, Primaquine, fava beans(favism) can aggravate G6PD deficiency

Active space

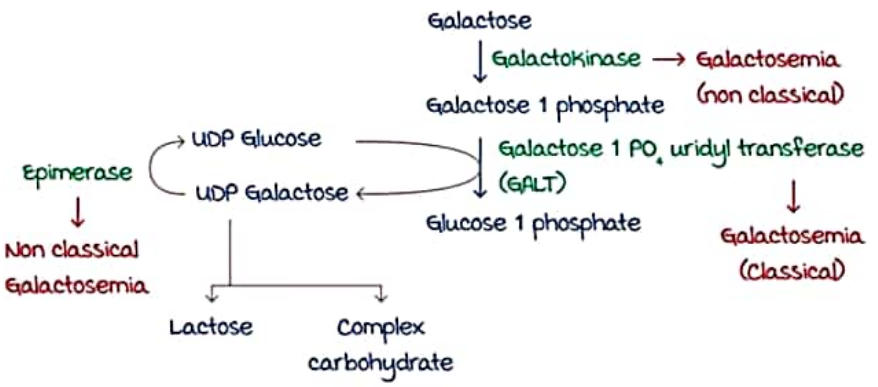
- Transketolase
  - → thiamine is the coenzyme
  - Erythrocyte transketolase is a sensitive indicator of thiamine status
  - Wernicke-Korsakoff syndrome: thiamine deficiency
    ↓
    ↓ transketolase

Active space

# GALACTOSE METABOLISM

## Metabolism of galactose

00:00:20




## Classic galactosemia

00:04:47

- Biochemical defect: Galactose 1 phosphate uridyl transferase
- ∴ There will be ↑ Galactose 1 phosphate
  ↓
  Allosteric inhibitor of
  Glycogen phosphorylase
  ↓
  ↓↓ Glycogenolysis
  ↓
  Fasting Hypoglycemia
- Symptoms:
  - Age of onset 1-2 weeks of life
  - Breast feeding initiate symptoms : Lactose → Galactose
  - Failure to thrive, vomiting, feeding difficulties.
  - Seizures, coma
  - Intellectual disability
  - Hepatomegaly, Liver failure → Jaundice
  - Oil drop cataract

    ∵ Gal 1 $Po_4$ → Galactose $\xrightarrow{\text{Reduction}}$ Galactitol, Dulcitol
    ↓
    Cataract
  - Neonatal sepsis: mc by E. coli

Active space

- Diagnosis:
  - Benedicts test +ve
  - Glucose oxidase test –ve
  - Mucic acid test +ve
  - Galactose tolerance test contraindicated
  - Enzyme studies
  - Genetic mutation

- Treatment:
  - Lactose free diet → Breast feeding absolutely contraindicated
  - By 4–5 yrs of age Galactose 1 phosphate pyrophosphorylase gets activated

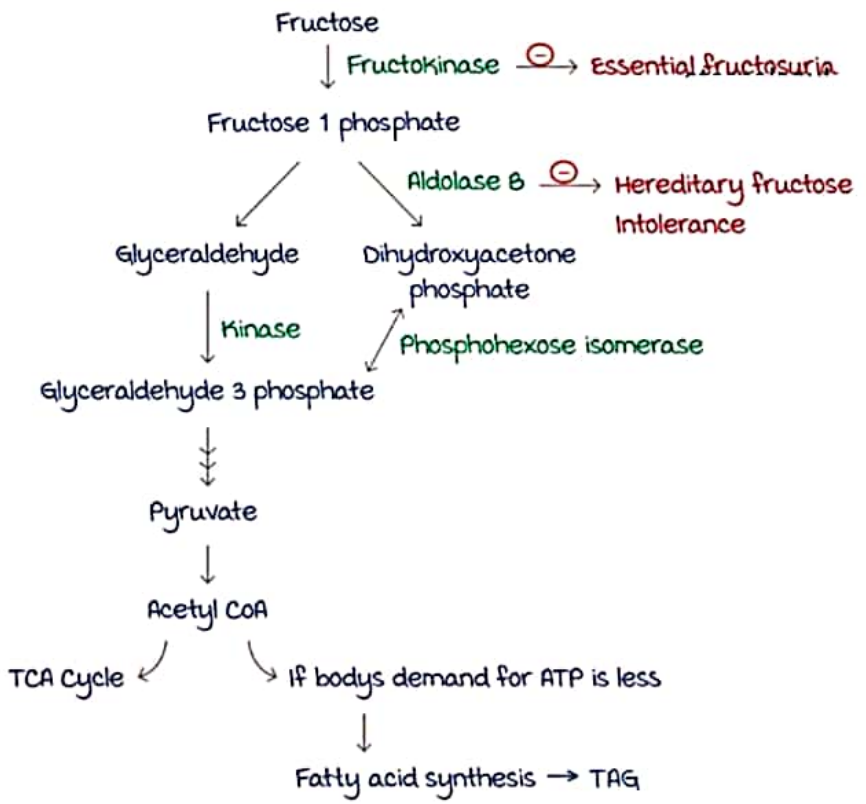
Galactose 1 $po_4$ ⟶ Galactose

## Non-classic galactosemia 00:13:17

- Benign condition
- Enzyme deficiency:
  - UDP Galactose epimerase
  - Galactokinase
- Only 1 manifestation in Galactokinase deficiency → Cataract

# FRUCTOSE METABOLISM

## Metabolism of fructose 00:00:49




- Acute fructose loading is harmful to the body
  - Hyperlipidemia
  - Hyperuricemia → Gout

## Hereditary fructose intolerance 00:09:39

- Biochemical defect: Aldolase B deficiency
  - ↑ ↑ Fructose 1 phosphate
  - ↓ Glycogen phosphorylase
  - ↓ Glycogenolysis
  - Fasting hypoglycemia

Active space

- Clinical features:
  - Age of onset → ≈ 6 months (during weaning period)
  - Vomiting, feeding difficulties
  - Failure to thrive
  - Convulsions, coma
  - Liver failure, hepatomegaly
  - Jaundice
  - No cataract

## Diagnosis of hereditary fructose intolerance 00:13:51

- Urine reducing substance ⊕
  - Benedict's test ⊕
  - Glucose oxidase test ⊖
  - Test for ketosis :
    - Rapid furfural test ⊕
    - Seliwanoff's test ⊕
- Enzyme studies
- Genetic mutation studies

- Treatment:
  - Fructose free diet

## Essential fructosuria 00:15:25

- Benign condition
- Biochemical defect: Fructokinase
- Renal threshold of fructose is low

↓

Excretion of fructose in urine

# TCA CYCLE

## TCA cycle: Introduction 00:00:08

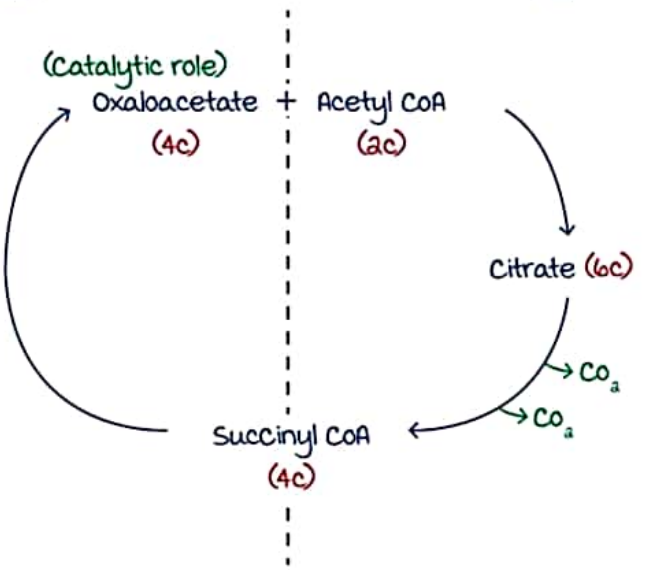
- Tricarboxylic acid cycle/final common oxidative pathway
- Elucidated by Hans Krebs
- Also called Krebs cycle / citric acid cycle
- It is the Final common oxidative pathway
- Carbohydrate → pyruvate —PDH→
- Protein → Amino acid —Catabolism→ Acetyl CoA → TCA cycle
- Lipids → Fatty acid —β oxidation→
- Acetyl CoA is oxidized
- Generate reducing equivalents
- Site: All organs with mitochondria
- Organelle: Mitochondrial pathway
- All enzymes in mitochondrial matrix except succinate dehydrogenase

  ∴ It is part of ETC → complex II (inner mitochondrial membrane)

## TCA cycle: Overview 00:07:09

2nd Half: Regenerate oxaloacetate

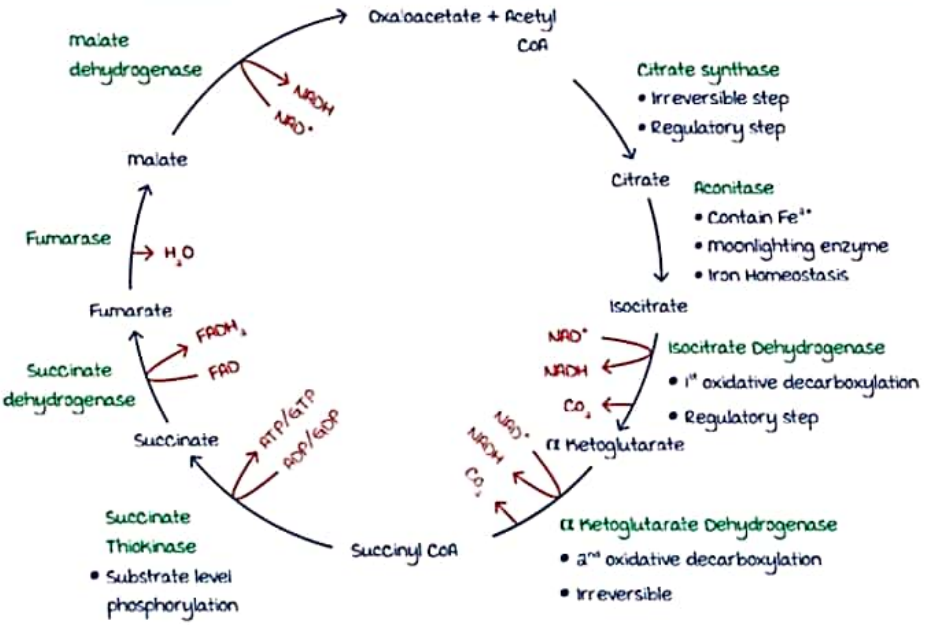
1st half: Oxidation of Acetyl CoA




Active space

## Steps of TCA cycle

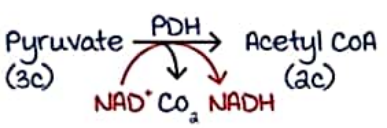
00:10:49



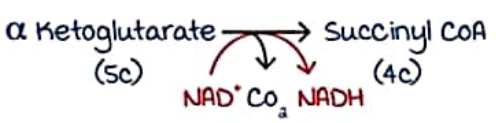

## Oxidative decarboxylation

00:22:09

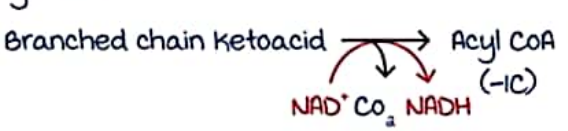
- PDH




- α KGDH




- Branched chain Ketoacid dehydrogenase




- CoA (pantothenic acid)
- NAD (Niacin)
- FAD (Riboflavin)
- Lipomide
- Thiamine

} Coenzymes

Active space

## Inhibitors of TCA cycle 00:33:18

- Fluroacetate $\xrightarrow[\ominus]{\text{Non-competitive}}$ Aconitase
- Arsenite $\xrightarrow[\ominus]{\text{Non-competitive}}$ α KGDH
- Malonate $\xrightarrow[\ominus]{\text{Competitive}}$ SDH

## Energetics of TCA cycle 00:35:35

- 3 NADH = 3 X 2.5 ATP = 7.5
- 1 FADH2 = 1 X 1.5 ATP = 1.5
- 1 ATP = 1

  Total = 10 ATP

- ∴ No of ATP liberated by 1 TCA cycle → 10 ATPs

## Significance of TCA cycle 00:39:08

- Acetyl CoA is completely oxidised
- Truly Amphibolic pathway
  - Catabolic role → Acetyl CoA oxidized
  - Anabolic role → OA →
    - Glucose
    - Citrate → FA
    - α KG → Glutamate → GABA
    - Succinyl CoA → Heme synthesis
- Anaplerotic reactions
  - Filling up reactions
  - Replenishment of depleted intermediates of TCA cycle
  - Ex: • pyruvate $\xrightarrow{\text{pyruvate carboxylase}}$ Oxaloacetate
    - Valine
    - Isoleucine
    - Methionine
    - Threonine

    } → Succinyl CoA

Active space

## Carboxylation reaction 00:46:08

- Pyruvate (3C) → OA (4C)
- Acetyl CoA (2C) → malonyl CoA (3C)
- Propionyl CoA (3C) → methyl malonyl CoA (4C)

All these reaction require →
- ATP
- Addition of 1 carbon
- Biotin
- Ligases

## Regulation of TCA cycle 00:48:30

- High ATP/ADP ratio → ⊖ TCA
- High NADH/NAD ratio → ⊖ TCA
- NADH, ATP → ⊖ ICDH
- In brain regulation at the level of PDH
- ↑ $Ca^{2+}$ in muscles → ⊕ ⊕ All dehydrogenases
- No normal regulation of TCA cycle

Active space

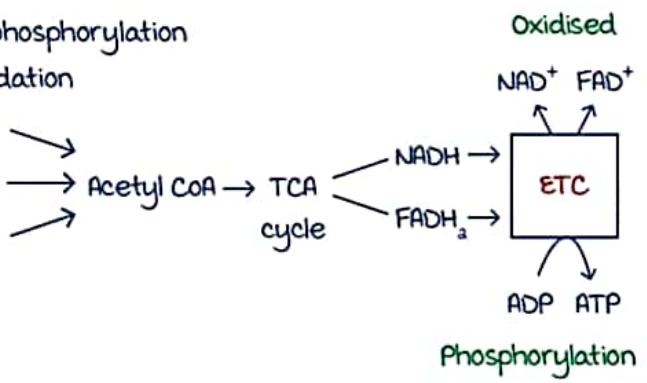
# ELECTRON TRANSPORT CHAIN

## ETC: Basics 00:00:20

- Oxidation: Loss of electrons
- Reduction: Gain of electrons
- Redox couple: Compound that can exist in oxidised as well as reduced State
  Eg: $NAD^+$ / NADH, FAD / $FADH_2$
- Redox potential : Ability to transfer electron / gain electron
- More Redox potential → more ability to gain electrons
- In ETC, series of redox couples arranged in ascending order of redox potential
- $e^-$ jump from low redox potential → High redox potential
  → Exergonic reaction → Liberate free energy

## Concept of ETC 00:07:20

- It is oxidative phosphorylation
- Coupling of oxidation
- Carbohydrate
- Protein
- Lipids




## ETC: Complex I & Q 00:10:36

- Location: Inner mitochondrial membrane
- Complex I:
  - NADH-Q oxidoreductase (NADH dehydrogenase)
  - Components: • FMN
    • Iron sulphur complex
- Coenzyme Q:
  - Mobile $e^-$ carrier
  - Also called ubiquinone or Q10
  - 10 Isoprene units

Active space

## ETC: Complex II, III, IV 00:16:48

- Complex II:
  - Succinate Q oxidoreductase (succinate Q reductase)
  - Components: • FAD
    • Iron sulphur complex
- Complex III:
  - Q-cyt c oxidoreductase / cyt b-C1 complex
  - Components: • Cyt b
    • Cyt $C_1$
    • Reiske Fe- sulphur complex
- Complex IV:
  - Cyt C oxidase
  - Final $e^-$ acceptor is $O_2$
  - Irreversible
  - Components: • Heme a $a_3$ / cyt a $a_3$
    • Cu A-Cu B

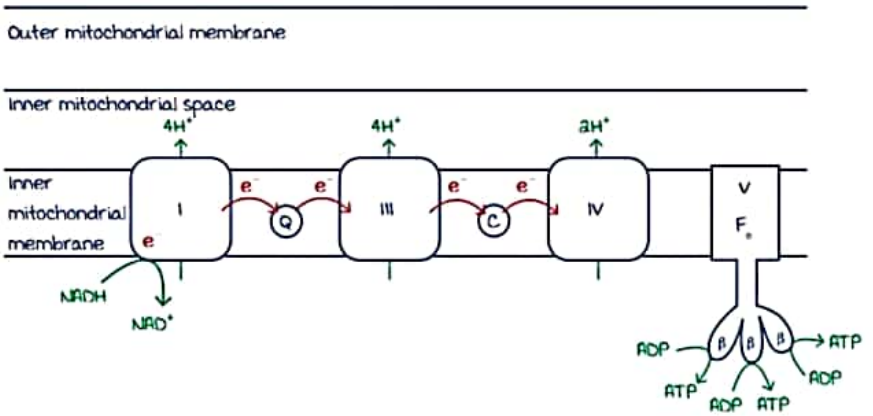
## ETC: Complex V 00:24:39

- ATP synthase
- Complex V: • $F_0$ subcomplex
  • $F_1$ subcomplex
- $F_0$ subcomplex:
  - made of 10 C disc proteins
  - Hydrophobic
  - Spans inner mitochondrial membrane
  - Proton channel
- $F_1$ sub unit:
  - made of 9 subunits → • 3 $\alpha$
    • 3 $\beta$ → ATP synthesising subunit
    • $\gamma, \delta, \varepsilon$
    ↑
    Rotatory subunit

Active space

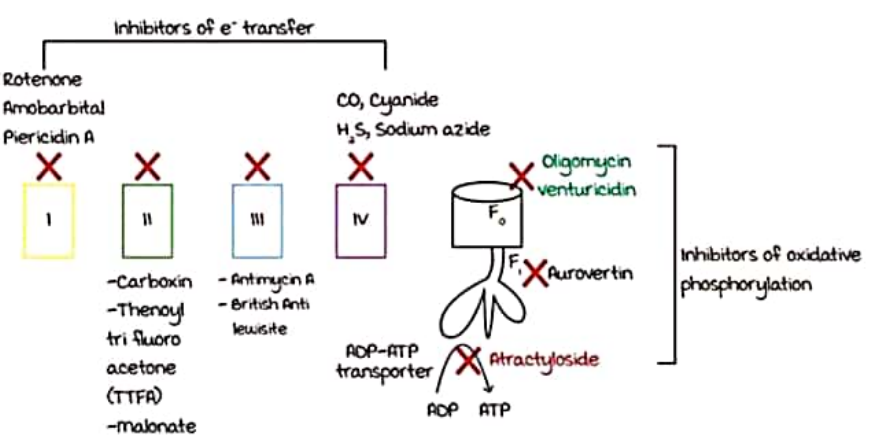
## Oxidative phosphorylation 00:29:57

Oxidation phase + phosphorylation phase




- Oxidation phase:
  - $e^-$ jumping
  - Release of free energy
  - Pump $H^+$ to intermembrane space
- Phosphorylation:
  - Create potential difference ( due to pumping of protons)
    ↓
  - $H^+$ moves to mitochondrial matrix through $F_o$ subunit
    ($F_o$ subunit- proton channel)
    ↓
  - Rotation of γ subunit
    ↓
  - Conformational change in β subunit : ADP → ATP
- This theory is called → Chemiosmotic theory
  By Peter Mitchell

## Inhibitors of ETC 00:41:43




Active space

## Uncouplers 00:50:53

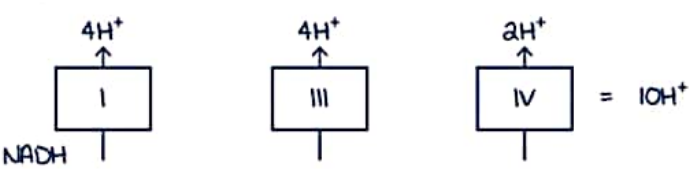
- Chemical uncouplers:
  - 2, 4 dinitrophenol
  - Dinitrocresol
  - FCCP ( fluoro carbonyl cyanide phenyl hydrazine )
  - Aspirin high dose
- Physiological uncouplers:
  - Thermogenin ( UCP - 1 )
  - Thyroxine
  - Long chain Fatty acid
  - Unconjugated bilirubin

## Ionophores 00:54:05

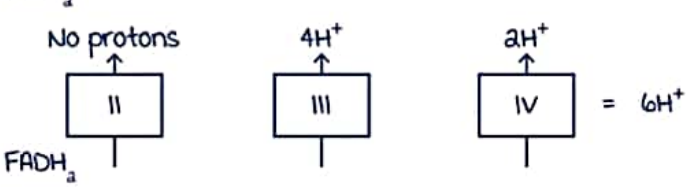
- Channel formers
- Dissipates proton gradient → inhibit $e^-$ transport
- Valinomycin
- Nigercin
- Gramicidin

## NADH & $FADH_2$ at ETC 00:56:06

- NADH:




- $FADH_a$:




- ∴ NADH > $FADH_a$
  (10) (6)

  ∴ NADH → $10H^+$ → 2.5 ATP

  $FADH_a$ → $6H^+$ → 1.5 ATP

Active space

- In brown adipose tissue 1 NADH → 0 ATP
  - ∵ Thermogenin inhibits phosphorylation
  - Functions:
    - Generate heat in hibernating animals & neonates
    - Prevent hypothermia
    - Non shivering thermogenesis

## High energy compounds 01:01:40

- Produce free energy > 7 Kcal
- Eg: • Phosphoenol pyruvate (highest energy)
  - Carbamoyl $PO_4$
  - 1, 3 BPG
  - Creatine $PO_4$

Active space

# CHEMISTRY OF LIPIDS

- Lipids : Heterogenous group of compounds soluble in nonpolar solvents ( ether, chloroform ) and insoluble in water
- Related more physically than chemically

## Classification of lipids

00:04:58

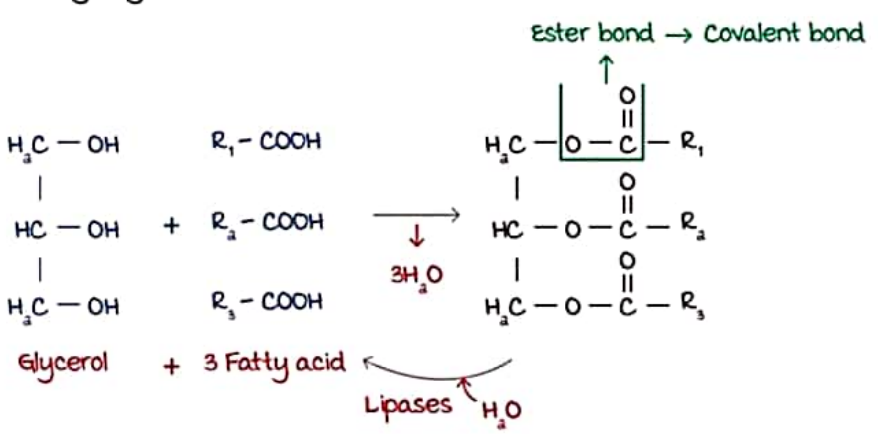
* Bloor's Classification

1. Simple lipids
2. Complex / Compound lipids
3. Derived lipids
4. Miscellaneous lipids

1. Simple lipids:

- Esters of fatty acid and alcohol (m/c : Glycerol)
- Eg:
  - i) Fats → • Triacyl glycerol
    • Solids at room temp
  - ii) Oils → • Liquid at room temp
  - iii) Waxes → • fatty acid + High Molecular weight alcohol

Triacyl Glycerol (Neutral fat)




2. Compound lipid / complex lipid

Fatty acid + Alcohol (glycerol) + other group

Active space

- If the other group is
  - $PO_4$ → Phospholipid
  - Carbohydrate → Glycolipid
  - $SO_4$ → Sulfolipid
  - Protein → Lipoprotein

3. Derived lipids:

* Derived from simple or complex lipid
* Precursors of other group
* Eg: • TAG $\xrightarrow{\text{Lipase}}$ 3 Fatty acid + Glycerol
  (Fatty acid, Glycerol → Derived lipids)
  - Fat soluble vitamins
  - Hormones
  - Steroids
  - Ketone bodies

## Fatty acid

00:19:08

- General formula: R-COOH
  ↓
  Aliphatic hydrocarbon chain

Classification of fatty acid

- Based on number of carbons on "R"
  I. Short chain fatty acids (SCFA) : $C_2$-$C_6$
  II. Medium chain fatty acids (MCFA) : $C_8$-$C_{14}$
  III. Long chain fatty acids : > $C_{16}$
  Very long chain FA (VLCFA) → > $C_{20/22}$
- Based on presence of double bond
  I. Saturated fatty acids → No double bond
  II. Unsaturated fatty acids → a) MUFA: 1 double bond
  b) PUFA: > 1 double bond

Active space

Saturated fatty acids:

i) SCFA – liquids in room temperature

| | | No. of C | Source |
|---|---|---|---|
| 1) Acetic acid | $CH_3COOH$ | 2 | → Vinegar |
| 2) Propionic acid | $CH_3-CH_2COOH$ | 3 | Butter |
| 3) Butyric acid | $CH_3(CH_2)_2COOH$ | 4 | |
| 4) Valeric acid | $CH_3(CH_2)_3COOH$ | 5 | |
| 4) Capric acid | $CH_3(CH_2)_4COOH$ | 6 | |

ii) MCFA – liquids in room temperature

| | | |
|---|---|---|
| 1) Lauric acid | 12 | Coconut oil/ Milk (richest) Butter |
| 2) Myristic acid | 14 | |

(iii) LCFA – solids in room temperature

| | | |
|---|---|---|
| 1) Palmitic acid (most abundant) | 16 | Animal fat |
| 2) Stearic acid | 18 | |

Unsaturated fatty acids

i) MUFA

| | | |
|---|---|---|
| 1) Palmitoleic acid | 16 | mustard oil/ Rapeseed Oil (richest) |
| 2) Elaidic acid | 18 | |
| 3) Oleic acid | 18 | |

ii) PUFA

| | No. of C | No. of double bond | source |
|---|---|---|---|
| 1) Linoleic acid | 18 | 2 | Safflower Oil |
| 2) Alpha Linolenic acid | 18 | 3 | Flax Seed Oil |
| 3) Gamma linolenic acid | 18 | 3 | Oil of evening primrose Borage oil |
| 4) Arachidonic acid | 20 | 4 | Animal fat |
| 5) Timnodonic acid | 20 | 5 | Fish Oil |
| 6) Cervonic acid | 22 | 6 | Breast Milk |

Active space

- Richest source of PUFA: Safflower oil

  2nd : Sunflower oil

  Least : Coconut oil

Essential fatty acids

- Linoleic acid → most essential Fatty acid
- α- Linolenic acid

Semiessential fatty acids

- Arachidonic acid
- Gamma linolenic acid (GLA)

} Derived from Linolenic acid

## Naming of double bond

00:45:10

Δ Numbering:- Δ 3

8 7 6 5 4 3 2 1

$CH_3-CH_2-CH_2-CH_2-CH=CH-CH_2-COOH$

ω .....γ β α

1 2 3 4 5 6 7 8 : $-\omega_5$ FA

- Based on ω classification there are three main group

| $\omega_3$ FA | $\omega_6$ FA | $\omega_9$ FA |
|---|---|---|
| Alpha linolenic acid | GLA | |
| Timnodonic acid | ↑ | |
| Cervonic acid | Linoleic acid | |
| | ↓ | |
| • ↓ Inflammation | Arachidonic acid | |
| • ↓ Cardiovascular risk | ↓ | |
| | Gives rise to prostaglandins and leukotrienes | |
| | ↓ | |
| | • mediators of inflammation | |
| | • ↑ Cardiovascular risk | |

Active space

## Significance of omega 3 fatty acid 00:51:48

1) ↓ inflammation
2) ↓ Cardiovascular risk
3) ↓ risk of ADHD
4) ↓ risk of Rheumatoid Arthritis
5) ↓ risk of Alzheimer's disease and cancer

## Cervonic acid / Docosahexaenoic acid 00:53:18

- $\omega_3$ FA
- Fish oil / breast milk
- Needed for infant/ foetal brain development and retina
- ↓ DHA antenatal → risk of Retinitis pigmentosa
- Can pass transplacentally

## Cis & Trans fatty acids 00:55:58

- Cis fatty acids → most naturally occurring fatty acids in body
- Kinking / bending seen in cis form at double bonds
- Cis form ↑ fluidity of the membrane
- Trans form is linear

Trans fatty acid

- Sources:
  - Partial hydrogenation: Vanaspati / Dalda / Cake Butter / Margarine
  - Bakery food
  - Fried rice
- more unsaturation – Liquid at room temperature
- Oxidative cleavage or hydrolytic cleavage

↓

Short fatty acids which are volatile

↓

Rancidity

Active space

Liquid ← CH=CH
↓ ← $H_2$
Solid ← $CH_2$-$CH_2$

"Partial Hydrogenation"
↓ Hardening of fat
↓ Rancidity
↑ Shelf Life

(2) Reheating of oil (deep fried items)
(3) High British thermal units – ↑ trans fatty acid
↓
fast food

## Ill effects of fatty acids 01:09:50

* ↑ LDL
* ↑ TG
* ↑ atherosclerosis
* ↓ fluidity
↓
↑ Rigidity →
↓
Insensitive to ligands

↑ DM & metabolic syndrome
↑
↑ insulin resistance
↑
insulin receptor
↑
↓ sensitivity of receptor

## Polarity of different lipids 01:12:41

Non polar
① Triacyl glycerol (neutral fat)
② Cholesterol ester

Amphipathic
① Fatty acids
② Phospholipid
③ Cholesterol

Active space

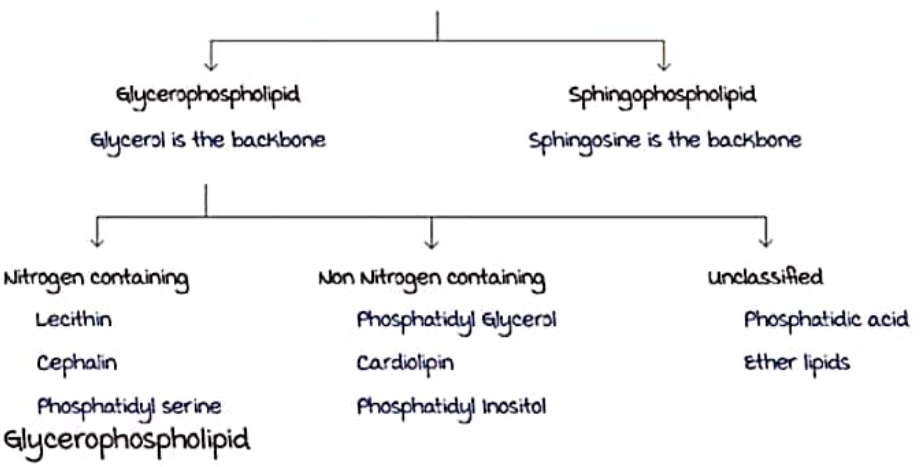
# PHOSPHOLIPIDS

* Phospholipids are compound lipids with phosphoric acid

* Parts: 1) Alcohol
  2) Fatty acid
  3) $PO_4$
  4) Base - Nitrogen containing or non nitrogen containing

## Classification of phospholipids

00:02:54




Glycerophospholipid

Glycerol

Ester linkage

$CH_2 - O - CO - R_1$

$CH - O - CO - R_2$

} Hydrophobic tail consists of Fatty acid

$CH_2 - O - PO_4$ - Nitrogenous / Non nitrogenous base

## Phosphatidic acid

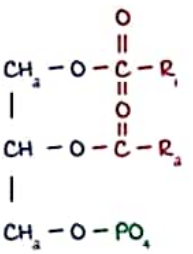
00:08:57

* Simplest glycerophospholipid
* All glycerophospholipids are derived from phosphatidic acid
* Glycerol + 2 Acyl group + $PO_4$

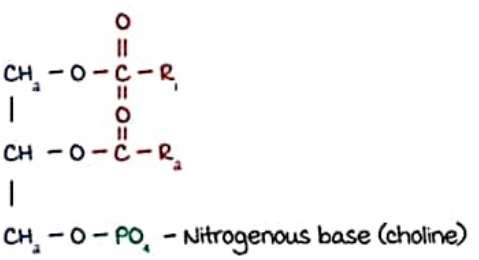
Diacyl glycerol

Active space

* No nitrogenous base



## Lecithin 00:12:27




Diacyl glycerol + $PO_4$ + Nitrogenous base ⇒ Lecithin
(Phosphatidic acid) (choline) (Phosphatidyl choline)

Significance

i) most abundant phospholipid in cell membrane
ii) most abundant phospholipid in Lung surfactant
iii) Store house of Choline

## Cephalin , Phosphatidyl serine, Phosphatidyl glycerol 00:18:23

Cephalin

* Phosphatidic acid + Nitrogenous base ⇒ Cephalin
(Ethanolamine) (Phosphatidyl ethanolamine)

Phosphatidyl serine

* Phosphatidic acid + Nitrogenous base
(Serine)
* mediator of apoptosis / Programmed cell death

Phosphatidyl glycerol

* Phosphatidic acid + Glycerol

## Phosphatidyl inositol 00:23:00

* Present in cell membrane
* mediator / source of second messengers in hormonal

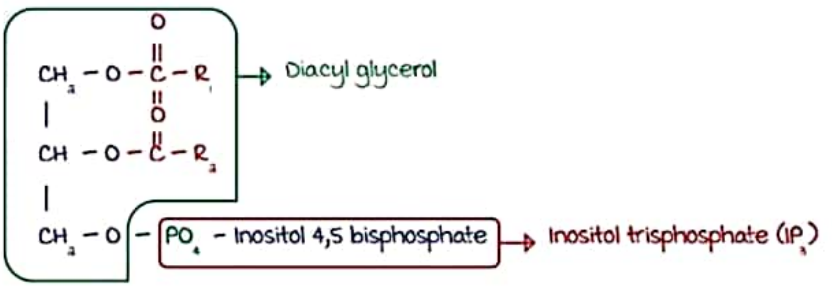
Active space

* Glycerol + 2 Acyl group + $PO_4$ + Inositol

  Diacyl glycerol

* $PIP_2$ (Phosphatidyl Inositol 4,5 bisphosphate)

*




## Cardiolipin 00:27:27

* Diphosphatidyl Glycerol
* No nitrogenous base

Significance

* Isolated first from cardiac muscle, hence the name
* Only antigenic phospholipid

  Cross react with antibodies raised against Treponema pallidum

  ∴ False +ve results in test for syphilis

* Present in inner mitochondrial membrane

  Disorders associated with defect in cardiolipin are:

  1) Cardioskeletal myopathy (Barth syndrome)
  2) Aging
  3) Heart failure
  4) Hypothyroidism

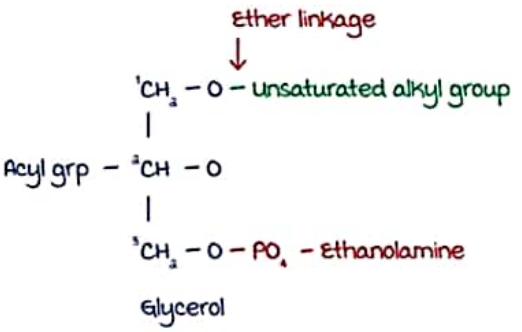
## Ether lipids 00:34:04

i) Plasmalogen

ii) Platelet Activating Factor

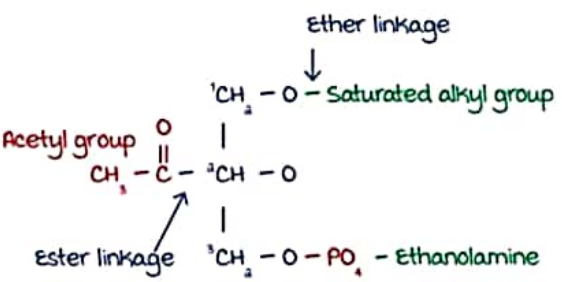
Active space

Plasmalogen




" Nitrogen containing glycerophospholipid"

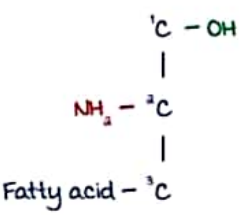
Platelet activating factor




" Nitrogen containing glycerophospholipid"

## Sphingophospholipid 00:39:52

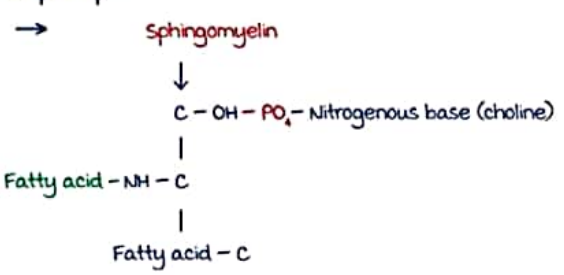
Sphingosine




* Amino alcohol
* Derived from serine + palmitic acid

## Sphingomyelin 00:42:35

* Only one sphingophospholipid




* Sphingosine + Fatty acid + $PO_4$ + choline

Ceramide

Active space

Significance

1) Cell membrane

2) Specialised structures in cell membrane – lipid rafts

3) Myelin sheath of Nervous tissue

4) White matter of brain

Active space

# GLYCOLIPIDS

*A/K/A Glycosphingolipids.

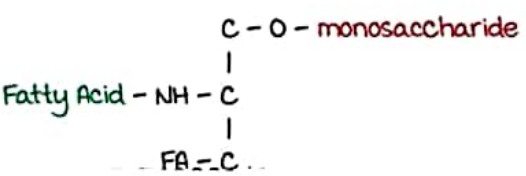
* Non phosphorylated sphingophospholipid

* Compound lipids with carbohydrate

| Phospholipid | Glycosphingolipid |
|---|---|
| * Glycerol or Sphingosine | * Sphingosine |
| * $PO_4$ present | * No phosphate group |

## Cerebroside

00:04:33




* If the monosaccharide is – Glucose : Glucocerebroside

Galactose : Galactocerebroside

Glucocerebroside

- Present in non-neural tissues

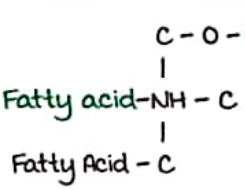
Galactocerebroside

- Present in neural tissue
- Fatty acid attached to Sphingosine : Cerebronic acid (24 C)

## Globoside

00:07:30

ceramide + disaccharide / oligosaccharide



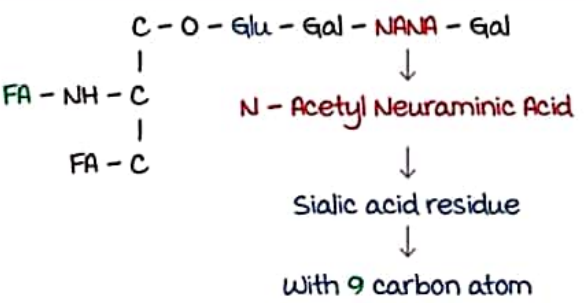

- Lactosyl ceramide
- Globotriaosyl ceramide

Active space

## Ganglioside

00:08:53

*




Ceramide + oligosaccharide (NANA)

* Named as G M$_n$

Ganglioside | No: assigned based on chromatography

Monosialo containing

GM$_1$

* Ganglioside that act as receptor for cholera toxin in human intestine

GM$_3$

* Simplest ganglioside
* Sphingosine + Fatty acid + Galactose – Glucose – NANA

(Sphingosine + Fatty acid) = Ceramide

Active space

# SPHINGOLIPIDOSES

* Metabolic disorder associated with defect in degradation of sphingosine containing compounds: Sphingophospholipids
  Glycosphingolipids

## Basic biochemical defect 00:03:09

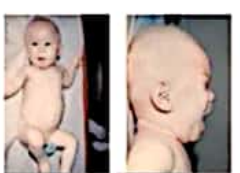
* Lysosomal storage disorder
* Defect in lysosomal hydrolase
  ↓
  Defect in degradation of sphingosine containing compounds
  ↓
  Accumulation of lipid substrate in lysosomes (intralysosome)
  ↓
  They all have N-Acyl Sphingosine ⇒ Ceramide

## GM, gangliosidoses 00:08:13

* $GM_1$ ganglioside —(β – Galactosidase, ✕)→ $GM_a$ ganglioside
* $GM_1$ ⇑⇑

Clinical features

1) Facies :
   - Low set ears
   - Long philtrum
   - Depressed nasal bridge
   - Frontal bossing
2) Hepatosplenomegaly
3) Angiokeratoma
4) Developmental delay
5) Blindness
6) Deafness
7) Cherry red spot in macula



Frontal bossing, depressed nasal bridge, long philtrum, low set ears

Cherry red macular spot

Active space

## $GM_2$ Gangliosidoses 00:12:01

* $GM_2$ —(β – Hexosaminidase, ✕)→ $GM_3$
* ↑↑ $GM_2$

1) Tay sach's disease
   - Defect in : β – Hexosaminidase A
   - α subunit is defective

2) Sandhoff's disease
   - Defect in : β – Hexosaminidase A & B
   - β submit is defective

Clinical features

* Tay sach's disease: 1) Developmental delay
  2) Neurological deficits
  3) ↑ Startle reflex (hyperacusis)
  4) Cherry red, spot in macula
* Sandhoff's disease: • All above features
  ⊕
  Hepatosplenomegaly
  Cardiac abnormalities

## Krabbe's disease 00:19:58

* Galactocerebroside —(β – Galactocerebroside β – Galactosidase, ✕)→ Ceramide
* ↑↑ Galactocerebroside
* Severe Neurological deficits
* No Hepatosplenomegaly
* Cherry red spots +/–

Active space

## Gaucher's disease 00:24:27

* m/c lysosomal storage disorder

* Biochemical defect :

Glucocerebroside —(β – Glucosidase/Glucocerebrosidase ✕)→ Ceramide

* ⇑ Glucocerebroside
  ↓
  Present in Non neural tissue (Reticulo endothelial system)
  ∴ No mental retardation (Intellectual disability)

* Organomegaly

| Krabbe's disease | Gaucher's disease |
|---|---|
| * ↑ Galactocerebroside ↓ | * ↑ Glucocerebroside ↓ |
| • Neural tissue | • Non Neural tissue |
| • Severe neurological deficit | • No neurological deficit |
| • No visceromegaly | • visceromegaly ++ |

Clinical features

* ↑ Glucocerebroside in RES
  ↓
  Hepatosplenomegaly

* No mental retardation

* No cherry red spot
  Exception : Type II Gauchers (Pseudo cherry red spot)

* Accumulation of Glucocerebroside in Bone marrow:
  - Pancytopenia
  - ↓ Thrombocytes → Bleeding manifestation
  - Pain and pathological # long bones

Active space

Treatment

* Enzyme replacement therapy (ERT)
  - Acid β glucosidase (Imiglucerase)
  - (Other ERT): i) Velaglucerase α
    ii) Taliglucerase α
* Substrate Reduction Therapy
  - Miglustat → ⊖ Glucosyl ceramide synthase
* Bone marrow transplantation

Diagnosis

* X-ray Femur: Erlenmeyer flask deformity
* Bone marrow biopsy: Gaucher cell

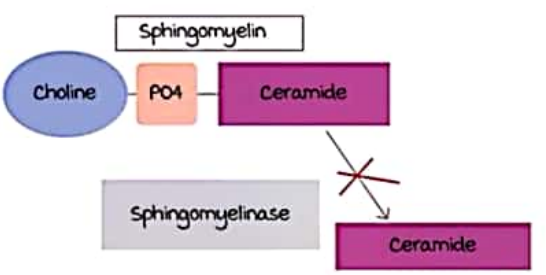
↓

Crumpled tissue paper or wrinkled paper appearance

## Niemann – Pick disease

00:36:01

* Enzyme defect: Sphingomyelinase
* ↑↑ Sphingomyelin
* Cherry red spot ⊕
* Zebra body inclusions




## Farber's disease

00:38:22

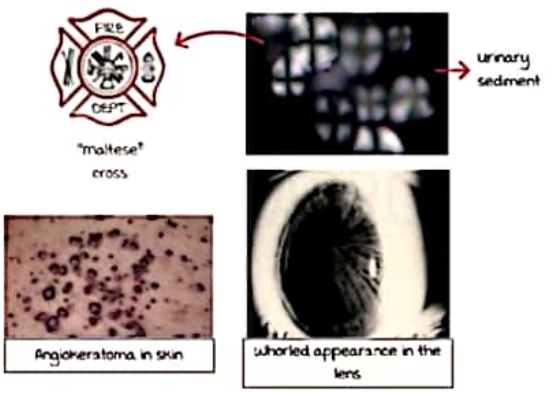
Ceramide —(Ceramidase ✕)→ Sphingosine + fatty acid

* Resemble Rheumatoid arthritis :
  Pain, Swellings, nodules ⊕ in joints

Active space

## Fabry's disease

00:40:42




* X-linked recessive disorder
* Biochemical defect: α galactosidase
* ↑↑ Globotrioasyl ceramide

Clinical features

* Angiokeratoma in bathing trunk areas
* Hypohydrosis
* Fabry's crisis: Agonising pain and inflammation of proximal joints
* Urinary sediments : Lipid inclusions excreted in urine

↓

maltese cross appearance

* Corneal and lenticular opacities – whorled appearance in lens

Treatment

* Enzyme replacement therapy :
  1) Recombinant α – Galactosidase
     (OR)
     Agalsidase β (OR) or Fabrazyme
  2) Agalsidase α

Active space

## Wolman's disease 00:45:59

* Defective enzyme: Acid lipase
* ↑ TAG, cholesterol esters in histiocytic foam cell
* Lysosomal storage disorder
* Not a sphingolipidosis

- Watery green Diarhoea
- Failure to thrive
- Relentless vomiting
- Hepatosplenomegaly
- Calcification of Abrenals


metachromatic leukodystrophy

* Enzyme defect: Arylsulfatase A

Pathognomic feature

## General characteristics of sphingolipidoses 00:48:35

* All are Autosomal recessive except Fabry's disease (X linked Recessive)

* Sphingolipidoses with no cherry red spot on the macula
  - Fabry's disease
  - Gauchers disease

* Sphingolipidoses with no mental retardation : • Fabru's disease
  • Gauchers type I

* With no hepatosplenomegaly: • Fabry's disease
  • metachromatic Leukodystrophy
  • Krabbe's disease

* With corneal clouding: • Fabry's disease
  • GM1 gangliosidoses

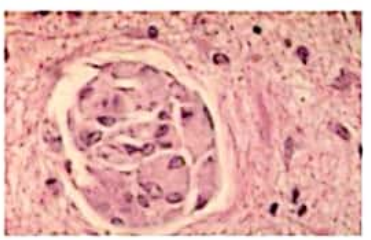
Active space

* Zebra body inclusions: Niemann Pick's disease

  Globoid cell inclusion: Krabbe's disease

- Zebra body inclusion
  Niemann Pick's disease

Globoid cell inclusion
Krabbe's disease



Active space

# FATTY ACID OXIDATION: BETA OXIDATION

Stages of fasting

1) 1-4 hrs after food : Well fed state

2) 4-16 hrs after food : Early fasting
→ Liver glycogenolysis

3) 16-48 hrs after food : Fasting state
→ Gluconeogenesis
→ β Oxidation of fatty acid ( FA )
↳ i) Provide ATP
ii) Acetyl CoA activates pyruvate carboxylase

4) 2 days – 5 days without food: Prolonged fasting / Starvation
→ Fatty acid oxidation
→ Ketone body synthesis

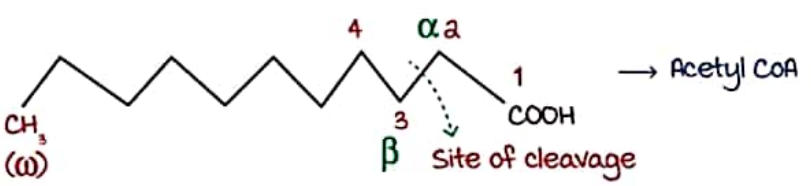
5) > 5 days: Prolonged starvation

## Different types of fatty acid oxidation 00:10:36

1) β oxidation – Saturated fatty acid (Palmitic Acid)
2) Oxidation of very long chain fatty acid
3) Oxidation of unsaturated fatty acid
4) Oxidation of odd chain fatty acid
5) Minor pathways of oxidation → a) α –oxidation
b) ω –oxidation

## β – oxidation of fatty acid 00:13:36

* Successive cleavage and release of a two carbon unit
→ Acetyl CoA




Active space

* m/c fatty acid that undergo β oxidation: Palmitic acid ($C_{16}$)
* m/c fatty acid oxidation → β - oxidation
* Sites of β-oxidation : 1) Liver
  2) Adipose tissue
  3) muscle

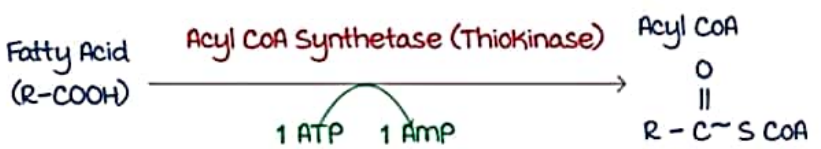
Organelle: Mitochondria

## Steps of β-oxidation

00:23:42

1) Activation of fatty acids
2) Transport of activated fatty acid to mitochondria
3) β - oxidation

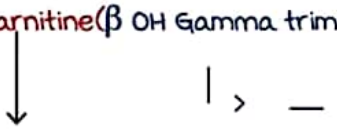
1) Activation of FA




* Requires a high energy $PO_4$
* Belongs to Ligase
* Only energy requiring step
* Takes place in cytoplasm
* Enzyme located in outer mitochondrial membrane

2) Transport of activated FA to mitochondria

* Transporter is Carnitine(β OH Gamma trimethyl ammonium butyrate)



synthesized in muscle[ Requires lysine + methionine(SAm) ]
* Fatty acids with carbon atom < 14 does not require carnitine

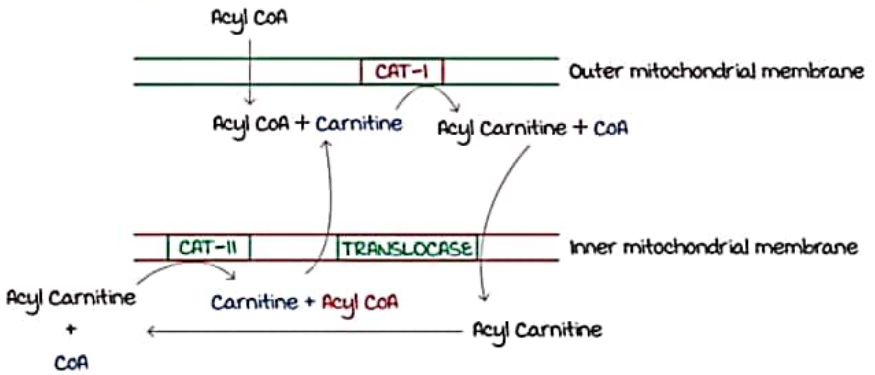
* Enzymes :

i) Carnitine Acyl Transferase I (CAT - I) / Carnitine palmitoyl transferase I (CPT - I) in outer mitochondrial membrane

ii) CAT-II / CPT II
iii) Carnitine Acylcarnitine Translocase } In inner mitochondrial membrane

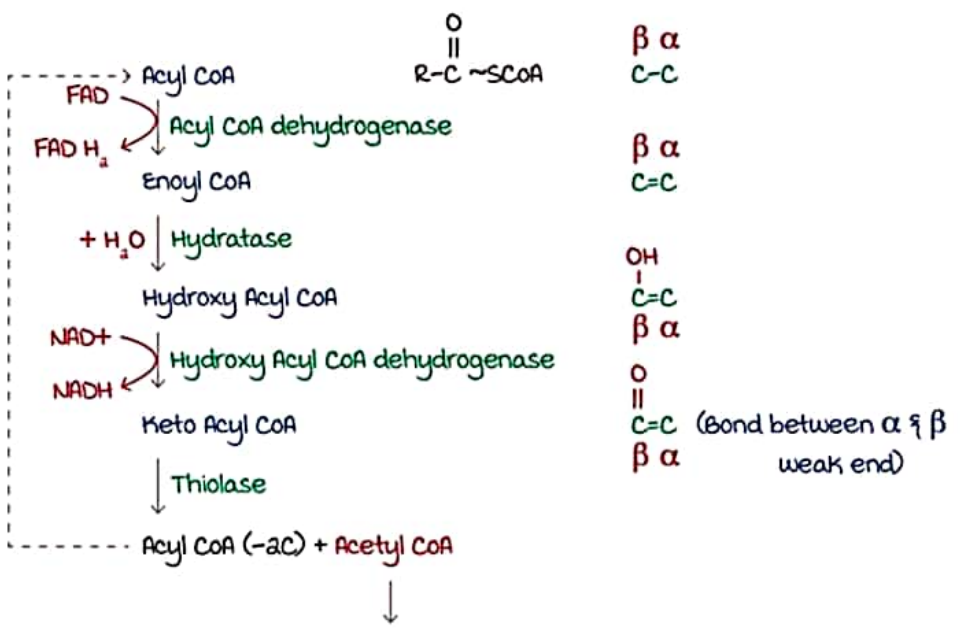
Active space

* Gateway of β oxidation → CAT-I / CPT-I




## 3) β-OXIDATION

* Happens inside Mitochondria




## Energetics of β – oxidation

00:56:31

* Palmitic Acid ($C_{16}$)

1) No. of β Oxidation $= \left(\frac{\text{No. of carbon atoms}}{2} - 1\right)$

$= \frac{16}{2} - 1 = 7$

2) No: of Acetyl CoA $= \frac{\text{No. of carbon atoms}}{2}$

$= \frac{16}{2} = 8$

Active space

* 1 β – oxidation = 1 NADH + 1 $FADH_2$

= 2.5 + 1.5 = 4 ATPs

* 1 Acetyl CoA → (TCA) → 10 ATPs

* Palmitic Acid: 7 β oxidation + 8 Acetyl CoA

= 7 × 4 + 8 × 10

= 108 ATPs

- Net ATP = 108 – 2 = 106 ATP

↓

Acyl CoA Synthetase requires 2 high energy $PO_4$

↓

2 ATP equivalence

## Regulation of β-oxidation

01:07:11

* Well fed state
  - Hormone: – Insulin
  - High insulin / glucagon ratio
  - ↑fatty acid synthesis
  - Intermediate – Malonyl CoA

↓

Allosteric inhibitor of CPT-I

* Fasting state
  - Hormone : – glucagon
  - Low insulin / glucagon ratio
  - ↓ Malonyl CoA → CPT I is open/active

Active space

# FATTY ACID OXIDATION: DISORDERS

## Jamaican vomiting sickness 00:01:15

* Ackee fruit contains toxin: Hypoglycin
* Inhibits Acyl CoA dehydrogenase

Ackee fruit
Jamaica (west africa)

∴ Inhibits β –oxidation
↳ i) ↓↓ ATP
ii) ↓ Acetyl CoA
} ↓ Gluconeogenesis

* Fasting hypoglycemia
* ↓ Acetyl CoA → ↓ Ketone body synthesis
∴ Non Ketotic fasting hypoglycemia

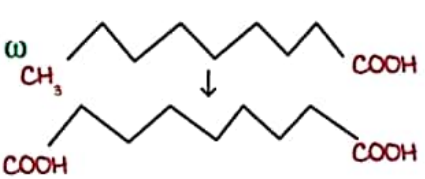
Clinical features
* Sudden onset of vomiting
* Fasting hypoglycemia
* No Ketone bodies
} – Coma convulsion death

## Medium chain Acyl CoA dehydrogenase deficiency 00:09:56

* m/c metabolic disorder associated with fatty acid
* Biochemical defect :

↓ MCAD → ↓ β –oxidation → ↓ ATP
↓ Acetyl CoA
↓ Gluconeogenesis

* Fasting hypoglycemia
* No Ketone bodies
* ↑ ω oxidation → ↑ dicarboxylic acid




Double headed fatty acid
or Dicarboxylic acid

Active space

Clinical features

- Seizure
- Coma
- Death
- Causes SIDS

Treatment

- Frequent meals – High carbohydrate
  Low fat

Active space

# MINOR FATTY ACID OXIDATION

## Very long chain fatty acid oxidation 00:01:30

* Occur in Peroxisome/ Glyoxysome
* modified β – oxidation
* Releases Acetyl CoA + $H_2O_2$

  ↓

  detoxified by Catalase
* Only upto Octanoyl CoA (8C)

  ↓

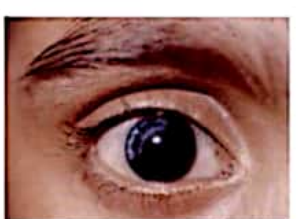
  To mitochondria where further ↓. oxidation happens
* Associated disorder : Zellweger syndrome

  Biochemical defect: – Peroxisomal protein targeting disorder

Clinical features

1) Mongoloid facies
2) Hypertelorism
3) High forehead
4) Upslanting palpebral fissure
5) Epicanthal folds
6) Brushfield spots in the iris


* Resembles Down's Syndrome



Brushfield spots in the iris

Diagnosis

1) ↓ No: of peroxisomes
2) Peroxisomal ghosts
3) ↑ VLCFA in plasma
4) ↑ Pipecolic acid in plasma
5) ↑ phytanic acid in plasma

Active space

## Unsaturated fatty acid oxidation 00:13:50

* Site: Mitochondria
* Modified β Oxidation
* Normal β Oxidation until double bond comes between α & β
* Acyl CoA dehydrogenase bypassed for every double bond in even position

Acyl CoA
↓
$FADH_2$ ← Acyl CoA dehydrogenase
↓
Enoyl CoA

* ∴ 1.5 ATP less for every double bond in even position

## Odd chain fatty acid oxidation 00:18:13

* Site: - Mitochondria
* β - Oxidation
* Products: Acetyl CoA + Propionyl CoA
  (Propionyl CoA: Glycogenic part of fat)
* Propionyl CoA (3C)
  ↓ Propionyl CoA Carboxylase: • Ligase
  D methyl Malonyl CoA (4C) • Require Biotin & ATP
  ↓ Racemase
  L methyl Malonyl CoA (4C)
  ↓ Mutase
  Succinyl CoA (4C) → (TCA) → Glucose

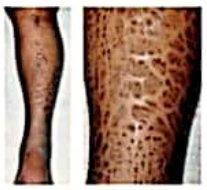
## α Oxidation 00:24:48

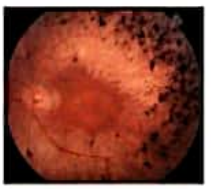
* Site: - Peroxisome & Endoplasmic reticulum
* No ATP is produced.
* ↓ β oxidation
* Occur to those fatty acid with a branch in β - carbon
* Phytanic acid: - Source → 1) Dairy product
  2) Green leafy vegetables
* 1 carbon group is released
* Defect in α oxidation: - Classic Refsums disease

Active space

Classic Refsums disease

* Defective enzyme: Phytanoyl CoA hydroxylase
  or
  Phytanoyl CoA oxidase
* Peroxisomal targeting disorder
* Clinical Features: –
  - Icthyosis
  - Retinitis pigmentosa
  - ↓ vision
  - Peripheral neuropathy
  - Ataxia

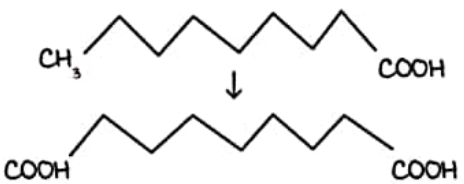

Icthyosis


Retinitis pigmentosa

## ω Oxidation

00:31:04

* ↓ β oxidation
* No ATP is generated
* Site: Microsomes (smooth endoplasmic reticulum)
* Enzyme: Mixed function oxidase




Double headed Fatty acid / Dicarboxylic acid

Active space

# KETONE BODIES

* Without food →
  i) 4-16 hrs (Early Fasting) → Glycogenolysis
  ii) 16-48 hrs (Fasting) → Gluconeogenesis
  iii) 2-5 day (Starvation) → β oxidation → Acetyl CoA
  ↓ (Ketone body synthesis)
  Metabolic fuel for extra hepatic tissues
  - Brain generate 20 % of energy from Ketone bodies.

## Starvation ketosis 00:04:10

I) Glycogen depleted
II) Gluconeogenic substrates depleted
III) β oxidation → Ketone bodies

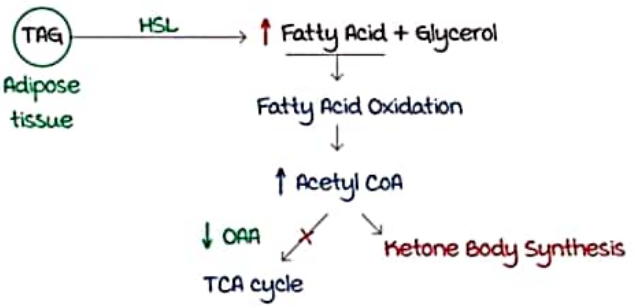
* Depletion of oxaloacetate ( due to ↑↑ Gluconeogenesis )

Triacylglycerol
↓
Fatty acid
↓ Fatty acid oxidation
Acetyl CoA
↓↓ OAA → ✗ TCA cycle
↘ Ketone body synthesis

## Diabetic ketoacidosis 00:07:42

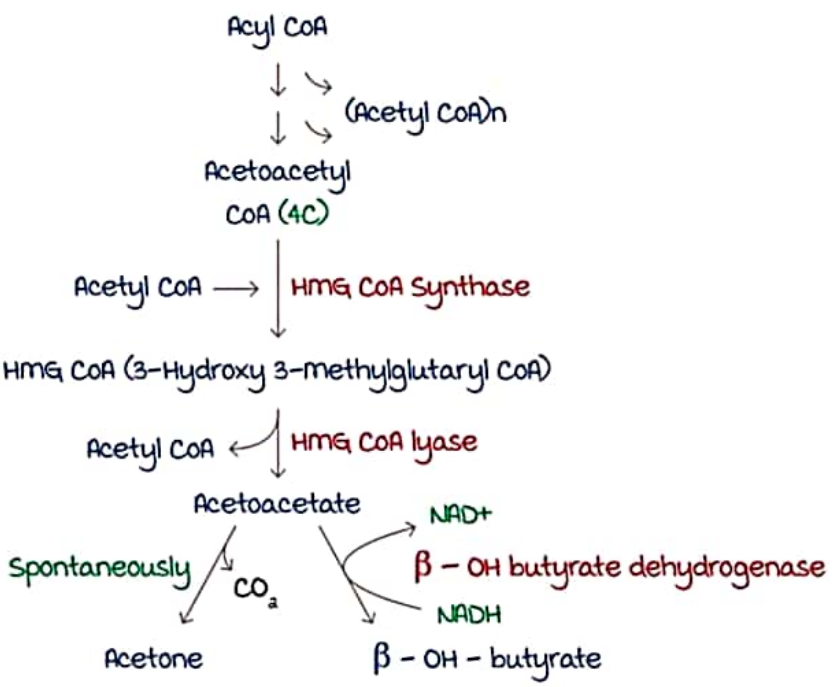
↓ Insulin → ↑↑ Glucose in blood vessels
But cell lacks glucose → Because GLUT 4 is not acting
↓
↑↑ Gluconeogenesis
∴ Depletion of oxaloacetate
↓
Insulin inhibits hormone sensitive lipase (HSL)
But ↓↓ Insulin → Activation of HSL

Active space




## Pathway of ketone body synthesis 00:14:27

* Occurring only in the liver inside mitochondria

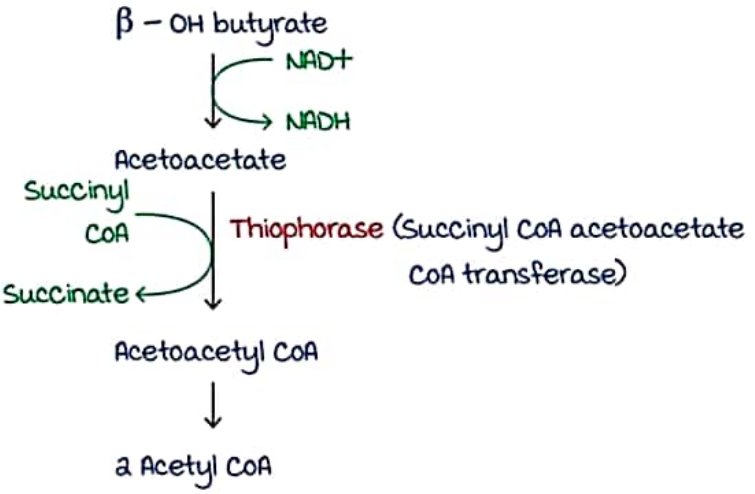



- Starting substrate → Acetoacetyl CoA
- Rate limiting enzyme → HMG CoA Synthase
- Ketone bodies: i) Acetoacetate : – 1° Ketone body
  ii) Acetone
  iii) β – OH butyrate } 2° Ketone bodies

Active space

## Pathway of ketone body utilization 00:23:32

* Occurs in extrahepatic tissues
* 2 organs that cannot utilize ketone bodies are
  1) Liver
  2) RBC




* Acetone is volatile → Excreted through lungs (Fruity smell)
* Energetics:
  - From acetacetate → 2 Acetyl CoA → TCA cycle
    ↓
    2 x 10 = 20 ATP

    Net ATP = 20–1 = 19 ATP

  - From β – OH butyrate → NADH + 2 Acetyl CoA
    = 2.5 + 20 = 22.5 ATP

    Net ATP = 2.5 + 19 = 21.5 ATP

## One liners in ketone body synthesis 00:33:22

* MC Ketone body in normal person:
  β OH butyrate: Acetoacetate = 1:1
* MC Ketone body in Ketosis:
  β OH butyrate: Acetoacetate = 6:1
* Spontaneously formed Ketone body = Acetone
* Neutral Ketone body:- Acetone

Active space

* Enzyme common to cholesterol synthesis & Ketone body synthesis → HMG CoA synthase

* Enzyme that utilise Ketone body → Thiophorase

## Test for ketone body 00:37:24

1) Rothera's test
   * Positive test: Purple Ring
   * Positive in : Acetoacetate and acetone
   * β OH butyrate : Rothera's test Negative

2) Gerhardt's test
   * Positive only in acetoacetate

3) Ketostix
   * Dip stick test to detect Ketone bodies.

Active space

# FATTY ACID SYNTHESIS

* Occurs in well fed state
* High insulin glucagon ratio

FA + Glycerol → Triacyl Glycerol

SITE

* m/c FA synthesized in body → Palmitic Acid
* Site: Liver, adipose tissue, Kidneys, brain, lungs, lactating mammary gland
* Organelle: Extramitochondria in cytoplasm

## Steps of fatty acid synthesis 00:04:55

* Elucidated by Feodor Lynen
  Hence aka Lynen's spiral
* Starting material: Acetyl CoA (2C)
  ↳ Sources

1) Aerobic glycolysis → Pyruvate
   ↓ PDH (in mitochondria)
   Acetyl CoA

2) β – oxidation (in mitochondria)

I) Transfer of Acetyl CoA from mitochondria to cytoplasm

II) Acetyl CoA carboxylase

III) FA synthase complex reactions

I) Transport of Acetyl CoA

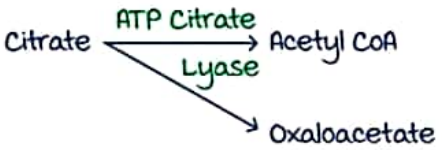
* Inside the mitochondria:

$$\underset{(2C)}{\text{Acetyl CoA}} + \underset{(4C)}{\text{Oxaloacetate}} \xrightarrow{\text{Citrate synthase}} \underset{(6C)}{\text{Citrate}}$$

* Citrate is a Tricarboxylic acid
* Tricarboxylic acid has a transpoter in the inner mitochondrial membrane → Tricarboxylic Acid Transporter

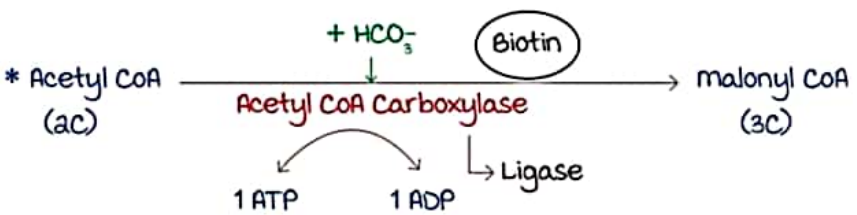
Active space

* Citrate comes out of mitochondria




2) Acetyl CoA Carboxylase

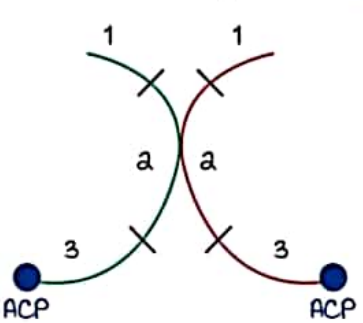
* It is a multienzyme complex




* Carboxylation reaction
* 1 carbon is added

3) FA synthase complex reactions

* Homodimer
* Each monomer unit has 6 enzyme activity + 1 Acyl Carrier Protein (ACP)
* ACP has Pantothenic Acid (Vit B5) in the form of 4 phospho pantetheine (-SH)
* Multifunctional enzyme
  - Single polypeptide has > 2 enzyme activity
* Shape: X – shaped (X-ray crystallography)




HOMODIMER

Each monomer unit has 3 parts:

- 1st unit (Domain): Condensing unit
- 2nd unit: Reduction unit
- 3rd unit: Releasing unit + Acyl carrier protein (ACP)

Active space

Condensing unit

1) Acetyl/Malonyl transacylase

2) Keto Acyl synthase

Reduction unit

1) Ketoacyl Reductase

2) Dehydratase

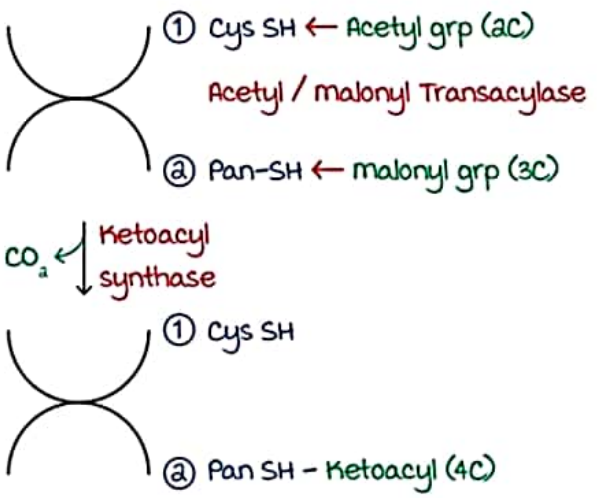
3) Enoyl Reductase

Releasing unit

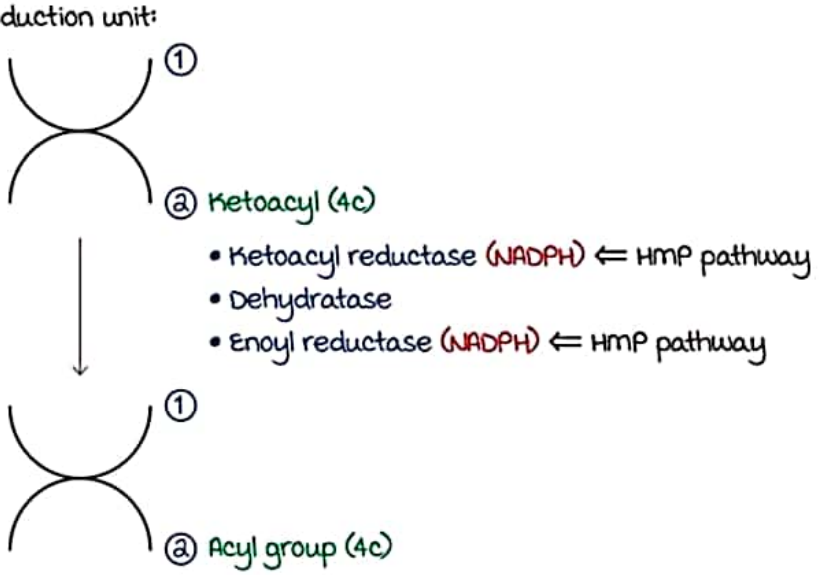
1) Thioesterase

- Acetyl CoA carboxylase is not a part of fatty acid synthase Complex

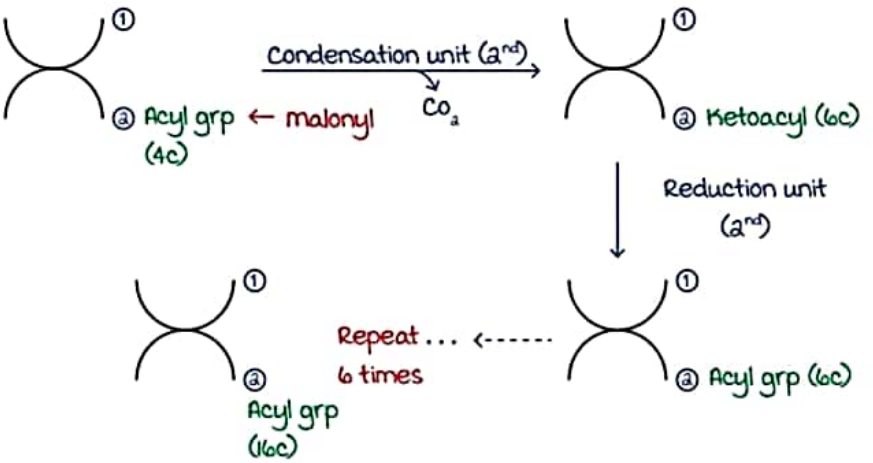
Condensation unit:




Reduction unit:

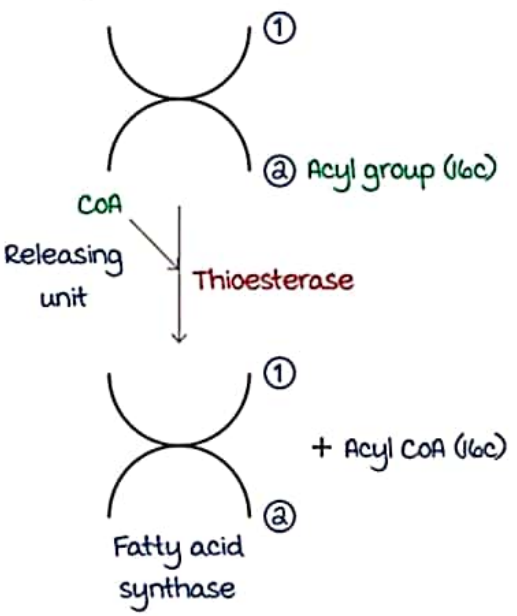



Active space




* (3C) malonyl CoA add (2C) for every repeat

Releasing unit




* Co-factor requirement: NADPH
$Mn^{2+}$

## Regulation

00:54:03

| Short term | Long term |
| --- | --- |
| • Allosteric regulation<br>• Covalent modification<br>• Compartmentalisation | Acetyl CoA<br>↓<br>↓ expression of enzymes that synthesize FA |

Active space

Allosteric regulation

* Rate Limiting Enzyme: Acetyl CoA carboxylase

* ∞ ∞ ∞ Acetyl CoA Carboxylase → dimeric form (Inactive)

↓ (+) citrate (–) Long chain FA

∞∞∞∞∞∞∞∞ → polymeric form (Active)

Covalent modification

* In well fed state
* Insulin
* Acetyl CoA carboxylase ⊕ in dephosphorylated state

Compartmentalisation

* β oxidation → Inside mitochondria
* FA synthesis → Outside the mitochondria

## Elongation of fatty acids 01:02:53

* Elongation of fatty acids occurs in
  i) Smooth ER ( By microsomal FA Elongase System){major}
  ii) Also by Mitochondrial FA Elongase{minor}
* ↑↑ myelination of brain

## Synthesis of unsaturated fatty acid 01:03:55

* Involve Desaturase and Elongase enzyme system in Endoplasmic reticulum
* Humans cannot insert double bond Δ9 (i.e b/w $C_{10}$ and terminal methyl group)

Active space

# CHOLESTEROL & BILE ACID SYNTHESIS

* cholesterol

Bile — Sterol ring

It is excreted through Bile

* Purely animal sterol
* Cannot generate energy

Use :

* Anabolic purpose
* Insulin → predominant role in controlling synthesis of cholesterol
* Cholesterol → cyclopentano perhydro phenanthrene ring (27 C)

## Pathway of synthesis of cholesterol 00:04:38

* Site: - All nucleated cells

Predominantly: -
- Liver, adipose tissue
- Adrenal cortex
- Gonads, instestine

* Organelle: SER & cytoplasm

Stages of cholesterol synthesis

* Synthesis of
  i) HMG CoA (6 C)
  ii) mevalonate (6 C)
  iii) Isoprenoid unit (5 C)
  iv) Squalene (30 C)
  v) Cholesterol

* 2 Acetyl CoA
  ↓ Thiolase
  Acetoacetyl CoA

Acetyl CoA → HMG CoA synthase (occurs in cytoplasm)

HMG CoA → 3 Fates:
i) Cholesterol synthesis
ii) Ketone bodies synthesis (mitochondrial HMG CoA synthase)
iii) Leucine catabolism

Active space

⊛ HMG CoA —(HMG CoA Reductase)→ Mevalonate (6C)

* Rate limiting enzyme
* Takes place in SER

⊛ Mevalonate (6C) —(Decarboxylation / Phosphorylation)→ Isoprenoid unit (5C)

⊛ 2 Isoprenoid units (5C + 5C) → Geranyl Pyrophosphate (10 C)

↓ ← Isoprenoid (5C)

2x Farnesyl Pyrophosphate (15C)

↓

Squalene (30C)

↓

↓

Lanosterol (1st cyclical compound)

↓

Zymosterol

↓

Desmosterol

↓

Cholesterol (27C)

## Functions of cholesterol

00:15:29

Cholesterol

- 50% → Bile Acids
- ↓ Vit D (7 dehydrocholesterol)
- → Steroid hormones
  - ↓
  - • Sex hormones
  - • Corticosteroids

## Regulation of cholesterol synthesis

00:17:49

1) Feedback regulation

Dietary cholesterol

↓

↓ Binding SREBP ( Steroid regulatory element binding protein ) at genes

↓

↓ expression of genes that synthesize HMG CoA reductase

- Long term regulation

Active space

II) Feedback Inhibition


III) Hormonal regulation

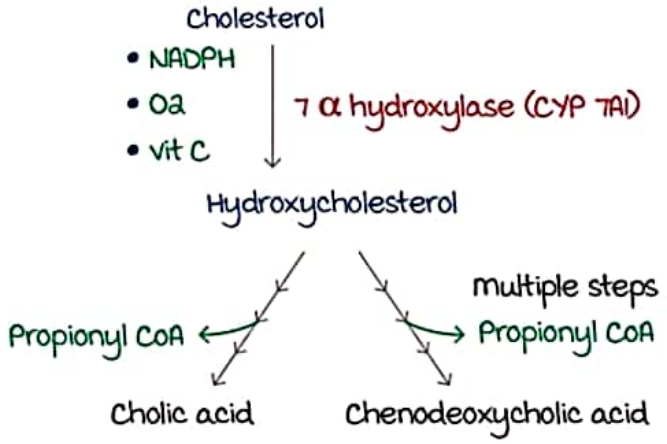
- Insulin ↑↑ cholesterol synthesis
- Well fed state
- Rate limiting enzyme : HMG CoA Reductase → Active in dephosphorylated state

## Bile acids 00:24:10

* Starting material → Cholesterol
* Bile acids → Excretory form of cholesterol

Pathway of synthesis of bile acid
I) Liver
II) Intestine

Liver: - Primary bile acids formed in liver




* Primary bile acids are conjugated with help of: I) Glycine
  II) Taurine

* Conjugated 1° bile acids are excreted through bile duct
  Bile duct is alkaline medium where bile acids are ionized by $Na^+/K^+$ to form Bile salts

* Conjugated bile acids are excreted through common bile duct to intestine

Active space

## Intestinal synthesis of 2° bile acids 00:30:16

* 1° bile acids undergo : • Deconjugation
  • Dehydroxylation

* Cholic acid → Deoxycholic acid → Enter enterophepatic circulation (98–99%)

Chenodeoxycholic acid → Lithocholic acid } 2° bile acids → Enter enterophepatic circulation (98–99%)

* Least enterohepatic circulation → Lithocholic acid

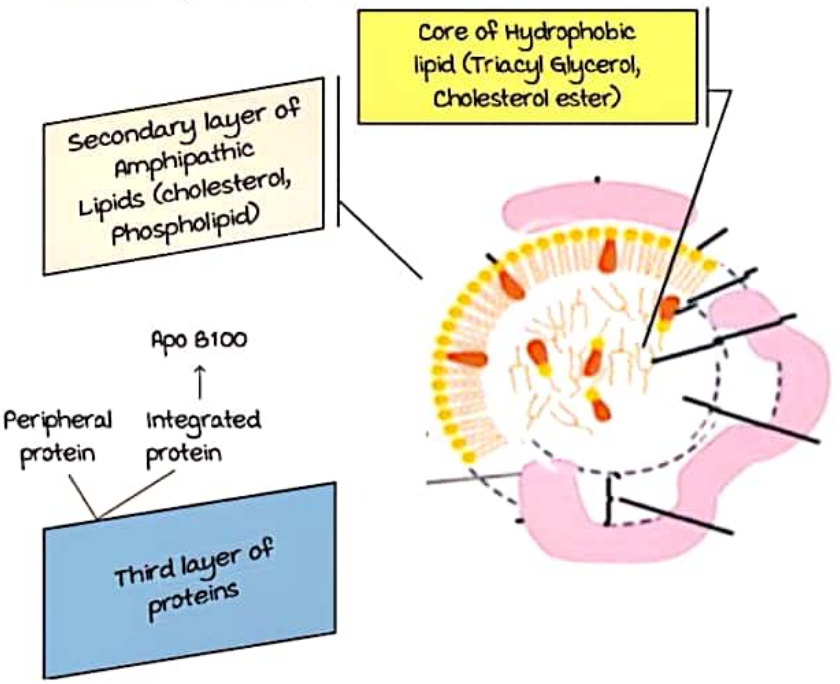
## Regulation of bile acid synthesis 00:35:28

* With help of Farnesoid X Receptor (FXR)
* Rate limiting enzyme: – 7 α hydroxylase (CYP 7A1)
* ↑ Bile Acid Pool → ↓ FXR → ↓ 7 α hydroxylase → ↓ Bile acid
* Chenodeoxycholic acid play a key role in regulation of FXR

Active space

# LIPOPROTEINS

* Compound lipids with proteins




## Chylomicrons

00:06:00

1) Carry exogenous / dietary TAG from intestine to peripheral organs
2) Maximum size
3) Maximum lipid content
4) Least protein content

} Least density: most bouyant

5) Maximum TAG / Exogenous TAG

* Apolipoproteins present in chylomicrons:
  - Uniquely present in chylomicron is – Apo B48
  - Other major apolipoproteins: Apo C II
    Apo E

## VLDL

00:10:16

* Assembled in Liver
* Carry endogenous TAG from liver to peripheral organs
* Apolipoproteins present: 1) Apo B100
  2) Apo C II
  3) Apo E

Active space

## LDL 00:11:32

* Formed from ILDL
* VLDL → IDL (Intermediate-density lipoprotein) → LDL
  (Lipoprotein cascade pathway)
* Maximum cholesterol / cholesterol ester
* Bad cholesterol
* Apolipoprotein: apo B100

## HDL 00:13:37

* Formed from intestine and liver
* Reverse cholesterol transport
* Good cholesterol
* Maximum protein content
* Maximum apolipoprotein content } maximum density
* Least lipid content
* Least size
* Maximum phospholipid
* Repository for apo E and apo C II
* Apolipoproteins: apo A,
* Enzyme activity → i) LCAT(Lecithin Cholesterol Acyl Transferase)
  ii) CETP (Cholesterol Ester Transfer Protein)

## LCAT &CETP 00:17:15

LCAT

Lecithin + Cholesterol
↓ LCAT
Cholesterol ester + Lysolecithin

CETP

* Transfer cholesterol ester from HDL to other lipoproteins
* Transfer TAG from other lipoproteins (IDL, LDL) to HDL

## LP (a) 00:22:05

* Has apo (a) and apo B100
* apo (a) linked to apo B100 by a disulphide bond
* apo (a) structural analog of plasminogen
* Inhibit clot lysis

* Risk factor for Thrombosis
* Indian population has high content of LP(a)

## Lipoprotein X

00:26:05

* Cholestasis: Cholesterol + Phospholipid

↓

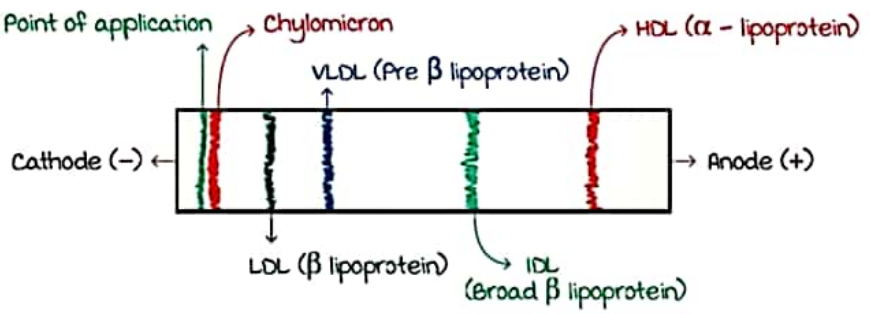
Lipoprotein X

⇓

Indicator of cholestasis

## Electrophoretic pattern of lipoproteins

00:27:56




* Band pattern
* Max protein = Max mobility

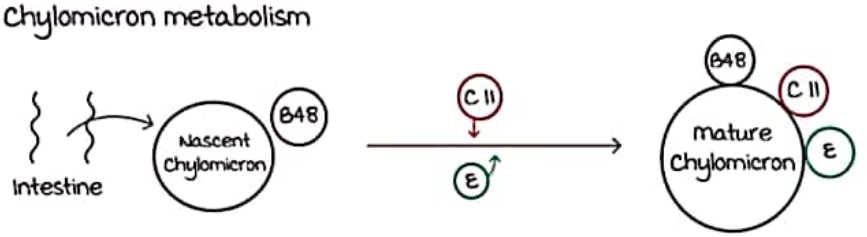
⇓

HDL

## Metabolism of lipoproteins

00:32:29

Chylomicron metabolism




↓

Passes through capillaries lining peripheral organs

↓

The vascular endothelium has LpL → Lipoprotein lipase
Heparan Sulphate is a GAG that anchors the LpL to vascular endothelium

* Action of LpL

TAG in Chylomicron → Glycerol + → Fatty Acid

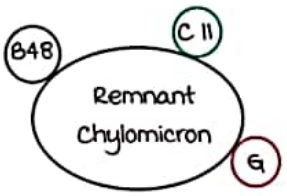
↓

Taken up by peripheral organs

Active space

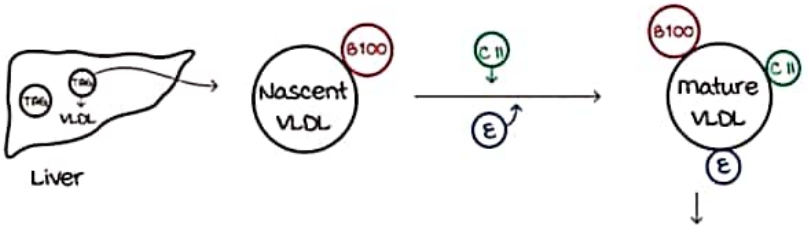
* Apo CII activates LpL

* Result:




- Taken up by liver
- apo E is ligand
- Receptor mediated endocytosis

VLDL & LDL METABOLISM




- Passes through capillaries lining peripheral organs with LpL in its endothelium

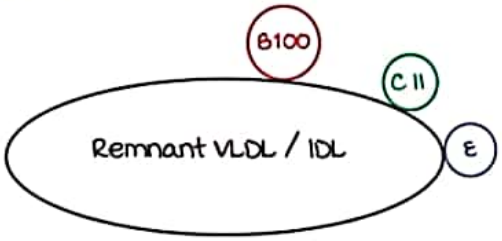
- Apo CII activates LpL

TAG ⟶ FA + Glycerol

↓

Taken up by peripheral organs

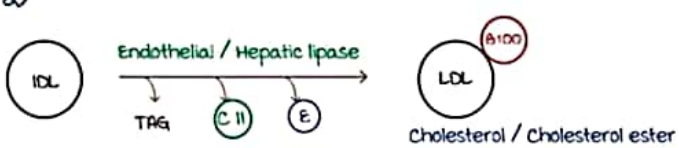
* Result:




↳ Fates:

1) Receptor mediated ondocytosis:
Taken up by liver
Apo E is ligand

2)




"Lipoprotein cascade pathway"

Active space

→ LDL
- 70% → Liver
- 30% → Extra hepatic organs

Apo B act as ligand

→ In presence of free radicals: LDL
↓
Oxidized LDL ← Taken up by macrophages
↳ Facilitate Atheroma formation in blood vessels

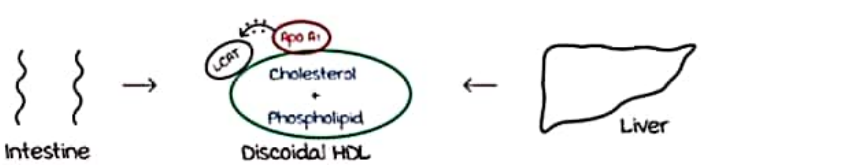
HDL METABOLISM

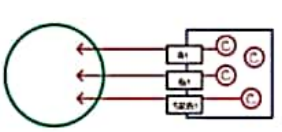
1) LCAT: Cholesterol (amphipathic lipid) ⟶ Cholesterol ester (hydrophobic)

2) Apo AI: Activates LCAT

3) Transporter:
- $ABCA_1$ (ATP Binding Cassette $A_1$)
- $ABCG_1$
- $SRB_1$ (Scavenger Recetor $B_1$)

} Transport cholesterol / Cholesterol ester from peripheral organs to HDL



Cholesterol ¶ cholesterol ester transported from peripheral organs to spherical $HDL_3$

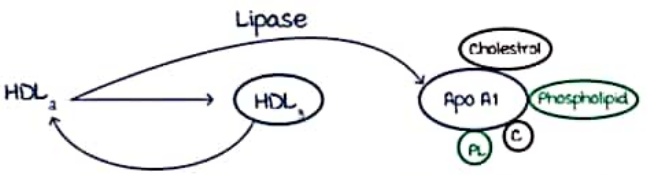
Spherical $HDL_3$ Peripheral organ

"Reverse Cholesterol Transport"

Spherical $HDL_3$ → Donates cholesterol to Liver → Cholesterol converted to bile acids ¶ excreted

Active space

PRE β HDL




Poorly lipidated HDL
(Pre β HDL)

* most potent HDL

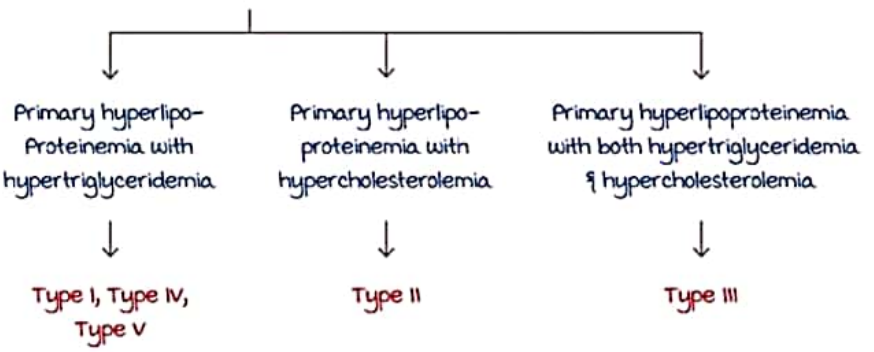
## Functions of apolipoproteins 01:15:15

- Apo C I → Inhibit cholesterol Ester Transfer protein
- Apo C II → Activate LpL
- Apo C III → Inhibit LpL
- Apo E → Ligand for chylomicron remnant / IDL, Arginine rich
- Apo B100 → Ligand for LDL
  → Assembly of VLDL
- Apo B48 → Assembly of chylomicron in intestine
- Apo A II → Inhibit LpL
- Apo A V → Facilitate binding of chylomicron & VLDL to lipoprotein lipase
- Apo D → Associated with human neurodegenerative diseases like parkinson's disease
- Apo E4 → Associated with Alzheimer's disease

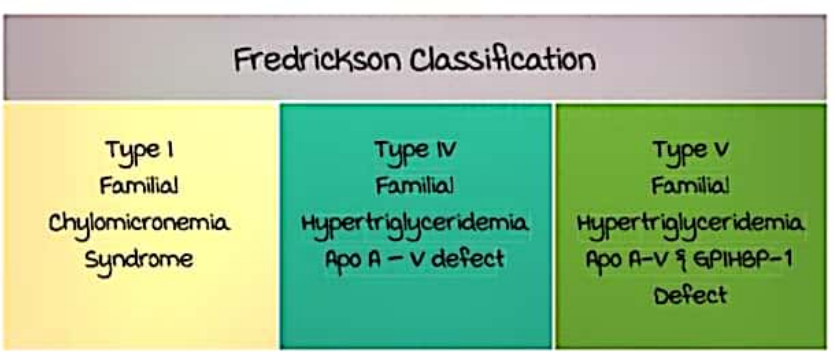
Active space

# HYPERLIPOPROTEINEMIA

* Classified by Fredrickson and Levy
* Primary hyperlipoproteinemia

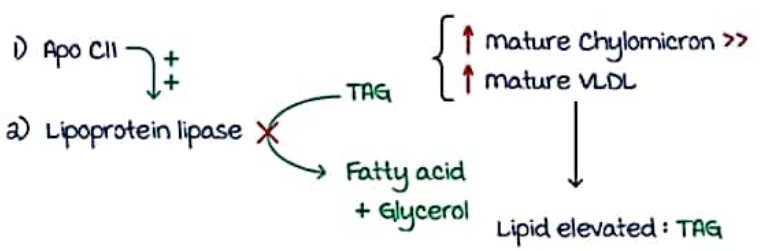



## Primary hyperlipoproteinemia with hypertriglyceridemia 00:06:43



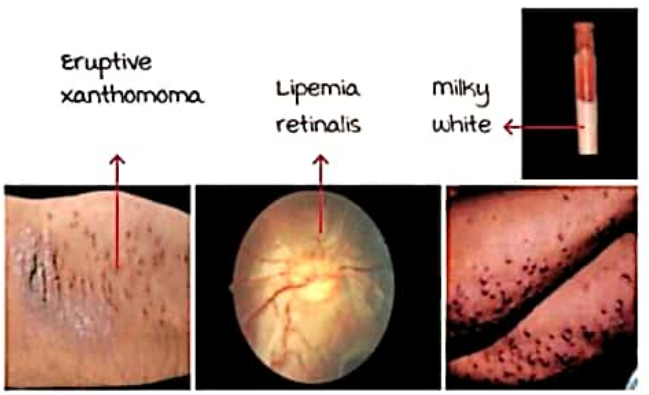

Type i hyperlipoproteinemia

* A/K/A Familial Chylomicromia Syndrome
* Biochemical defect




Active space

Clinical features




* Milky white plasma
* Eruptive xanthoma
* Lipemia retinalis
* ↑ TAG / 1000 mg /dl → Pancreatitis → Abdominal pain

Treatment

A Lipogene – "Tiparvovec"

- A gene therapy approach for Familial chylomicronemia
- Adeno associated Viral Vector expressing Gain of function LpL variant leading to skeletal myocyte expression of LPL

Type IV hyperlipoproteinemia

* Biochemical defect: Apo A V

↓

Facilitate the association of Chylomicron, VLDL with LPL

* ↑↑ Chylomicron, VLDL → ↑↑ TAG
* ∴ Familial Hypertriglyceridemia

Type v hyper lipoproteinemia

* 2 defects: i) Apo A V → ↑ TAG

ii) Glycosylated Phosphatidyl Inositol HDL Binding Protein 1 (GPIHBP1) → Helping export of LpL to vascular endothelium

Active space

## Primary hyperlipoproteinemia with hypercholesterolemia 00:19:47

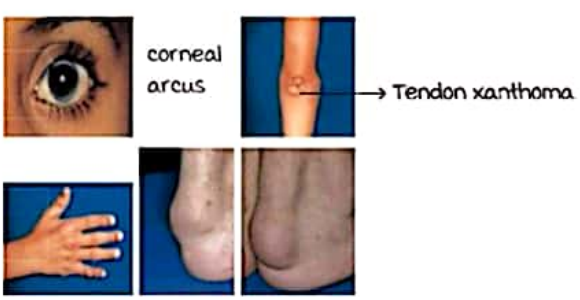
| Fredrickson Classification | | |
|---|---|---|
| Type IIa Familial Hypercholesterolemia (ADH I) most common | Type IIa Familial Defective Apo B (FDB) (ADH Type II) | Type IIa ADH Type III |

| Fredrickson Classification | |
|---|---|
| Type IIa Autosomal Recessive Hypercholesterolemia (ARH) | Type IIa Sitosterolemia |

## Familial hypercholesterolemia 00:23:03

* ADH ( Autosomal dominant hypercholesterolemia ) Type I
* most common
* Biochemical defect: LDL receptor
* Elevated lipoprotein: LDL
* Elevated lipid: Cholestrol + Cholestrol ester

Clinical features
* Corneal arcus
* Tendon xanthoma
* Plasma is clear
* ↑ risk of CAD
* ↑ risk of PVD




Treatment

New treatment in Familial Homozygous Hypercholesterolemia

A small molecule inhibitor of microsomal Triglyceride Transfer Protein-Lomitapide → ↓ VLDL → ↓ IDL → ↓ LDL

Artisense oligonucleotide to apo B (Apo B inhibitor) Mipomersen → ↓ LDL

Active space

## Sitosterolemia (Type II a)

00:28:33

* Primary hyperlipidemia with hypercholesterolemia
* Biochemical defect: ABCG$_5$, ABCG$_8$ } Actively secrete out plant sterol through intestine lumen & bile duct
* ↑ Plant sterol in cells

↓

↓ Transcription of LDL receptors

↓

↓ LDL receptors

↓

↑ LDL in plasma → ↑ Cholesterol in blood

ADH Type II

* Familial Defective apo B (FDB)
* Defect: Apo B 100

ADH Type III

* Defect: PCSK 9 → Secrete protein that accelerate lysosomal degradation of LDL receptors
* Gain of function mutation → ↓ LDL receptor → ↑ LDL

↓

↑ cholesterol

ARH

* Effect in LRAP (LDL Receptor Adapter Protein)
* ↓ed uptake of LDL

↓

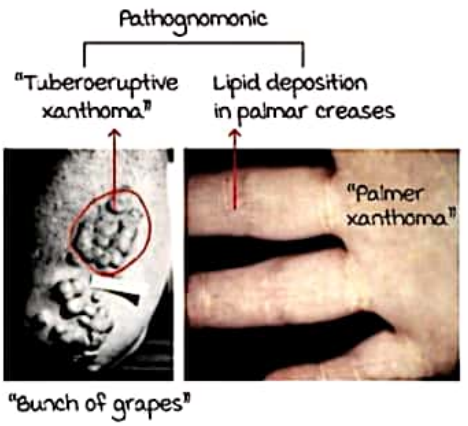
↑↑ cholesterol

Active space

## Primary hyperlipoproteinemia : both HTG & HC 00:41:43

* Fredrickson Classification:

Type III Familial Dysbetalipoproteinemia (FDBL)




* Biochemical defect: Apo E mutation

↓

↑↑ Chylomicron remnant & VLDL remnant

* Hence K/a Remnant Removal Disease

Broad β disease

* Elevated lipid → Both TAG & Cholesterol
* Slight ↑ risk of CAD
* Plasma clear

## Abetalipoprotenemia 00:49:39

* Hypolipoproteinemia
* Biochemical defect:

MTP (Microsomal Triglyceride Transfer Protein) is mutated

* ∴ ↓ Chylomicrons, ↓ VLDL
  ↓ IDL
  ↓ LDL
* Ⓝ HDL

Clinical features

* Acanthocytes
* Pigmentary retinitis
* Bleeding manifestation

↳ Chylomicron carry Fat Soluble vitamins (Vit K)

Active space

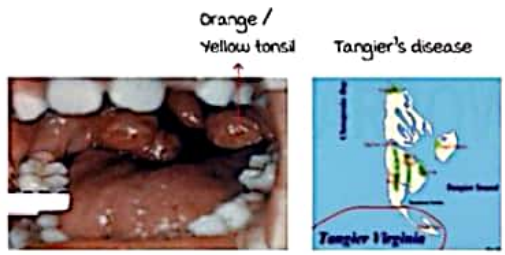
## Tangier's disease

00:55:48

* Hypolipoproteinemia
* Predominantly seen in tanggiers region of virginia
* Biochemical defect: ABCA$_1$ → ∴ ↓ HDL Other lipoproteins: Ⓝ

Clinical features

* Orange / yellow tonsil
* Hepatosplenomegaly
* Mono neuritis multiplex
* Low level of HDL




3) Lcat deficiency

| Complete (Norum's disease) | Partial (Fish eye disease) |
|---|---|
| ↑ Lecithin, ↑ Cholesterol<br>↓ Cholesterol, ↓ Lysolecithin<br>Progress to ESRD | • Benign<br>• Do not progress to ESRD |

| Disease | Molecular Defect |
|---|---|
| Type I<br>Familial Chylomiconemia syndrome | Lipoprotein Lipase<br>Apo CII |
| 1. Type IIa Familial Hypercholesterolemia | 1. LDL receptor |
| 2. FDB [Familial Defective apo B]<br>ADH Type II | 2. ApoB-100 |
| 3. Sitosterolemia | 3. ABCG5 and ABCG8 |

| Disease | Molecular Defect |
|---|---|
| Type III | ApoE |
| Abetalipoproteinemia | Microsomal Triglycerides Transfer protein |
| Tangier's disease | Mutation ABCA-1 |
| Fish eye Disease | Partial LCAT Deficiency |

Active space

# PLASMA LIPID PROFILE

## Fasting lipid profile 00:00:40

Biochemical parameters

1. Total cholesterol (TC)
2. Serum triglycerides (TG)
3. LDL cholesterol
4. HDL cholesterol

Adult treatment plan (ATP) IV guidelines

1. Total cholesterol: Fasting preferred
   - a) Desirable → < 200 mg/dl
   - b) Borderline High → 200-239 mg/dl
   - c) High → > 240mg/dl

2. Serum triglycerides: Fasting sample is taken
   - a) Normal level → < 150mg/dl
   - b) Borderline high → 150-199mg/dl
   - c) High → 200-499mg/dl
   - d) Very high → > 500mg/dl

3. LDL Cholesterol:
   - a) Optimum → < 100mg/dl
   - b) Near or above optimum → 100 – 129 mg/dl
   - c) Borderline high → 130-159mg/dl
   - d) High → 160-189mg/dl
   - e) Very high → > 190mg/dl

4. HDL Cholesterol:
   - a) Low: ≤ 40mg/dl
   - b) High: ≥ 60mg/dl

Active space

New parameters

1) Apo B/ Apo A, ratio

↓ ↓

- LDL HDL
- Normal ratio: 0.7 – 0.9
- Fasting sample is not needed

2) Lipoprotein (a)

- Has Apo (a) and Apo B100

↓

Analogous to plasminogen

↓

plasmin

↓

Fibrin clot → Fibrin degradation product

Risk factor for thrombosis

- Ideal level: 30mg/ dl

3) Total Cholesterol/ HDL ratio

- Ideal : 3.8-6

4) Non HDL Cholesterol

- Ideal level: < 130mg/ dl

## Calculation of lipid fractions

00:14:43

1) Total cholesterol = LDL + HDL + VLDL

2) VLDL $= \frac{\text{Serum triglycerides}}{5}$

3) LDL = Friedewald's formula

$= TC - (HDL) - (VLDL)$

$= TC - (HDL) - \frac{TG}{5}$

Active space

# LIPASES

* Break covalent bond – Ester bond
* Triacyl Glycerol (TAG) $\xrightarrow{3\,H_2O}$ Glycerol + 3 Fatty acids
* Class of Hydrolase

## Hormone sensitive lipase (HSL) 00:03:19

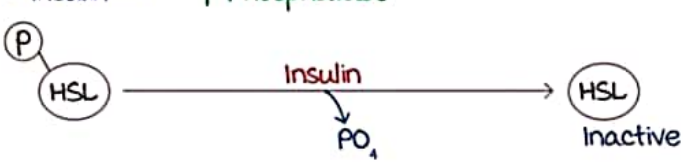
* Location: Adipose Tissue
* Function: Hydrolyse TAG stored in adipocytes
* During fasting state

TAG $\xrightarrow{HSL}$ 2 monoacyl glycerol + 2 Acyl CoA

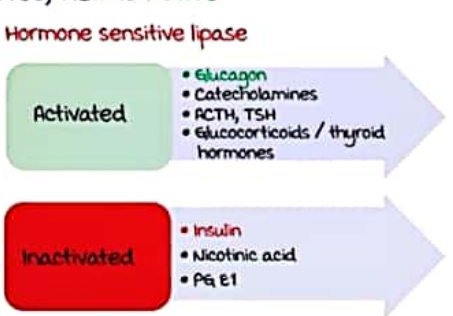
* Fasting state ↓ Glucagon ↓ ⊕ HSL

∴ Active in: 1) Fasting 2) Glucagon 3) Phosphorylated

* Insulin → ↑ Phosphatase




* In diabetes, HSL is Active




## Lipoprotein lipase (LPL) 00:14:53

* Anchored to endothelium of capillaries in Heart, Adipose tissue, Spleen, Renal medulla, Aorta, Diaphragm, Lactating mammary gland

Active space

* Anchored to wall by a GAG → Heparan sulphate
* Inj Heparin → LPL dislodged
* Activated by apo C II

Action

* Hydrolyse TAG in Chylomicron and VLDL
* In fed state
* Hormone: Insulin

↓

↑es the expression of LPL

## Hormone sensitive lipases vs lipoprotein lipase 00:20:30

| | HSL | LPL |
|---|---|---|
| * Location → | Adipocyte | Capillaries |
| * Action → | Hydrolyse TAG in Adipose tissue | Hydrolyse TAG in Chylomicron, VLDL |
| * Action → | Fasting ↓ Glucagon | Fed ↓ Insulin |

## Hepatic lipase 00:23:25

* Location: Sinusoidal surface of liver
* metabolism of Chylomicron remnant and conversion of $HDL_3$ to $HDL_a$

## Endothelial lipase 00:24:30

Action: $HDL_3$ → $HDL_a$

$HDL_3$ → Pre β HDL

* Pre β HDL:
  - Poorly lipidated, most active HDL
  - Absorb maximum cholesterol from peripheral organ

## Intestinal lipase 00:25:40

* Hydrolyses TAG (dietary) in intestine.

↓

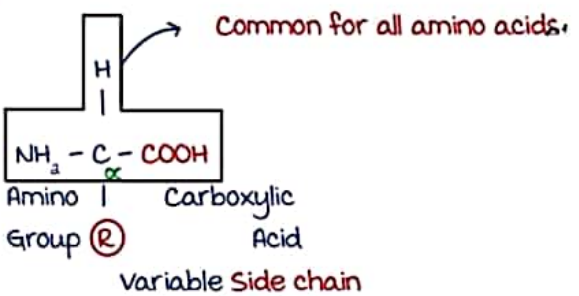
Fatty acid + Glycerol

Active space

# CHEMISTRY OF AMINO ACID : CLASSIFICATION

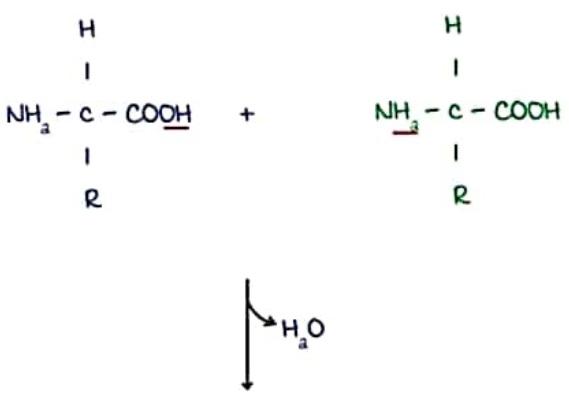
## Introduction to amino acids 00:03:25

- Genes
  ↓
  ↓
  Protein :
  → Regulatory functions
  → Signal transduction
  → Enzymes
- Amino acids are building blocks of proteins.
- Amino acid :

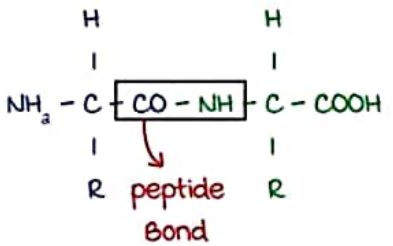



→ Most amino acids are : α amino acids.
→ Non – α amino acids are :
- β alanine
- β amino isobutyrate
- γ amino isobutyrate

## Classification based on side chain : Aliphatic, Hydroxyl Group containing, acidic amino acids 00:08:45




Active space

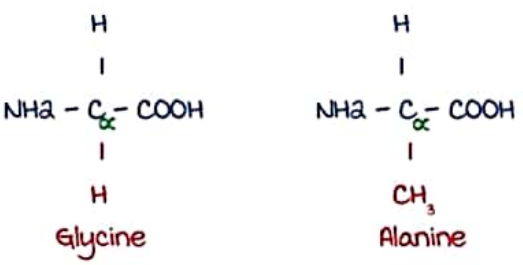



→ this peptide bond can only form a hydrogen bond.

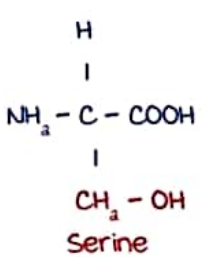
Classification based on side chain :

I. Aliphatic amino acid → simple → Glycine (GLY) G
Alanine (Ala) A

→ Branched chain → Leucine
Isoleucine
Valine

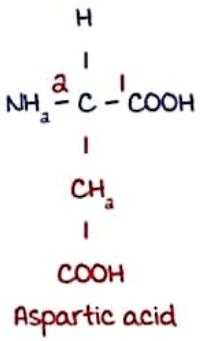



II. Hydroxyl Group containing amino acid → Serine (ser) S
Threonine (Thr) T
Tyrosine (Tyr) y

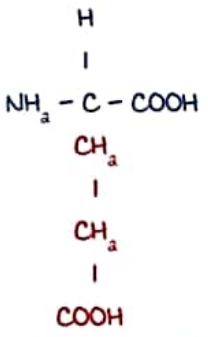



III. Acidic amino acid (- COOH) → Aspartic Acid (ASP) → Aspartate (ionised state)
Glutamic acid (Glu) → Glutamate ( ionised state )

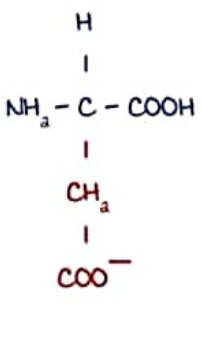
Active space



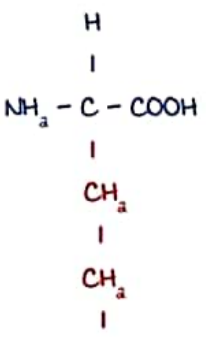
Aspartic acid



Glutamic acid



Aspartate



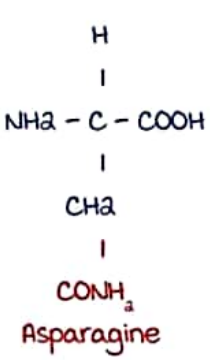
Glutamate

**Warning** : Not all points are covered in the notes, especially conceptual explanations. Please use the notes in conjunction with Marrow Edition 4 videos.

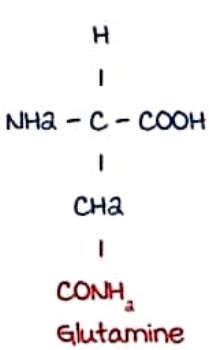
## Classification based on side chain : Amides, Sulphur Containing and basic amino acids

00:21:37

IV. Amides (– CONH$_a$) → Asparagine (Asn)
→ Glutamine (Gln)



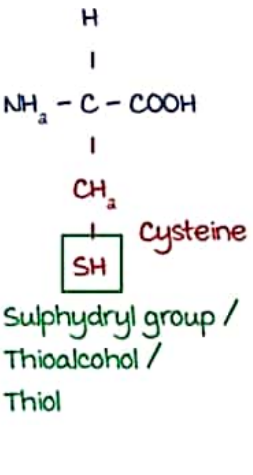
Asparagine

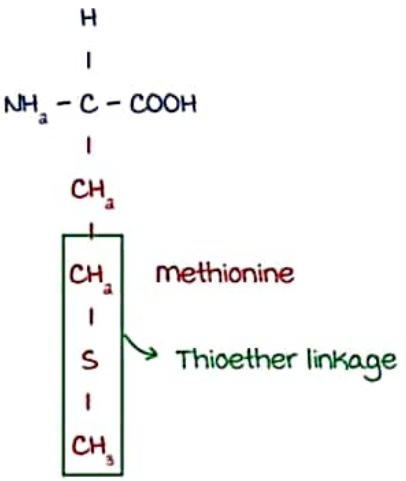


Glutamine

Active space

V. Sulphur containing amino acids → Cysteine (Cys)
→ methionine (met)




VI. Basic amino acid → Histidine (aromatic)
→ Arginine
→ Lysine

→ Exatra amino group in 'R'
→ Variable side chain.

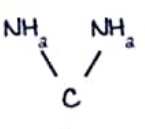
Histidine (His)


Imidazole

→ Five membered
→ 'a N' Containing

Arginine (ARG)

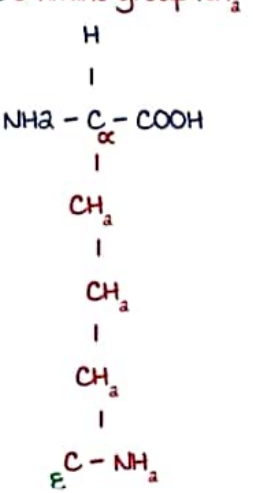
→ has Guanidium group




→ most basic
→ maximum Amino group

Lysine (Lys)

→ has ε Amino group NH$_a$



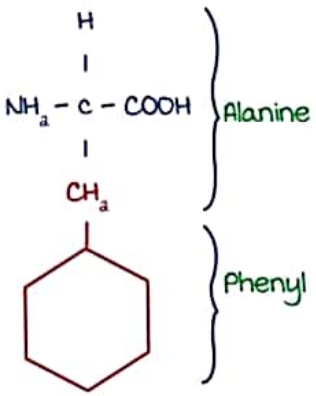

## Classification based on side chain : Aromatic and Imino groups

00:34:01

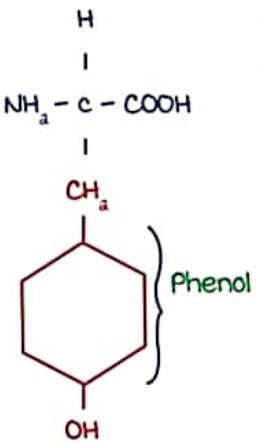
VII. Aromatic amino acid → Phenyl alanine (Phe)
→ Tyrosine (Tyr)
→ Histidine (His)
Thyptophan (Trp)

Active space

Phenyl alanine

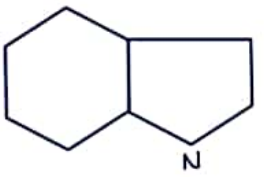



Tyrosine




Tryptophan

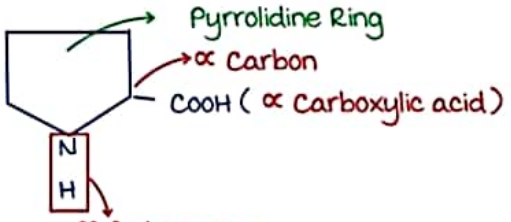
→ has 'Indole ring'
→ 2 rings in structure




Benzene + Pyrrole

VIII. Imino Acids

→ Proline




## Classification based on side chain characteristics

00:48:33

- Classification

**Polar**

**Charged**

**Positively**

→ Basic
Histidine
Arginine
Lysine

**Negatively**

→ Acidic
Aspartic Acid
Glutamic Acid

**Uncharged**

→ Hydroxyl group
Serine
Threonine

→ Amide
Asparagine
Glutamine

→ Simple
Glycine
(least polar)

→ Sulphur containing
Cysteine

**Non – Polar**

1. Branched chain Amino Acid
   Leucine
   Isoleucine
   Valine
2. Aromatic Amino Acid
   All exept Histidine
   Tyrosine :
   - least non polar among non polar aromatic amino acid
3. Imino Acid
   Proline
4. Simple Amino Acid
   Alanine
5. Sulphur Containing
   methionine

Active space

## Classification based on metabolic fate

00:57:55

- Classified into :

| Purely Glucogenic | Both Ketogenic and Glucogenic | Purely Ketogenic |
|---|---|---|
| → Converted to Glucose | → Phenyl Alanine | → Converted to Ketonebodies |
| → All other Amino acids | Isoleucine | |
| → Principal glucogenic Amino Acid ; Alanine | Tryptophan | → Leucine |
| | Tyrosine | Lysine |

## Classification based on nutritional requirement

01:02:15

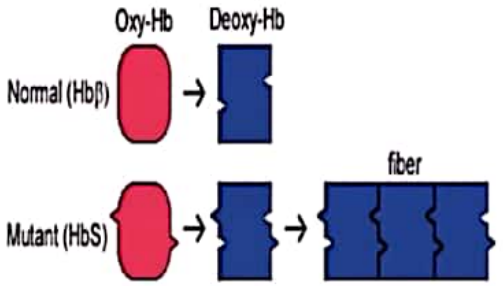
- Classified into

| Essential | Non – Essential |
|---|---|
| → Methionine | → All other Amino Acids |
| Threonine | |
| Tryptophan | |
| Valine | |
| Isoleucine | |
| Leucine | |
| Phenyl alanine | |
| Lysine | |
| → + Histidine | |
| + Arginine → Considered Semi Essential also . | |

One liners .

- Aromatic Amino acid that is not non polar : Histidine
- Aromatic amino acid that is least non polar : Tyrosine
- Simplest amino acid : Glycine
- Most abundant amino acid in protein : Alanine
- Most abundant amino acid in plasma / CSF : Glutamine
- Most non polar amino acid : Isoleucine
- Least non polar among the non polar amino acids : Proline
- 2nd lest non polar amino acid : Tyrosine
- Most polar amino acid : Arginine
- least polar among the polar amino acids : Glycine

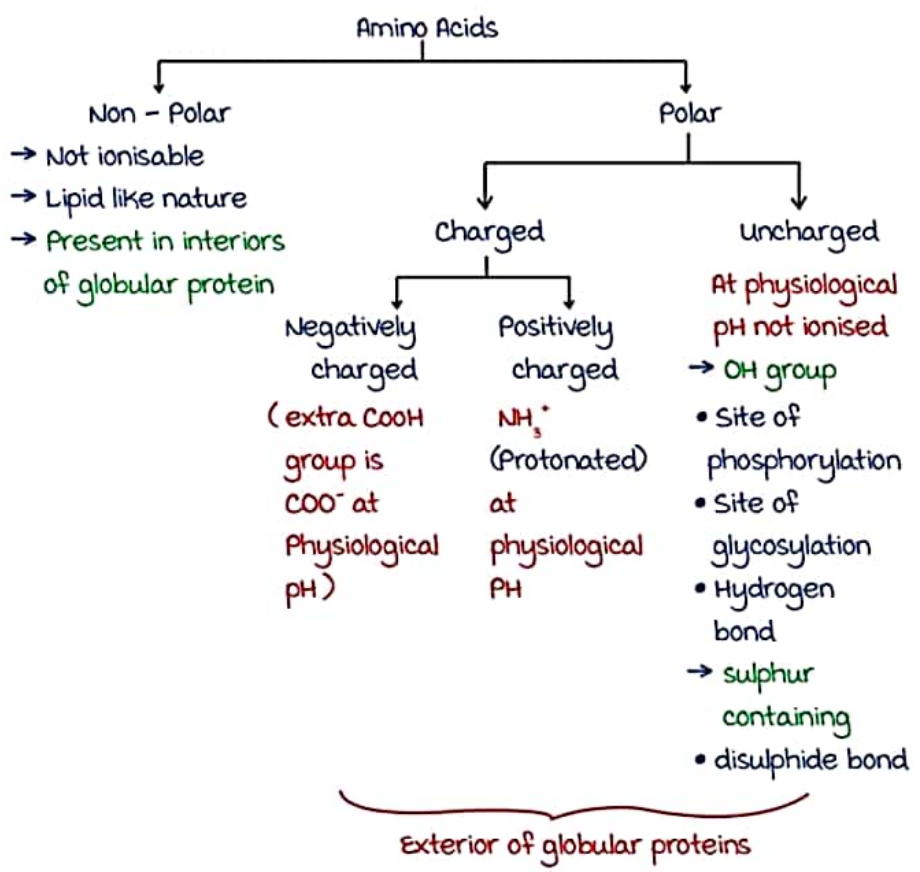
Polymerisation of Hbs :




- In Hbs, 6th position of beta globin chain, Glutamate is replaced by valine .
- In deoxygenated state ; polymerisation of Hbs happens
- Non - conservative / Non - Homologous mutation
  [ polar replaced by non - polar amino acid ]

## Polar and Non-polar amino acids

01:21:19




Active space

# CHEMISTRY OF AMINO ACIDS : DERIVED AMINO ACID

Derived amino acids

- Derived post translationally
- Do not have codon

Seen in the proteins

- Hydroxy proline } collagen
- Hydroxy lysine } vitamin C
- Gamma carboxy glutamate

In Gamma carboxylated protein

- Factor II, VII, IX, X,
- Protein C, protein S
- Osteocalcin – bone
- matrix GLA protein

not seen in the proteins

- Ornithine
- Arginino succinate
- Citrulline

} intermediate of urea cycle

- Homoserine
- Homocysteine

} in metabolism of sulfur containing amino acids

- Cystine – 2 cysteine join together to form disulphide bond

Insulin
Immunoglobulin

(cysteine – s –s – cysteine)

- Methyllysine – myosin
- Desmosine – Elastin

Active space

## Selenocysteine

00:07:14

- 21st protein forming amino acid (A.A)
- Codon – UGA
- Formed by cotranslational modification
- Precursor Amino Acids is serine
- Recoding – involved in formation

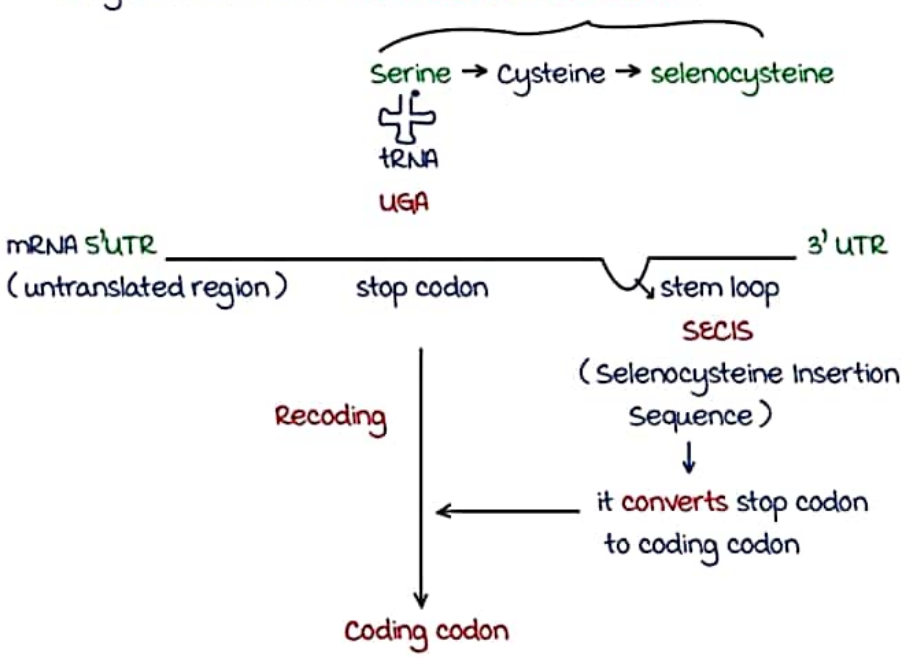
Enzymes / proteins containing selenocysteine

1) Glutathione peroxidase
2) Deiodinase
3) Thioredoxin reductase
4) Glycine reductase
5) Selenoprotein – P (protein)

## Formation of selenocysteine

00:10:20

Along with translation – cotranslational modification




Active space

## Pyrrolysine 00:15:44

- 22nd protein forming amino acid
- Codon – UAG
- Similar to selenocysteine
- Cotranslationaly modified
- Precursor Amino Acid is lysine
- seen in bacteria

## Beta - alanine 00:17:27

- Amino group attached to β – carbon
- Dipeptides that contain Beta alanine

↓

Carnosine – Beta alanine + Histidine
Anserine – Beta alanine + methyl histidine
(methylated carnosine)
} present in muscle for buffering action

- Homocarnosine – Does not contain Beta alanine

↓

Present in brain

- other compounds that contain Beta alanine

↓

Vitamin B5 / pantothenic acid

↓

Beta alanine as a part of pantothenic acid

↓

- CoA
- Acyl carrier protein (Fatty acid synthesis)

- Beta alanine is derived from – cytosine & uracil
pyrimidine degradation product

Active space

# PROPERTIES OF AMINO ACIDS

Amino acids :

1. Can absorb UV light
2. Exhibit isomerism
3. Can exist in different charged states
4. Exhibit buffering capacity
5. Titration curve

## UV light absorption

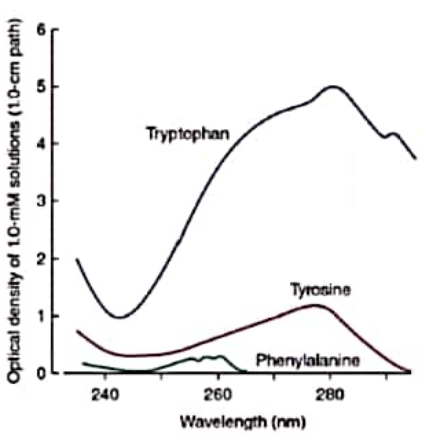
00:03:36

Amino acids :

- They are colourless → cannot absorb visible light
- absorb UV light of 250 - 290 nm
  maximum at : 280 nm

eg : Aromatic amino acids ( have conjugate ring )
- Tryptophan ( maximum )
- Phenyl alanine
- Tyrosine

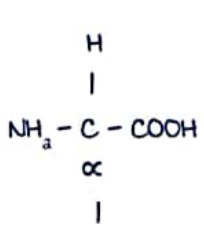
Application : Photospectrometry - measure concentration of proteins based on absorption of light spectrum




← Absorption spectrum of amino acids

## Isomerism of amino acids

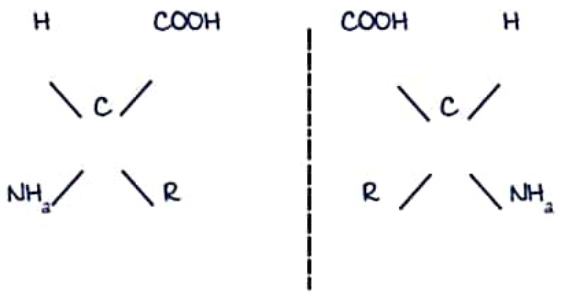
00:08:20



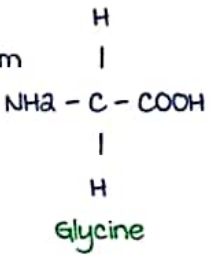

Cα - asymmetric carbon

Active space

∴ exhibit D and L isomerism
(mirror images)




Exception :
Glycine – no asymmetric carbon atom
– not optically active




Glycine

- Most amino acids in our body exist in L – form
  Reason : Most enzymes in our body can act on L – form .

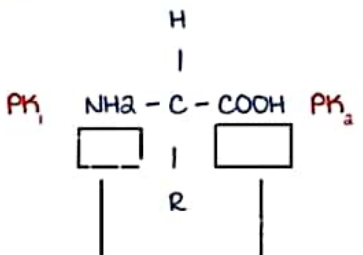
D – amino acid seen in free form are :
1. D Aspartate
2. D serine
} found in brain

Racemase → act on both D and L forms

## Amino acids exist in different charged state 00:14:34

Iso electric PH ( PI )
- average of ionization constants ( PK )
- in compounds with multiple ionisable groups .

In amino – acid .




When protonated :
$NH_3^+$
( positive )

When deprotonated
$COO^-$
( negative )

Ionization constant of any ionizable group : PK

Active space

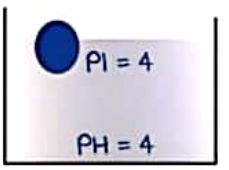
$$PI = \frac{PK_1 + PK_2}{2}$$

At PI, all compounds exist as zwitter ions / Ampholytes

## Properties of amino acid at different PH

00:18:54

1. pH of medium = PI
   - Positive charge = negative charge
     ∴ net charge → neutral
   - Amino acids exist as zwitter ions / ampholytes




Properties :

a) Maximum precipitability
b) Minimum solubility
c) No mobility in electric field
d) Least buffering capacity

Applications :

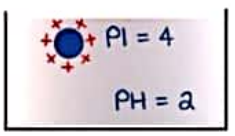
- Precipitation of albumin ( PH = 4.7 ) by addition of acetic acid .
- Isoelectric focusing
  method of separation of mixture of proteins based on absent mobility at isoelectric PH

2. PH < PI

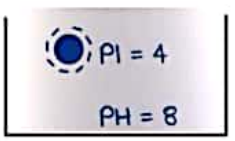
PH less → acidic → ↑ [ $H^+$ ] → protonated

↓

∴ amino acid positively charged




3. PH > PI




PH more → basic → [ $H^+$ ] less

↓

amino acid ← Deprotonated

negatively charged

Active space

Applications :

1. PI of albumin = 4.7
   in blood, pH = 7.4
   pH > PI ∴ albumin is negatively charged in stomach, pH = 2 – 3
   pH < PI ∴ albumin –npositively charged

2. Acidic Amino Acid : PI = 2 – 3 ∴ negatively charged
   Basic Amino Acid : PI = 8 – 9 ∴ positively charged

## Buffering action of amino acid

00:36:00

Buffers : solutions that resist change in pH

Henderson Hasselbach Equation :

$$pH = pKa + \log \frac{\text{base}}{\text{acid}} \Big/ \frac{\text{ionized state}}{\text{unionized state}}$$

- Buffers :
  - Weak acid + conjugate base

  eg : $CH_3COOH + CH_3COONa$

- When ionized = unionized, pH = pKa
  I.e partially ionized state .

Maximum buffering capacity at pH = pKa

| Dissociating Group | $pK_a$ Range |
|---|---|
| α-Carboxyl | 3.2–4.1 |
| Non-α COOH of Asp or Glu | 4.0–4.8 |
| Imidazole of His | 6.5–7.4 |
| SH of Cys | 8.5–9.0 |
| OH of Tyr | 9.5–10.5 |
| α-Amino | 8.0–9.0 |
| ε-Amino of Lys | 9.8–10.4 |
| Guanidinium of Arg | ~12.0 |

PKa of Imidazole of Histidine : 6.5 – 7.4

Blood PH : 7.4

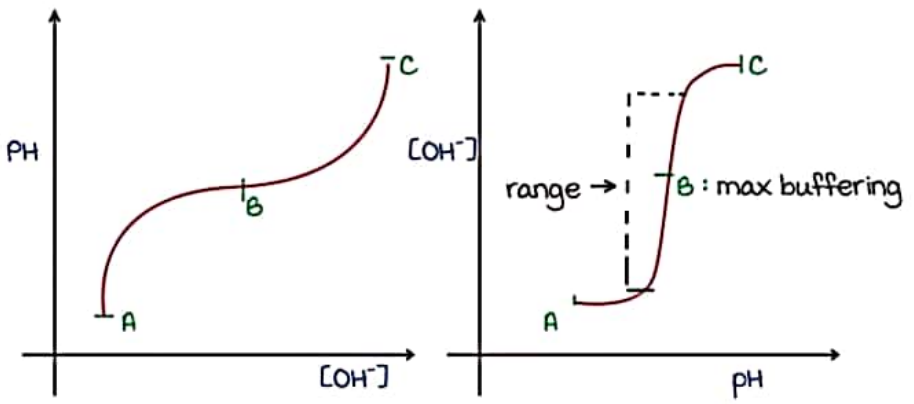
∴ Histidine – best buffer

Active space

## Titration curve

00:56:15

Graphical plot of alkali [ $OH^-$ ] added to the pH of the medium




eg : $CH_3COOH$, weak alkali added to it, analyse change in point

At point A : Unionised state

$[CH_3COOH]$

At point B : $\frac{[CH_3COO^-]}{[CH_3COOH]} = 1$. (Partially ionized)

$pH = pKA + \log 1$

$pH = pKa$

∴ maximum buffering point

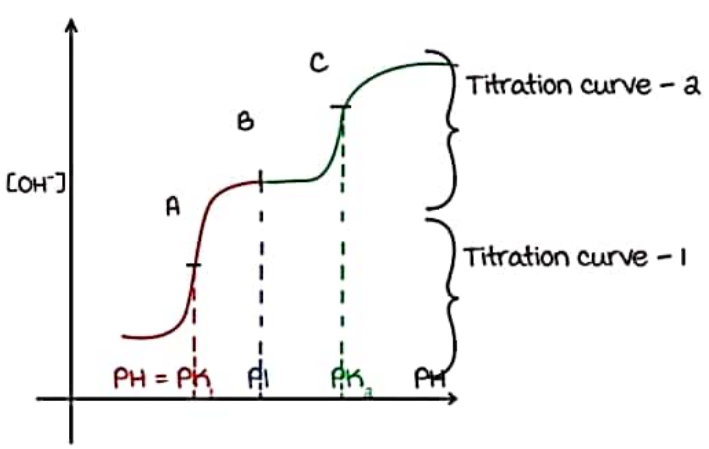
Maximum buffering range : pK +/− 1

At point C : completely ionized

↓

$[CH3COO^-]$

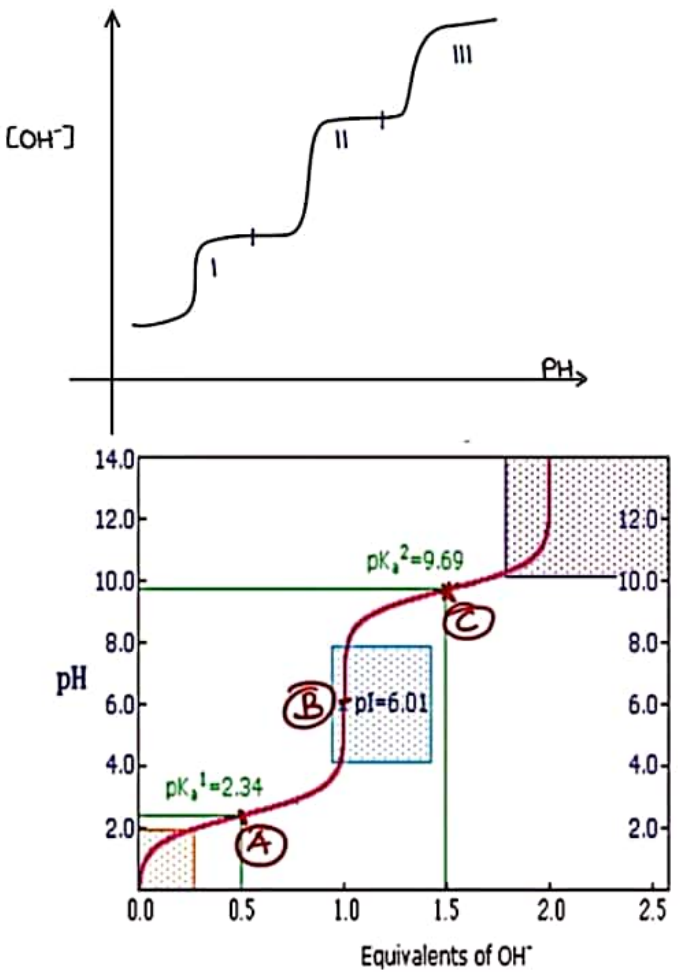
a. Compound with a ionizable groups ( eg : amino acid )




Active space

$$pI = \frac{pK_1 + pK_2}{2}$$

3. Compound with 3 ionizable groups




- Compound with 2 ionizable groups

  Point A : pH = $pK_1$

  partially ionized .

Point B : $pI = \frac{pK_1 + pK_2}{2}$

net charge → neutral

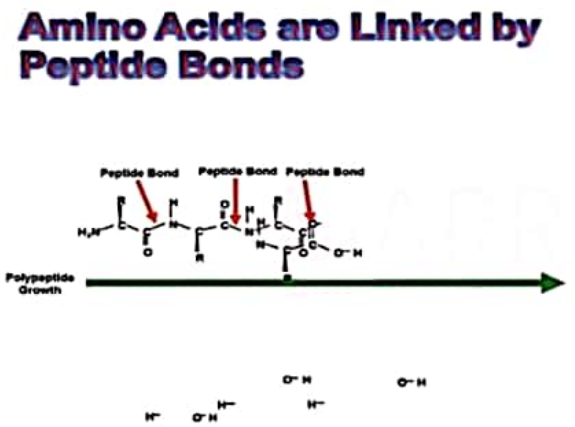
least buffering capacity

Active space

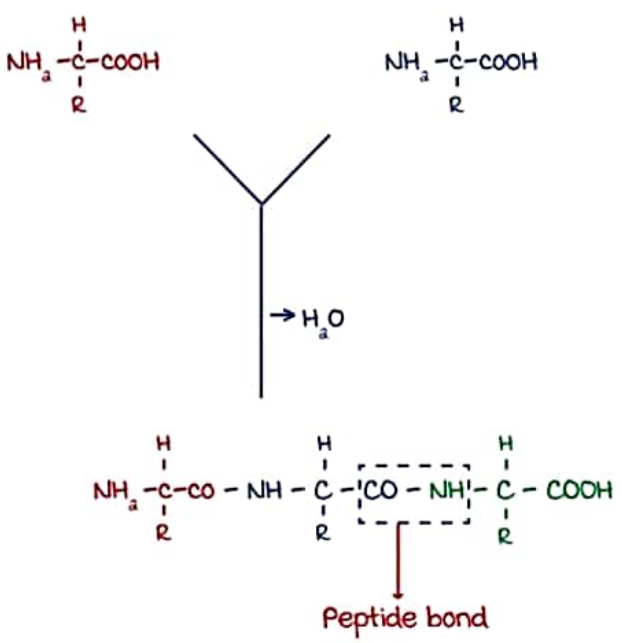
# PROTEIN FOLDING AND STRUCTURE

## Structure of proteins 00:00:30

1. Primary structure :




- amino acids are linked by peptide bond.




**Warning** : Not all points are covered in the notes, especially conceptual explanations. Please use the notes in conjunction with Marrow Edition 4 videos.

- → Peptide bond is " trans" in its configuration.
- → Partial double bond
- → Polar, uncharged
- → Can form only hydrogen bond
- → planar ( due to limited mobility of bond )

Active space

## Primary structure of insulin

00:07:10

Insulin :

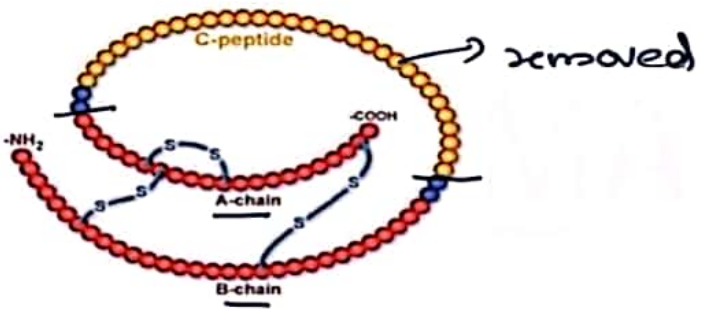
→ 1st hormone isolated.

By : Banting and Best

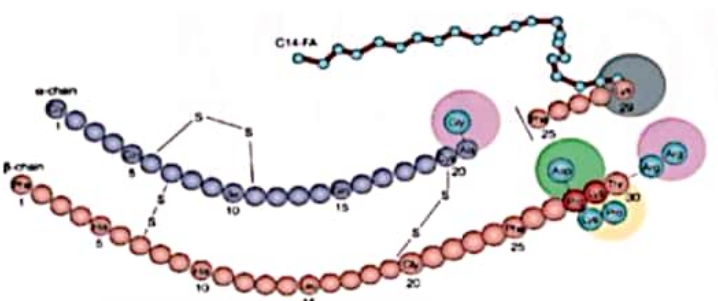
Noble prize : Banting and John McLeod ( director)

→ 1st protein to be sequenced.

By : Frederick sanger.




↓ Looses c-peptide

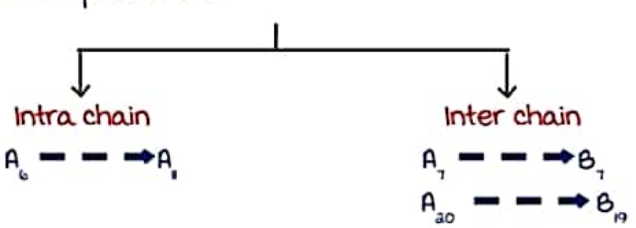


Primary structure

→ Insulin in its natural form has 51 amino acids.

***Two polypeptide chain***
***➢A chain -21 AA[A(E)kkis in Hindi]***
***➢B chain-30 AA***
***Disulfide Bonds***
***➢A6----------> A11***
***➢A7----------> B7***
***➢A20----------> B19***

→ Disulphide bond :




Active space

→ 8th , 9th, 10th amino acids among human and bovine species are different.

| AMINO ACID substitutions of A chain | | | |
|---|---|---|---|
| 8 | 9 | 10 | |
| Thr | Ser | Ile | Human |
| Ala | Ser | Val | Bovine |
| Thr | Ser | Ile | Porcine |

→ No variation in sequence (8th, 9th, 10th) between porcine and human amino acid chains.

→ 30th amino acid in B chain is different between Humans and bovine / porcine

| Amino acid at 30th position in B chain | |
|---|---|
| Thr | Human |
| Ala | Bovine |
| Ala | Porcine |

→ Human insulin should not undergo mutation at:

1) 8th, 9th, 10th amino acid of A chain.
2) 30th amino acid in B chain.
3) In disulphide bonds between :

$A_6$ — — → $A_{11}$
$A_7$ — — → $B_7$
$A_{20}$ — — → $B_{19}$

**KEY POINTS**
- Species variation restricted to 8,9,10 in A chain and C terminal AA of B chain
- Human and Porcine insulin differ only in 30 th AA in B-chain

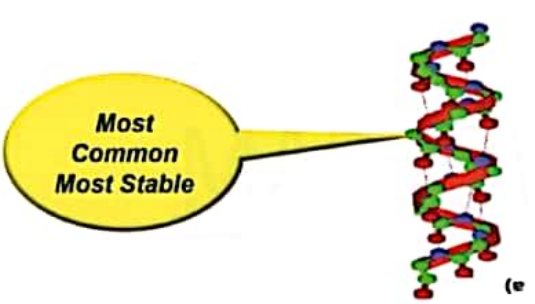
## Secondary structure

00:15:18

→ The folding of Short (3-30), contiguous segment of polypeptide in to geometrically ordered units.

Alpha Helix :

→ most human Proteins are made by ∝ Helix.



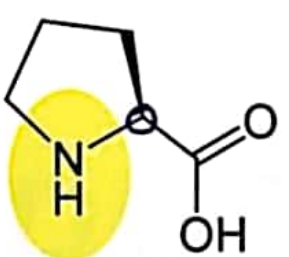

Active space

Protein : Secondary Structure : Alpha Helix :

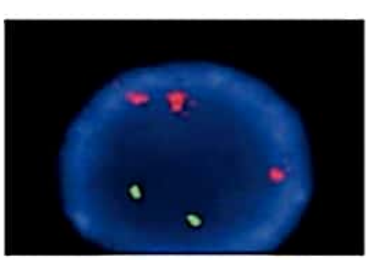
→ 1° Structure form "Right handed spiral"
→ Force that stabilize alpha Helix
↓
" Hydrogen bond " ( between every 4th amino acid)
Intra chain bond
↓
" Parallel to the peptide bond "

## Amino acids that disrupt stability of alpha helix 00:19:24

1) Proline
- Nitrogen is a part of rigid ring.
- No substituent hydrogen to participate in hydrogen bond.
- Induce "Kinks"




2) Glycine
- Has only one hydrogen, Small and flexible.
- Induce "bends"
- Very less found in alpha helix.



3) Bulky amino acids.
Eg : tryptophan

4) Charged amino acids.
( In series)

(-) (-) (-) (-)
aa1 – aa2 – aa3 – aa4

Active space

→ Amino acid which shows greatest tendency to form alpha helix – Alanine
"methionine" (maximum)

→ least (possible) tendency to form alpha helix – "Proline"

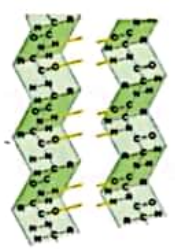
→ Proline is present in the first turn of alpha helix.

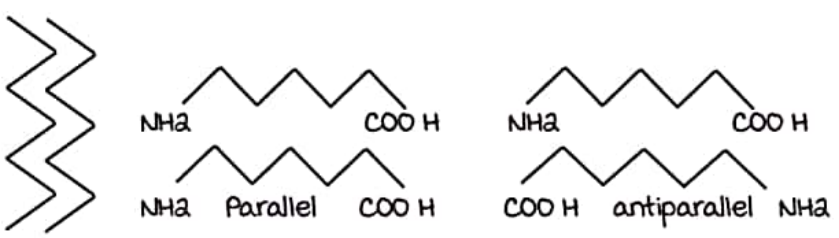
→ In one turn of ∝Helix
↓
3.6 amino acids / turn.

## Beta pleated sheet

00:26:00

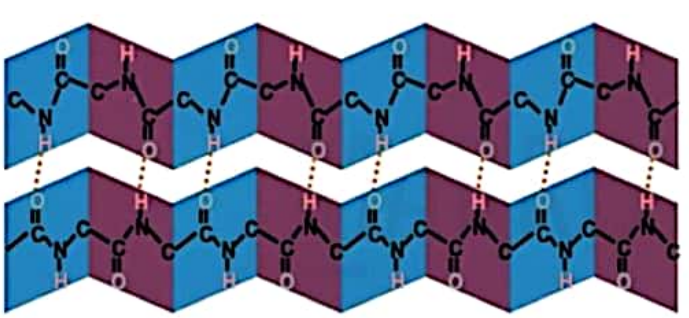
→ Polypeptide chain is almost fully extended
→ Has 'zig – zag' pattern






→ Adjacent strands in a sheet can run in the same direction (parallel) or opposite direction (anti parallel)

Protein secondary structure : Beta pleated sheet :



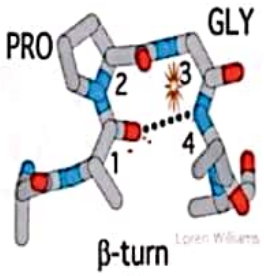
→ Interchain hydrogen bond
→ Perpendicular to peptide bond

Active space

→ Hydrogen bonds – between adjacent beta sheet.

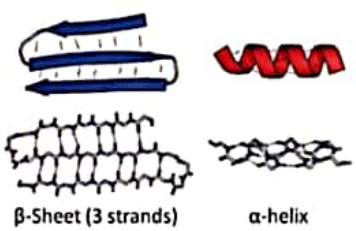
β turn :
- join two secondary structures.
- involves 4 amino acyl residue, in which first residue is hydrogen bonded to fourth.

→ Proline and glycine are often present in β turn.

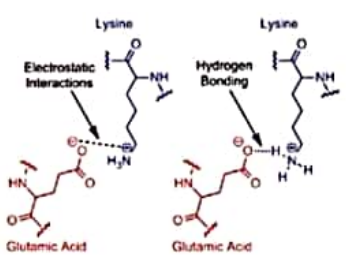



## Forces that stabilize secondary structure-non covalent bonds 00:31:06

1) Hydrogen bond



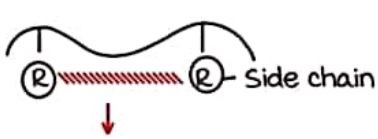

2) Ionic bond




3) Vander Waals forces

↓

Hydrophobic interaction

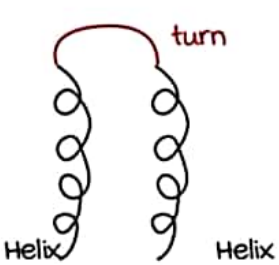



↓

Vander Waals forces.

→ Weak non- covalent forces.

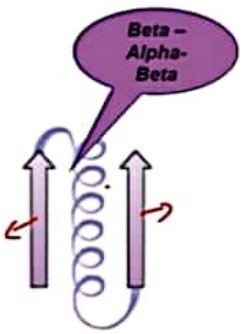
Super secondary structure .
→ A/K/a motifs
→ Combination of secondary structural elements.



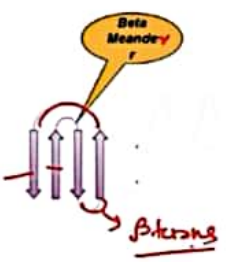

Active space

Examples :

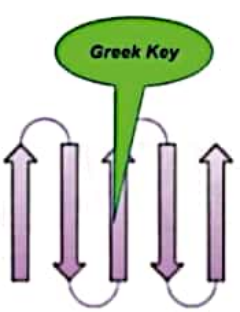
1) Beta - Alpha- Beta




2) Beta - meander




3) Greek Key



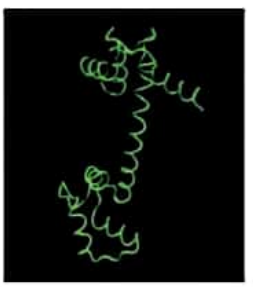

4) Beta barrel

Active space

## Tertiary structure, domain and quaternary structure 00:34:05

Tertiary structure :

→ Secondary structures get folded into a 3 dimensional conformation of a polypeptide

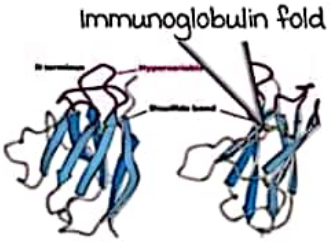


Domain:

→ Section of protein structure Significant to perform a particular chemical or physical task such as binding of a substrate.

Example : 1) Immunoglobulin fold

Immunoglobulin fold



2) Rossmann fold

↓

NADPH binding domain

Quaternary structure :

- When a protein has 2 or more Polypeptide subunits, Their arrangement in space is referred to as Quaternary

Forces that stabilise tertiary and quaternary structures:

- Primary non-covalent bonds.

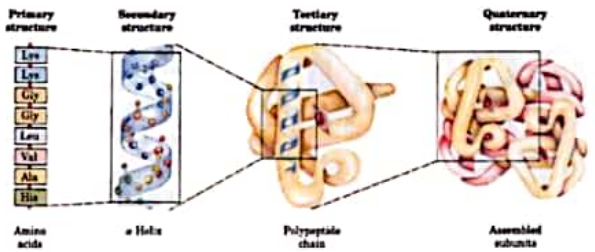
→ hydrophobic interaction.

- hydrogen bond.
- Electrostatic (Ionic) bond.

Active space

- vander waal's forces.
→ Some protein contain covalent bond (disulfide bond).

protein : level of organization :




## Protein folding 00:41:18

- Proteins are Conformationally dynamic molecule that can fold into functionally competent conformation.
- Auxiliary proteins assist - protein folding

↓

" molecular chaperones"

↓

They are → Heat shock proteins (HSP)

1) Hsp 70
2) Hsp 90
3) Hsp 40 ( Co Chaperone )
4) Hsp 60 - Chaperonin - large Multisubunit protein.

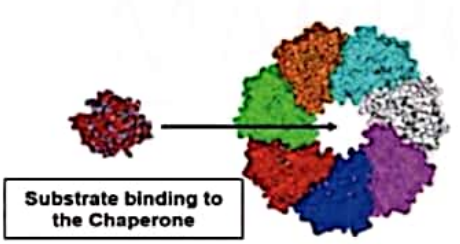
→ Bip ( immunoglobulin heavy chain binding protein )
→ Glucose regulated protein [ GRP - 94 ]
→ calreticulin
→ Calnexin

} Calcium binding protein.

## Enzymes assisting in folding and properties of chaperones 00:44:53

Enzymes assisting protein folding

1) Protein disulphide isomerase (PDI)
2) peptidyl prolyl isomerase (PPI)




Active space

Properties of chaperones:

1) Inducible by conditions that cause unfolding

↓

eg : Fever

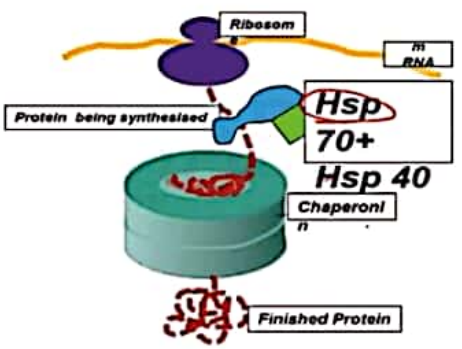
↓

↑ Heat shock proteins.

2) Binds predominantly to hydrophobic region of unfolded protein.

3) Associated with ATPase activity.

4) Part of quality control or editing mechanism.




## Protein degradation

00:48:36

Lysosomal degradation
- → ATP independent.
- → membrane proteins.
- → proteins with long life

Proteasomal degradation
- → ATP dependent
- → misfolded proteins
- → Short lived proteins.

Proteasomal degradation

ERAD – Endoplasmic reticulum Associated Degradation of proteins.

→ Ubiquitin is the key molecule in protein degradation.

↓

Binding of misfolded protein to Ubiquitin.

↓

" Kiss of death "

→ Ubiquitin bind to protein based on

↓

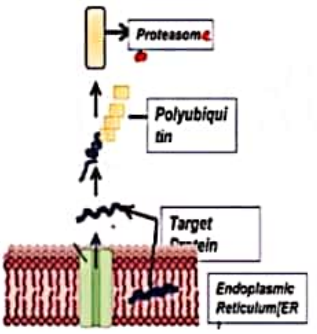
"N end Rule"

↓

Binds to PEST sequence in the amino terminal.

Active space

PEST – Proline
Glutamic acid
Serine
Threonine




Lysosomal degradation.
- ATP independent process.
- For long lived proteins and membrane proteins.

## Protein misfolding disease

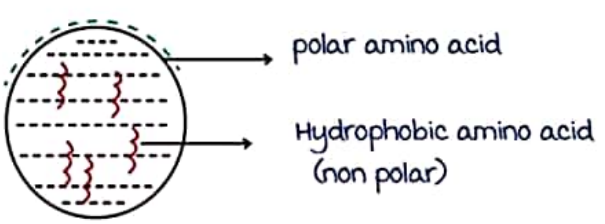
00:55:34

1) Prion diseases.
2) Prion related protein disease
3) Amyloidosis.

Alteration in protein confirmation :
$PrP^{c}$ the cellular isoform of prion protein
(rich in $\alpha$ helix)

↓

$PrP^{sc}$ (Scrapies) the disease causing isoform
[ rich in $\beta$ Sheets structure]




→ $\beta$ Sheets get aggregated.

Active space

## Prion related protein disease

01:00:31

→ Aggregated β Sheets – resistant to degradation.
→ Examples :
1) Alzheimer's disease
2) Parkinson's disease
3) Beta thalassemia
4) Cystic fibrosis.
5) Huntington's disease
6) Fronto temporal dementia (FTD)
7) Amyotropic lateral sclerosis (ALS)
8) Dementia with lewy bodies (DLB)

# FIBROUS PROTEINS

-Structural proteins

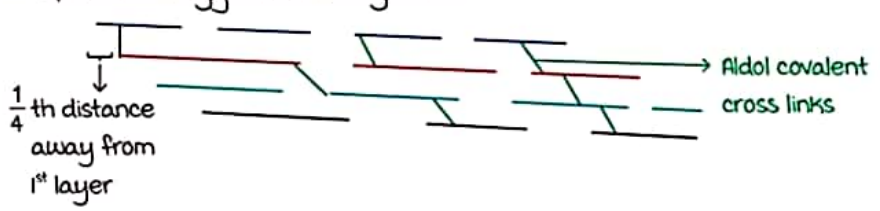
-collagen, elastin, keratin, fibrillin, laminin

## Collagen 00:02:07

- Most abundant fibrous protein present in extracellular matrix
- Most abundant protein in the body
- Highest density in: Cornea > Skin

Structure of collagen

- Triple helix
  - 3 Polyproline α chain
  - Single poly proline α chain → Glycine-X-Y repeat
    - Every 3rd amino acid → glycine
    - X, y → Hydroxy proline / Hydroxy lysine
    - Each α chain made of 1000 amino acids
  - Most abundant aa is glycine
  - 33% is Glycine
  - Recurring amino acid
  - Each α chain twisted in left handed direction
    3 α chains together twisted in right handed direction
- Quarter staggered arrangement




## Synthesis of collagen 00:10:32

* It can be
  - Intracellular:- RER of fibroblast
  - Extracellular:- Extracellular matrix

Active space

Intracellular events

- Hydroxylation of proline and lysine residue
  ↓ vitamin c , αKetoglutarate
  by prolyl/ lysyl hydroxylase (monooxygenase)
- Glycosylation of hydroxy lysine
- Intra chain and inter chain disulfide bond formation
- Formation of triple helix

} RER

↓

Golgi apparatus : • Procollagen packed into secretory vesicle
• Transported to extracellular matrix

Extracellular events

- Cleavage of N and C terminal polypeptide
- Assembly of collagen fibril into Quarter staggerred arrangement
- Formation of covalent crosslinks

## Types of collagen

00:18:27

| Type | Tissue |
|---|---|
| I(mc) | most connective tissues, including bone |
| II | Cartilage, vitreous humor |
| III | Extensible connective tissues such as skin, lung and vascular system |
| IV | basement membranes |
| V | Minor component in tissues containing collagen I |
| VI | most connective tissues |
| VII | Anchoring fibrils |
| VIII | Endothelium, other tissues |
| IX | Tissues containing collagen II |
| X | Hypertrophic cartilage |
| XI | Tissues containing collagen II |
| XII | Tissues containing collagen I |
| XIII | many Tissues |
| XIV | Tissues containing collagen I |
| XV | many Tissues |
| XVI | many Tissues |
| XVII | skin hemidesmosomes |
| XVIII | many Tissues |
| XIX | Rhabdomyosarcoma cells |

* major collagen present in bone: Type I (90%)
* major collagen present in dermis, ligaments and tendons: Type I (80%)
* major collagen present in cartilage: Type II (40-50%)
* Collagen type in dermo epidermal junction: Type VII
* major collagen present in Aorta: Type I & Type III (20-40% each)
* most abundant collagen: Type I
* Collagen in wound healing → Type I, II and III involved
  m/c: Type I collagen

## Type of collagen and associated disorders

00:23:26

| Type of collagen | Gene or enzyme | Disease |
|---|---|---|
| Type I | COL1A1 and COLIA2 | Osteogenesis imperfecta, Osteoporosis, Ehlers-Danlos syndrome (Type VII EDS) |
| Type II | COL2A1 | Chondrodysplasias Osteoarthritis |
| Type III | COL3A1 | Ehlers-Danlos syndrome (Type IV EDS)[most serious] |
| Type IV | COL4A3-COL4A6 | Alport syndrome (including both autosomal and X-linked forms) |
| Type V and Type I | COL5A1, COL5A2, COLIA1 | Classical EDS |
| Type III | COL3A1 Tenascin XB (TNXB) | Hypermobile EDS (Type III EDS) |
| Type VII | COL7A1 | Epidermolysis bullosa, dystrophic |
| Type X | COL10A1 | Schmid metaphyseal chondrodysplasia |
| Lysyl hydroxylase | Lysyl hydroxylase | Ehlers-Danlos syndrome (type VI EDS) Kyphoscoliotic EDS Scurvy |
| | Procollagen N-proteinase (also called as ADAM TS2) | Ehlers-Danlos syndrome (Type VII autosomal recessive) Dermatosparaxis type |
| Lysyl oxidase | Lysyloxidase (require Cu) | menkes disease(ATP7B) |

Active space

Villefranche classification of EDS

| Subtype | Defect in |
|---|---|
| 1. Hypermobility | Type III collagen, tenascin X |
| 2. Classical | Types I and V collagen |
| 3. Vascular | Types III collagen |
| 4. Kyphoscoliosis | Lysyl hydroxylase |
| 5. Arthrochalasis | Type I collagen |
| 6. Dermatosparaxis | ADAM metallopeptidase with thrombospondin type I motif (ADAMTS2) |

## Elastin

00:35:21

* Elastic recoil
* Lung, large arterial blood vessel, elastic ligaments

| | Collagen | Elastin |
|---|---|---|
| 1. Types | many | only 1 |
| 2. Triple Helix | + | − |
| 3. Gly-X-Y | + | − |
| 4. Presence of hydroxy lysine | + | − |
| 5. Glycosylation | + | − |
| 6. Cross links | Aldol | Desmosine |
| 7. Extension peptides | + | − |

Disorders associated with Elastin:

- William Beuren Syndrome
- Cutis Laxa

Note : Desmosine requires 4 lysines

## Keratin

00:38:54

* Protein present in the hair, nails and outer layer of skin
* Alpha helix cross linked by Disulphide bond
* Rich in cysteine
* Harder the Keratin, more Disulphide bond

Active space

## Fibrillin-1

00:40:18

* Large glycoprotein
* Structural component of microfibrils
* Scaffolds for deposition of elastin
* Mutation in gene for fibrillin-1 leads to marfan's syndrome

  Also: • Acromicric dysplasia
  • Galeophysic dysplasia

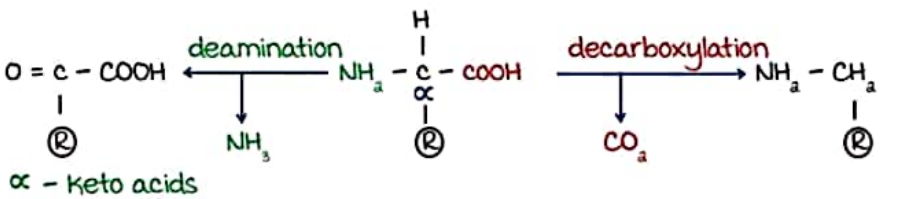
* Congenital contractural arachnodactyl:
  - Mutation in the gene of Fibrillin 2 [Chr 5]
  - This is important in deposition of microfibrils
  - Early in the development
  - Clinical features: contractures, arachnodactyly, dolichostenomelia

* Classical epidermolysis bullosa:
  - Mutation in Keratin-5

Active space

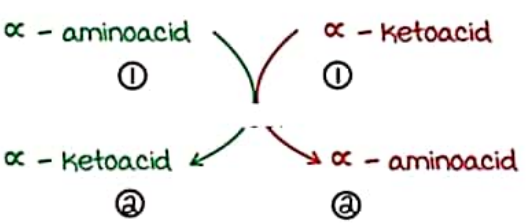
# GENERAL AMINO ACID METABOLISM

## General reaction of amino acids 00:01:51




Amine : biologically important amines

## Transamination 00:07:31

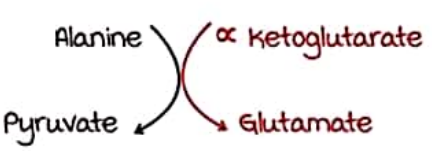



- Transfer of amino group from 1 amino acid to ∝ Ketoacid to form a new pair of amino acid and Ketoacid

- General properties :
  - Site : cytoplasm(mostly)
  - Co-enzyme : PLP ( vit $B_6$ )
  - Completely reversible
  - $NH_3$ is not released freely

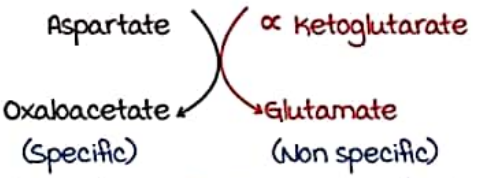
## Example of transamination 00:11:59

- ALT : Alanine Aminotransferase
  ( SGPT : Serum Glutamate Pyruvate Transminase )

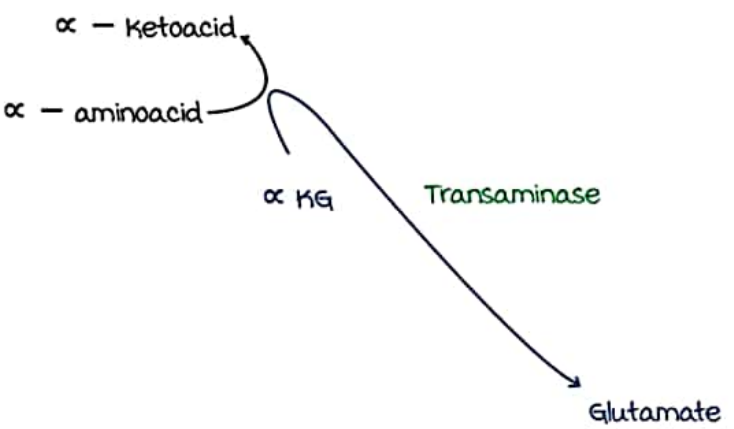



Active space

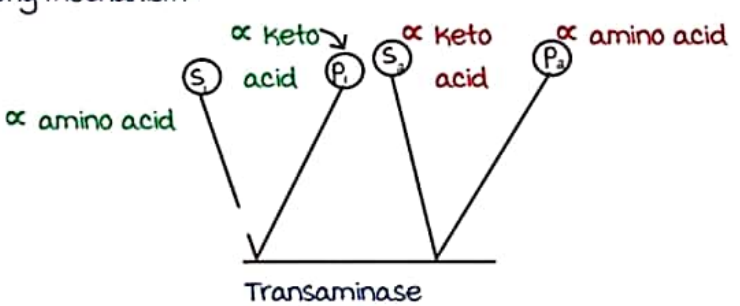
- AST : Aspartate Amino Transferase
  ( SGOT : Serum glutamate oxaloacetate transferase )




- Transamination is specific for one pair of substrates and non - specific for the other pair
  ∝ - aminogroup is getting concentrated as glutamate




- Ping pong mechanism :




There are 2 substrates and 2 products ( Bibi reaction )

- Biosynthesis of nutritionally non - essentital amino acids
  ∝ KG ⟷ Glutamate
  Oxaloacetate ⟷ Aspartate
  Pyruvate ⟷ Alanine

Active space

## Significance of transamination 00:26:12

- Reversible
- PLP is the coenzyme
- Cytoplasm
- Concetrates the ∝ amino group as glutamate
- Ping pong mechanism
- Biosynthesis of non - essentital amino acids

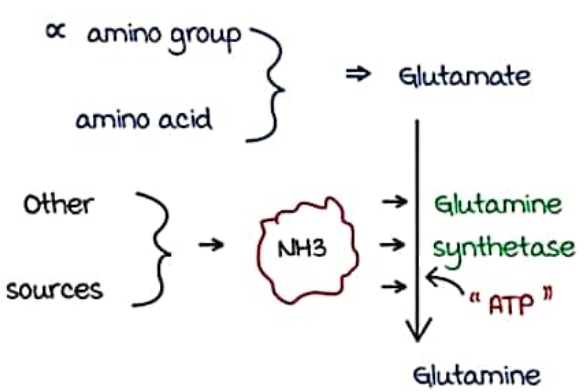
* Amino acids that do not take part in transamination
  - Lysine
  - threonine
  - proline
  - Hydroxyproline

* Non alpha amino group that take past in transamination
  - δ amino group of ornithine
    δ ornithine aminotransferase enzyme ( coenzyme : PLP )
    deficiency causes : gyrate atrophy of retina & choroid
    Rx : Restrict Arginine ( Source of ornithine )
    Supplement PLP

## Sources of ammonia 00:32:20

- Sources of ammonia :
  1. ∝ - amino group of amino acid
     → detoxified to glutamate by transmination
  2. amino sugar
  3. non - protein nitrogenous substances : Nucleotides
     phospholipids
     porphyrins
  4. gut bacteria

## Transport of ammonia in most organs including brain 00:34:33



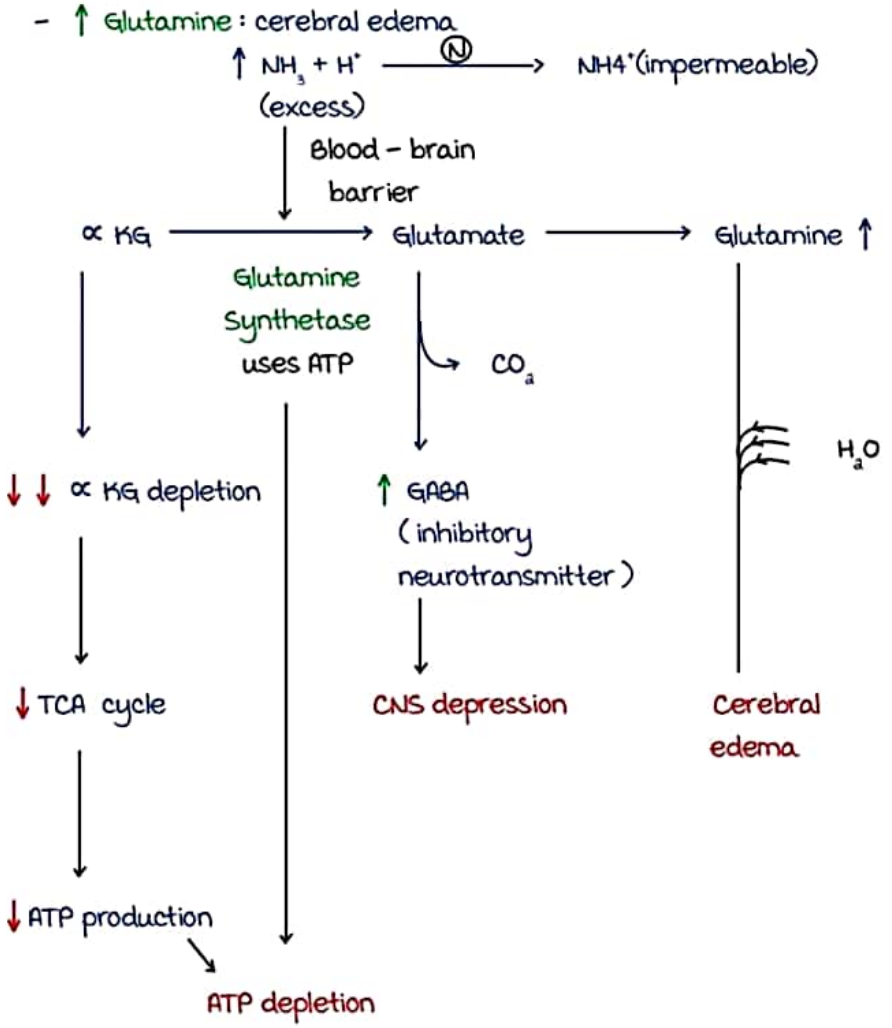

Active space

- Site : Mitochondria
- First line trapping of ammonia
- Detoxifies harmful ammonia in the brain
- Ligase reaction
- ATP is required
- Transport form of ammonia in most organs : Glutamine
  in hyperammonemia, plasma glutamine levels are high .

## Toxicity of Ammonia

00:34:33

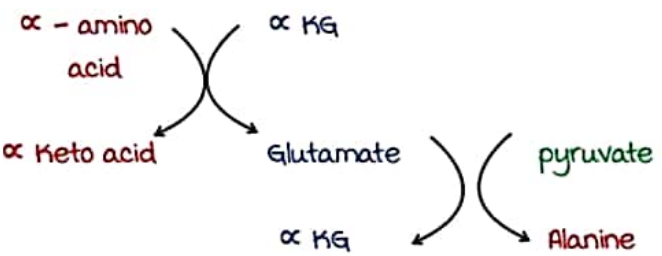
- ∝ KG depletion
- ↑ GABA : CNS depression
- ↓ ATP
- ↑ Glutamine : cerebral edema



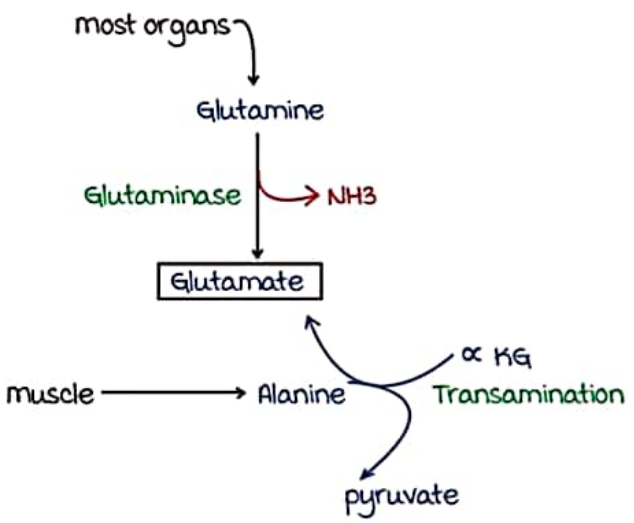

Active space

## Transport of ammonia from muscle, liver 00:49:19

- Transport form of ammonia from muscle – Alanine

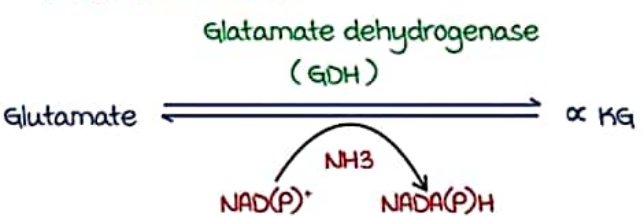



- Liver :

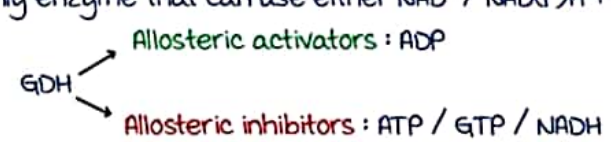



## Glutamate metabolism 00:53:26

- Ammonia accumulates as Glutamate in liver and undergoes oxidative deamination



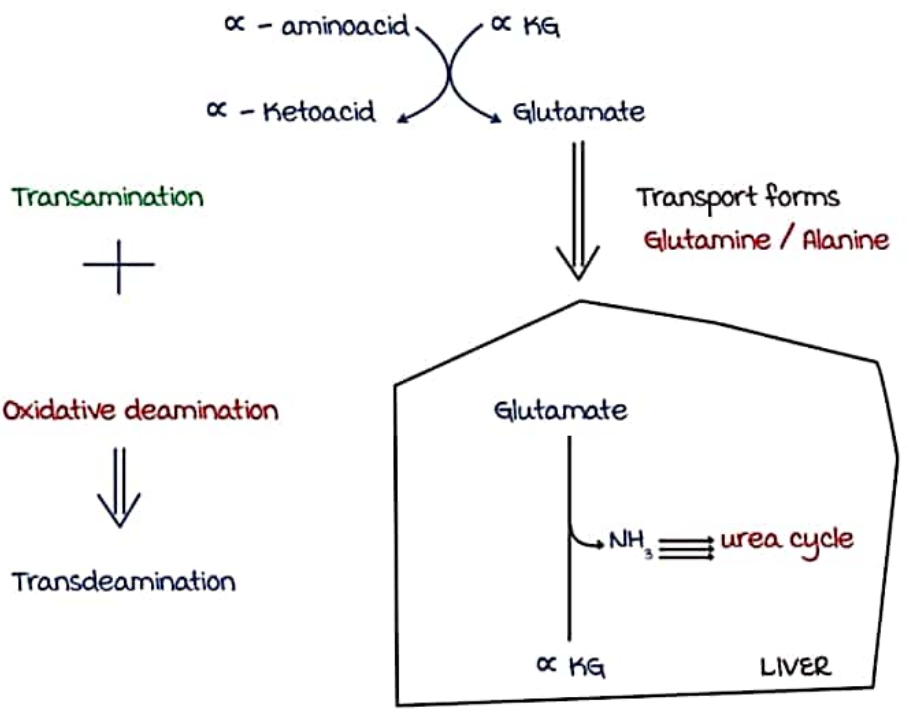

- $NH_3$ is released freely
  reversible reaction
  only enzyme that can use either NAD+ / NAD(P)H + → GDH




Active space

## Concept map - handling of Nitrogen

01:00:19



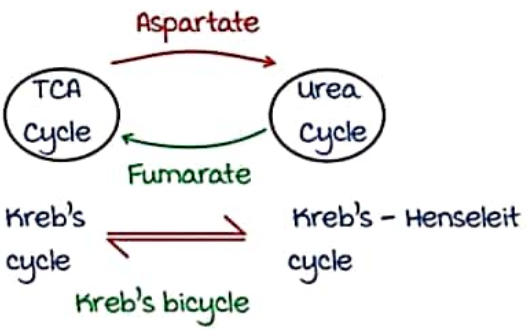

Active space

# UREA CYCLE PATHWAY AND DISORDERS

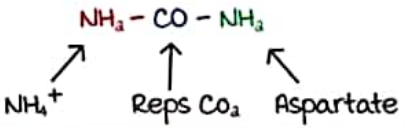
## Introduction

00:03:20

- It is also called Kreb's – Henseleit Cycle
- A/K/A Ornithine cycle– Ornithine is generated here
- Urea Bicycle :




- Urea :




- Compounds consumed in Urea cycle
  1. $NH_4^+$
  2. Aspartate
  3. $CO_2$

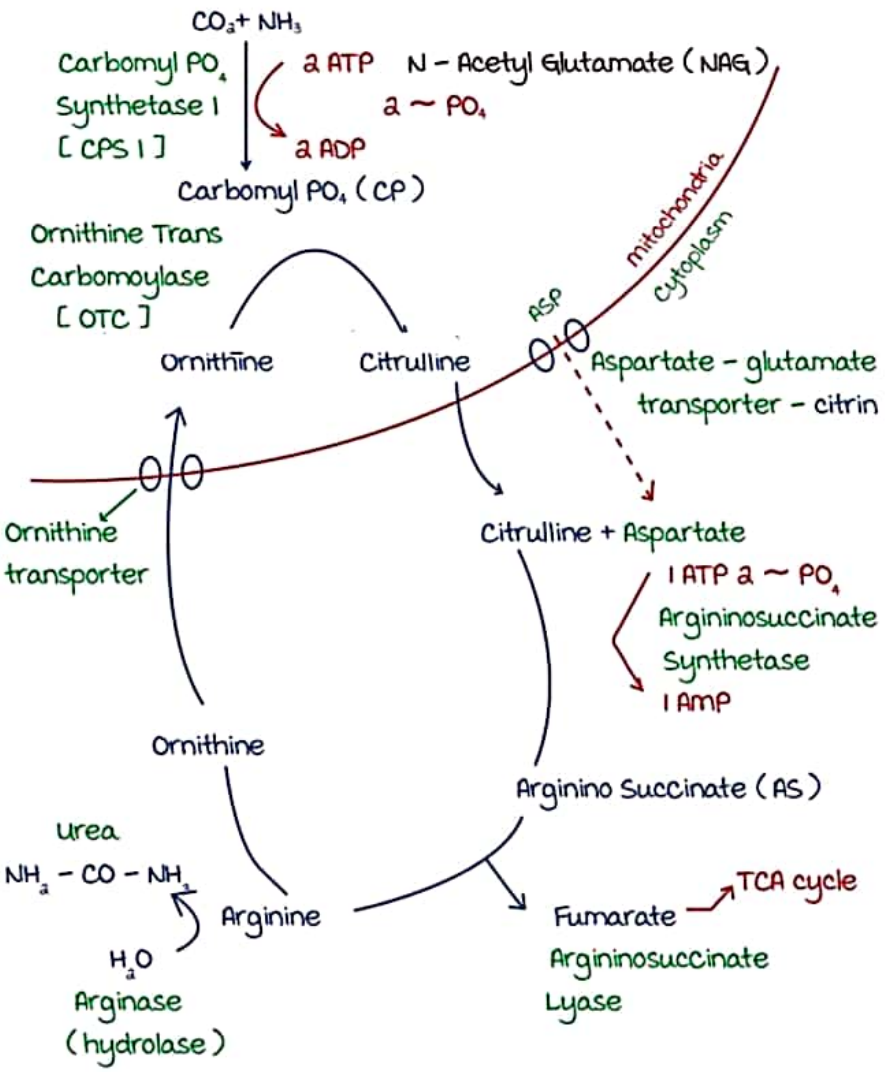
- Site : Liver
  Organelles : Both cytoplasm and mitochondria

  Other cycles in both cytoplasm and mitochondria are :
  - Pyrimidine synthesis
  - Urea cycle
  - Heme synthesis
  - Gluconeogenesis

Active space

## Reaction

00:09:40




- CPS I : Rate limiting enzyme
  Pace maker enzyme
  2 ATP - ( ~ $PO_4$ )
  Biotin independent carboxylation . ( Other eg : CPS I
  CPS II
  Gamma carboxylation
  malic enzyme )
- All enzymes with letter A is in cytoplasm .

Active space

## Energetic and regulation

00:20:53

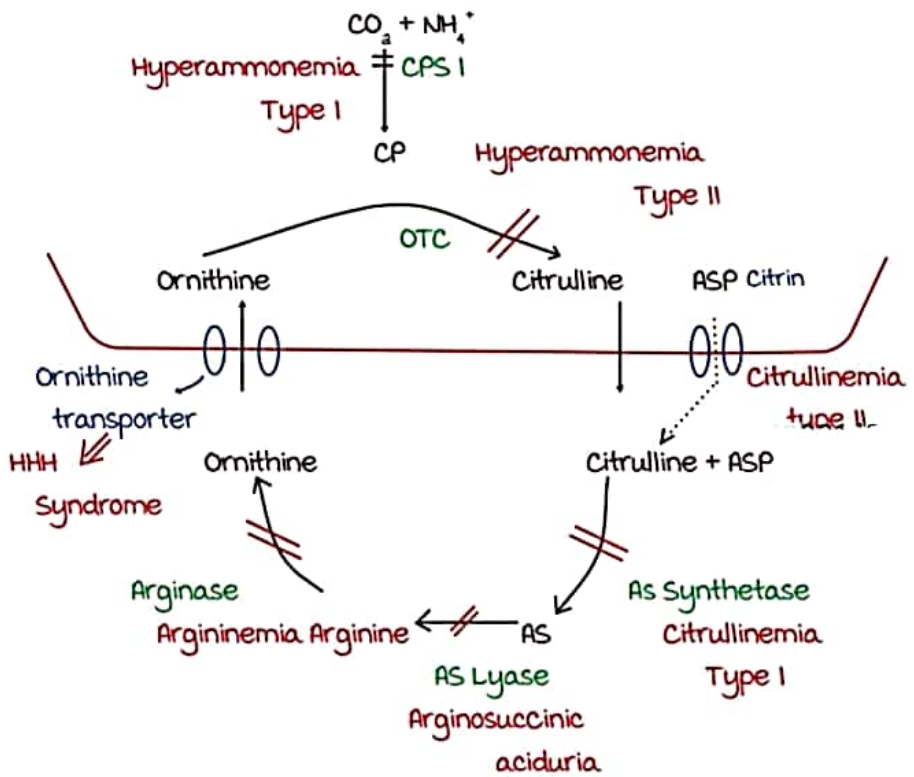
- CPS I → 2 ATP — 2 ~ $PO_4$
- ASSynthetase → 1 ATP — 2 ~ $PO_4$

  3 ATP directly — 4 ~ $PO_4$ , 4 ATP equivalent

- Regulation
  - High protein diet → ↑ urea cycle enzyme synthesis
  - NAG iss an allosteric activator of CPS I
  - Compartmentation : (1st 2 reactions) → Rest in cytoplasm
    mitochondria

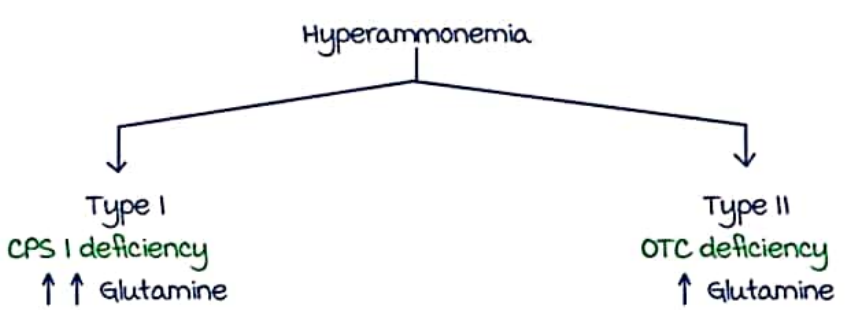
## Urea cycle disorders

00:24:09



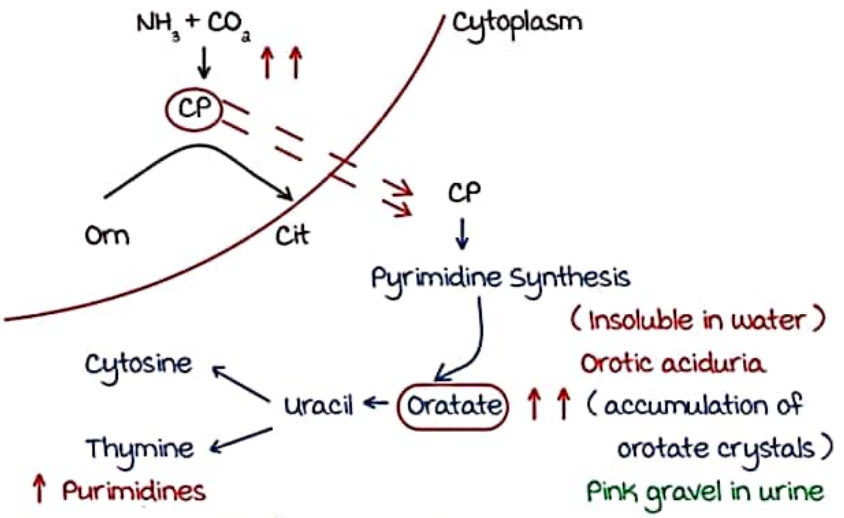

## Hyperammonemias

00:30:20



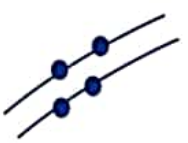

Active space

- Type II :




- Distinguishing factors of HA Type II are :
  - Orotic aciduria
  - Increased pyrimidine synthesis
- X - linked partially dominant (or recessive)
- most common urea cycle disorders [ 40% ]

- Trichohexis nodosa : dry brittle hair
  See in argininosuccinic aciduria



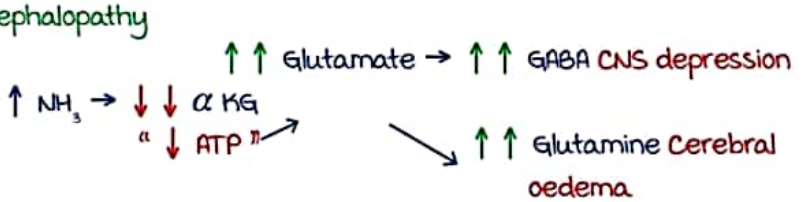
- HHH Syndrome
  - defect in Ornithine transporter / Ornithine permease
  - gene : ORNT - 1
  - Hyperammonemia
    Hyperornithinemia
    Homocitrullinemia / nuria
  - Homocitrullinemia is because CP combines with Lysine

- Arginemia
  - Least hyperammonemia
    - nitrogen is alredy adducted
    - arginase enzyme isoform is active
  - Progressive spactic diplegia => Scissoring of lower extremities

## Clinical features

00:43:17

- Encephalopathy



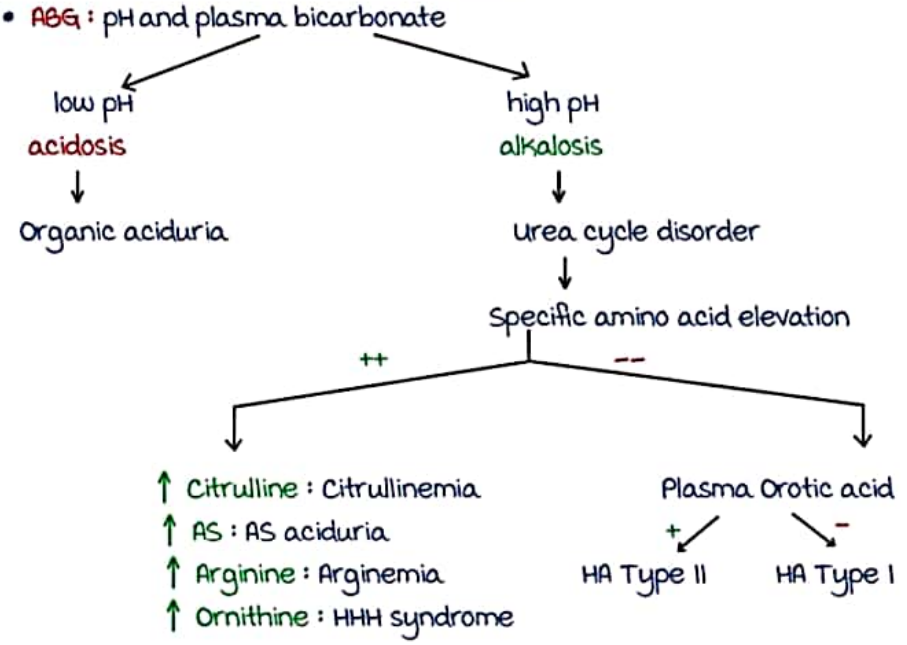

Active space

- Hyperammonemia
- Respiratory alkalosis

  ↑ $NH_3$ → Hyperventilation → Respiratory alkalosis
  Tachypnoea
- In neonates ,
  - feeding difficulties
  - lethargy
  - tachypnoea
  - Convulsions
  - if untreated ,deep coma

## Investigations 00:47:22

- Plasma $NH_3^+$
  - Tested by the methods : Ammonia selective electrode
    Glutamate dehydrogenase enzymatic method .
- ABG : pH and plasma bicarbonate

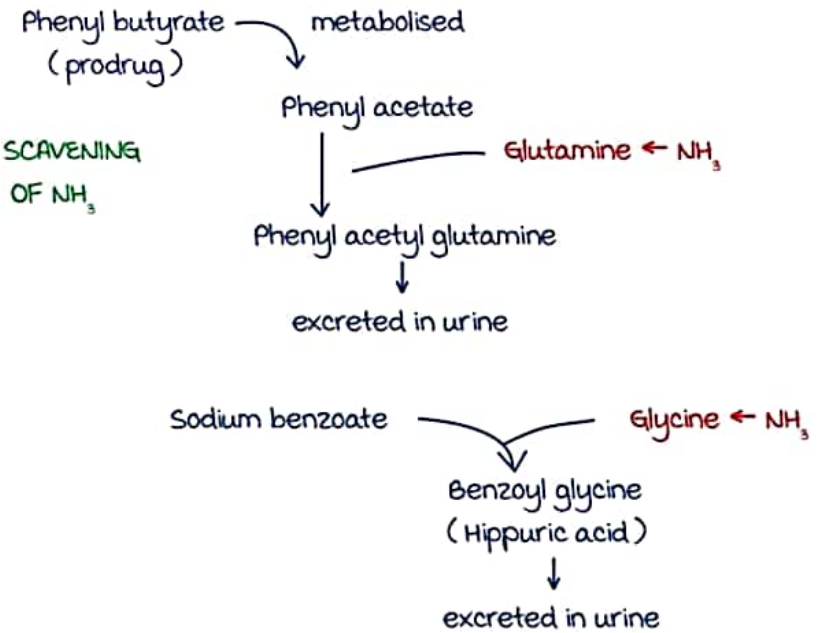



## Treatment 00:52:27

- 1st line treatment : Arginine
  - provides ornithine ,a positive regulator of urea cycle
  - allosteric activator of NAG synthase which ↑ NAG , a positive regulator of urea cycle
  - essential amino acid
  - contraindicated Arginase defect disorders

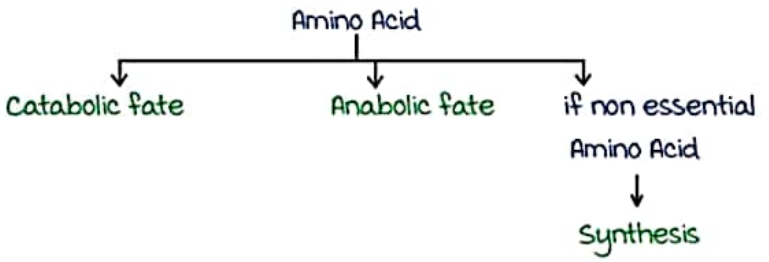
Active space

- 2nd line : Acylation therapy
  - Sodium benzoate or phenyl butyrate is used
  - Mechanism of action :



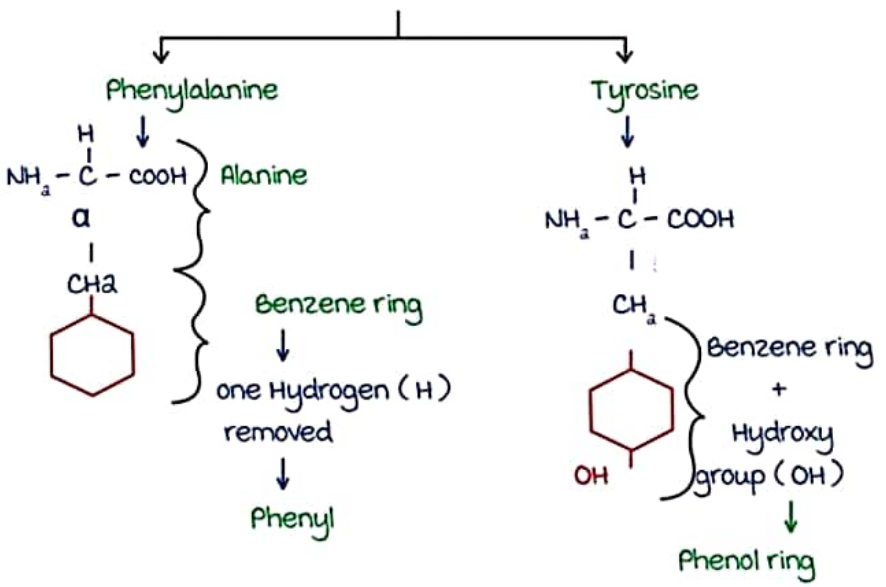

Active space

# AROMATIC AMINO ACIDS




## Phenylalanine and tyrosine structure

00:05:55

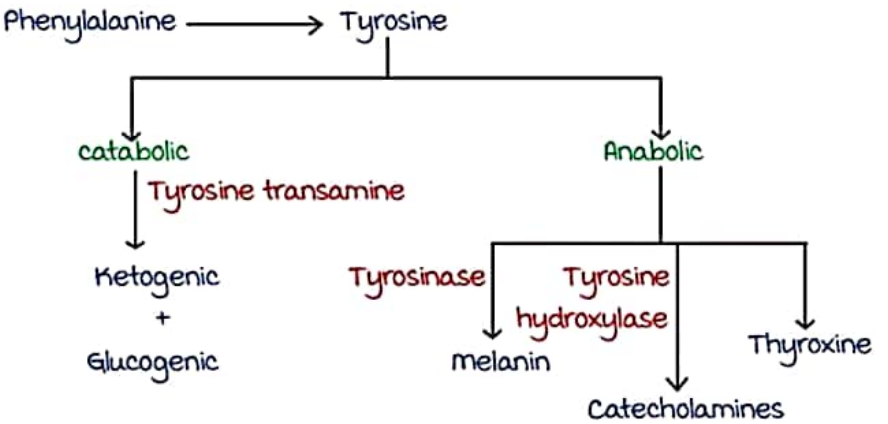



- Essential Amino Acid ( AA )
- Non polar AA
- Both Ketogenic & glucogenic

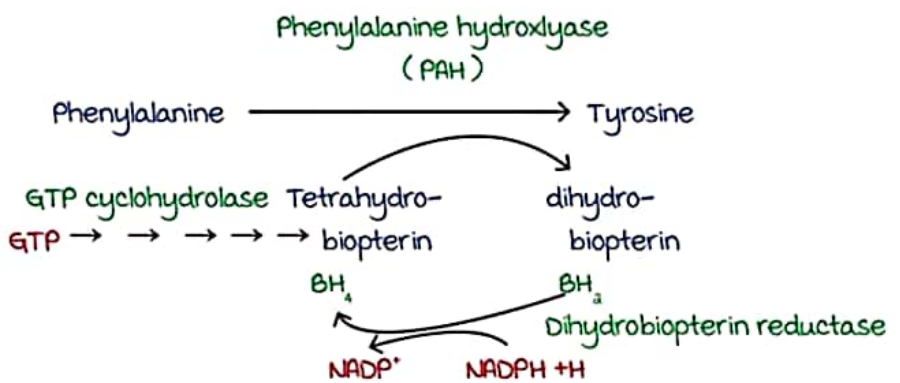
- Non essential AA
- Non polar AA
  ( because of OH group there is some polar nature )
  ↓
  Least non polar AA among non polar aromatic AA
- Both Ketogenic & glucogenic

Active space

## Metabolism of phenylalanine-overview 00:09:48




## Conversion of phenylalanine to tyrosine 00:12:13




Propereties of this reaction

I) Irreversible reaction

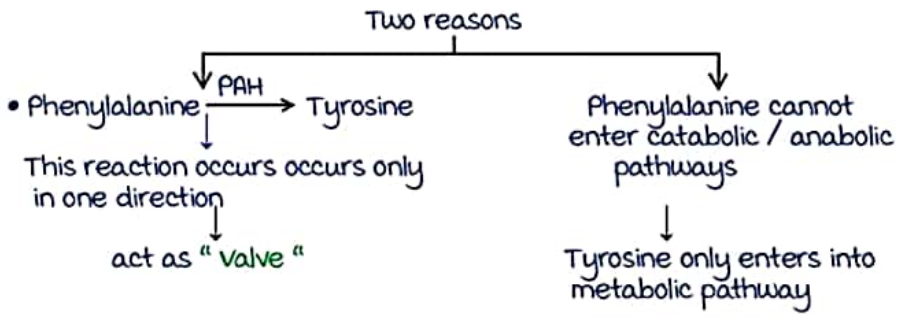
II) Phenylalanine hydroxylase is – monoxygenase (requires $BH_4$)

Clinical correlation

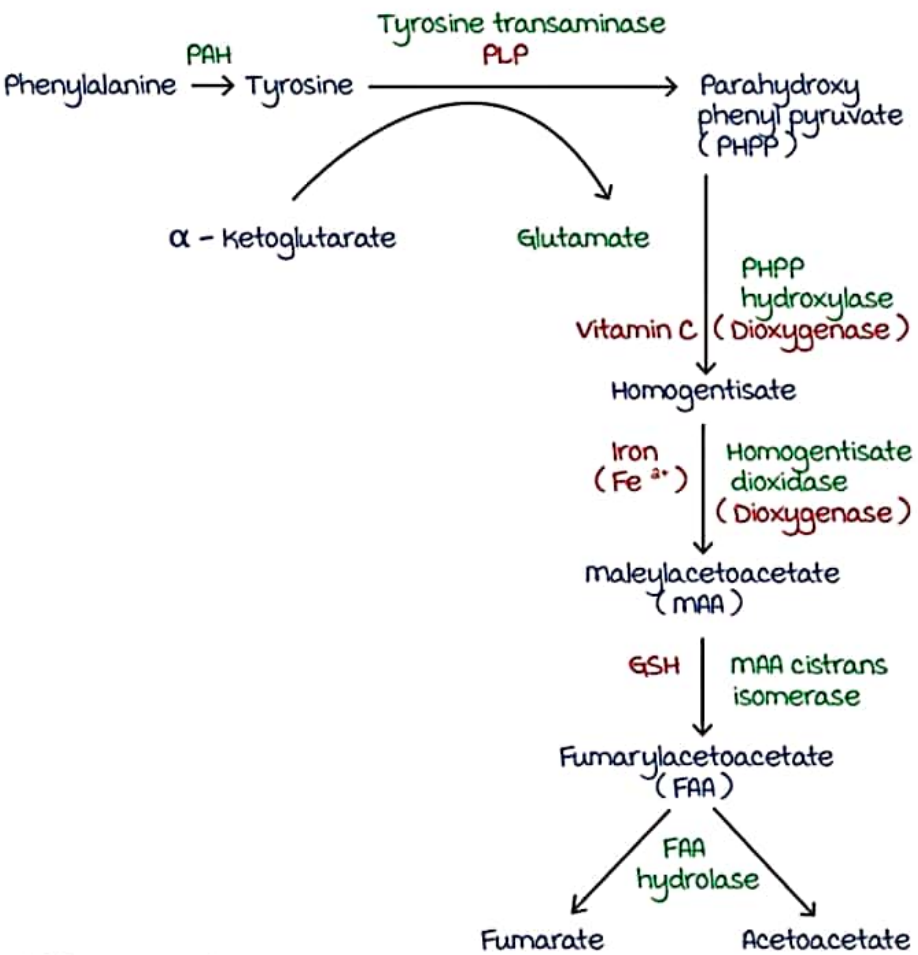
1) Phenylketonuria – Type I (classic) defect in phenylalanine hydroxylase
2) Phenylketonuria – Type II & III (non classic) defect in Dihydrobiopterin reductase
3) Phenylketonuria Type IV & V (non classic) defect in formation of tetrahydrobiopterin.

Active space

## Phenylalanine and tyrosine - twin amino acids 00:17:37




## Catabolic fate of phenylalanine and tyrosine 00:20:07




Clinical correlation

1) Alkaptonuria – defect in homogentisate dioxidase

2) Phenyketonuria– defect in phenylalanine hydroxylase

Active space

## Type I and II tyrosinemias

00:26:17

- Tyrosinemia – A / K / A – Tyrosinosis

| Type I tyrosinemia / Hereditary tyrosinemia / Hepatorenal tyrosinemia | Type II tyrosinemia oculocutaneous / Richner Hanhart syndrome |
|---|---|
| • M . C tyrosinemia<br>• Resemble porphyria<br>• Treatment – Nitisinone / NTBC<br>• Cabbage like body odour<br>• Enzyme deficiency – FAA hydrolase | clinical manifestation<br>ocular → corneal ulcer<br>cutaneous → Non pruritic hyper Keratotic plaque (Soles & palms)<br>• Enzyme deficiency – Tyrosine Transaminase |

## Type - III tyrosinemia

00:31:22

- A / K / A – Neonatal tyrosinemia
- Least common.
- Enzyme deficiency – PHPP hydroxylase
- It is associated with Hawkinsinuria

↓ Mutant PHPP hydroxylase

↓ Partially active

↓ Produces Hawkinsin

↓ C / F – Swimming pool body odour

## Phenylketonuria - biochemical defect

00:34:07

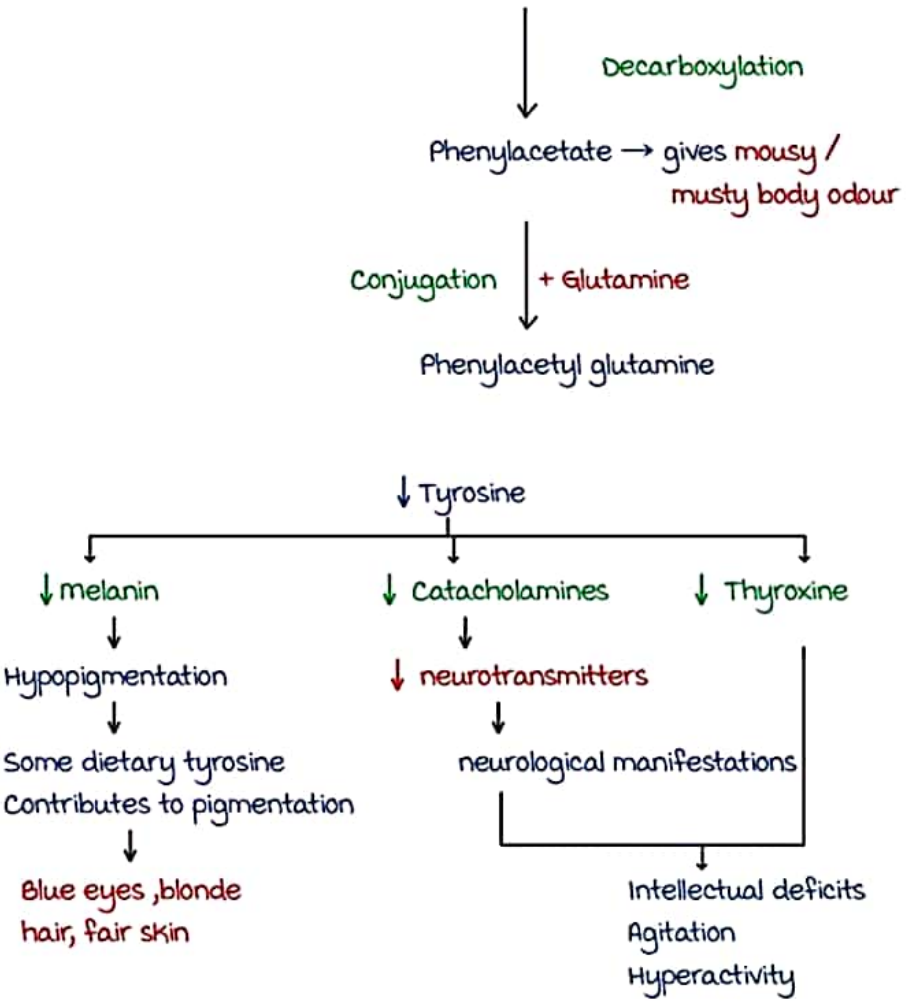
Phenylalanine $\xrightarrow[\text{classic PKU}]{\text{PAH}}$ Tyrosine

- Phenylalanine → enters alternate metabolic pathway

Phenylalanine $\xrightarrow{\text{Transamination}}$ Phenylpyruvate ($\xrightarrow{\text{Reduction}}$ phenyl lactate)

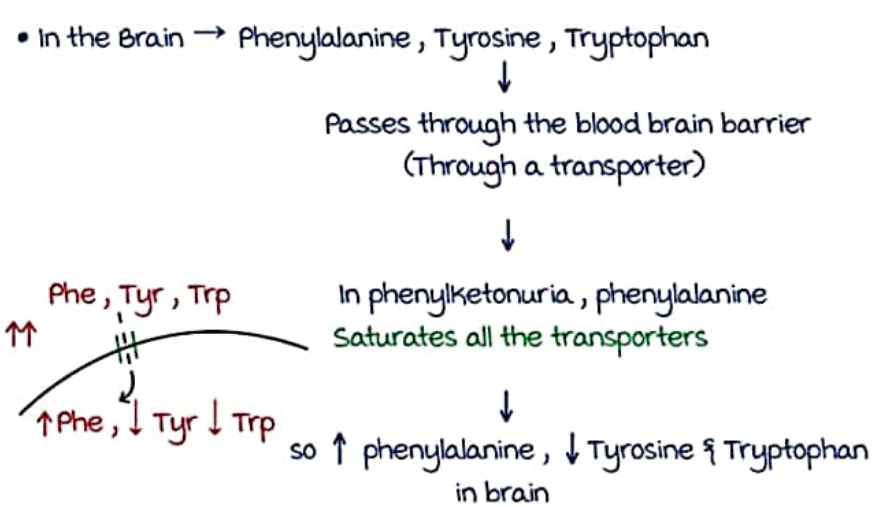
(it is phenylketoacid)

Active space




## Phenylketonuria - cause of neurological manifestations

00:43:17




Active space

↑ Phenylalanine → Tyrosine
↓ Tyrosine → ↓ Neurotransmitter
↓ Tryptophan → ↓ Serotonin

These results in neurological manifestation.

- infancy – phenylketonuria

↓

C / F – Severe vomiting

↓

may be diagnosed as a case of
congenital hypertrophic pyloric stenosis

## Phenylketonuria - laboratory diagnosis

00:47:43

1) Guthries bacterial inhibition test

↓

Blood sample – " Heel prick "

↓

Bacillus subtilis requires phenylalanine for growth

↓

if phenylalanine in blood – bacterial cultures seen

2) Ferric chloride test – in urine

↓

To 1 ml of urine

↓

Add ferric chloride reagent

↓

gives transient blue green colour

↓

means ferric chloride – positive

↓

due to presence of phenylpyruvate

3) Blood phenylalanine – 2 – 6 mg / dl
> 20 mg / dl – bad prognosis

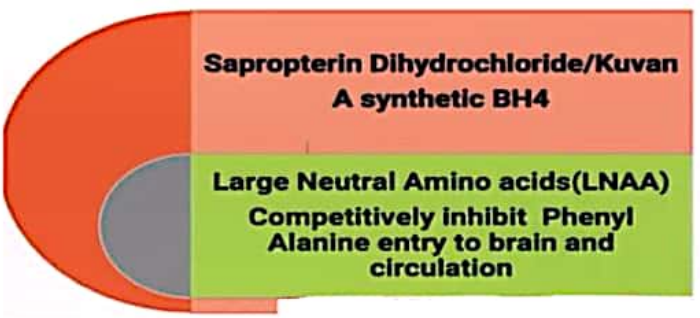
Acid space

4) Tandem mass spectrometry / Fluorometric analysis – Gold standard investigation
5) Enzyme studies
6) Genetic mutation
7) Phenylalanine hydroxylase probe

## Phenylketonuria - treatment 00:53:02

1) Dietary restriction of phenylalanine

**Latest treatment of PKU**

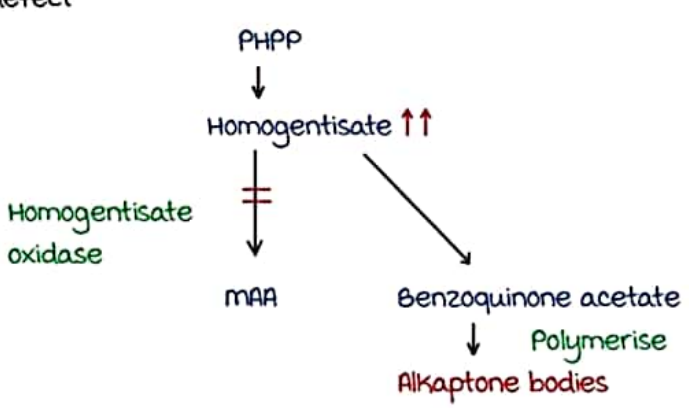



- Recombinant enzyme therapy – phenylalanine ammonia lyase
  ↓
  under trial

## Alkaptonuria 00:55:09

- It is a part of Garrod's tetrad – studied by " Archibald Garrod "
  ↓
  C – Cystinuria
  A – Alkaptonuria
  A – Albinism
  P – Pentosuria
  → IEM (Inborn errors of metabolism)

Biochemical defect




Active space

Accumulates in cartilagenous tissues of

- intervertebral disc → Ochronosis → C / F – low backache seen in middle aged
- Nose
- Pinna
- Sclera

→ Blackish spots

## Alkaptonuria - diagnosis & treatment 01:00:37

- Homogentisate → Excreted in urine
  - ↓ Freshly excreted urine ( Normal in colour )
  - ↓ Later urine gets oxidised
  - ↓ Black discoloration ( From top to bottom in a tube ).
  - ↓ in infants – Black / reddish discoloration of diaper

- In Alkaptonuria – No mental retardation
  - ↓ because phenylalanine is converted to tyrosine

Laboratory diagnosis

- Alkalanisation of urine – ↑ darkening
- Ferric chloride test
- $AgNo_3$ ( silver nitrate ) test – Positive
- X – ray spin – parrot beak appearance
  Bamboo like spine

Treatment

- Nitisinone / NTBC → inhibit → PHPP hydroxylase

PHPP → homogentisate ↓ ↓

Active space

## Segawa syndrome 01:05:99

- Enzyme defect – GTP cyclohydrolase

↓ $BH_4$ synthesis
↓
but normal phenylalanine levels

- Autosomal dominant
- m.c in females
- C/F – Dystonia with diurnal variation
  ↓
  sudden weakness of lowerlimb
  more in the evenings

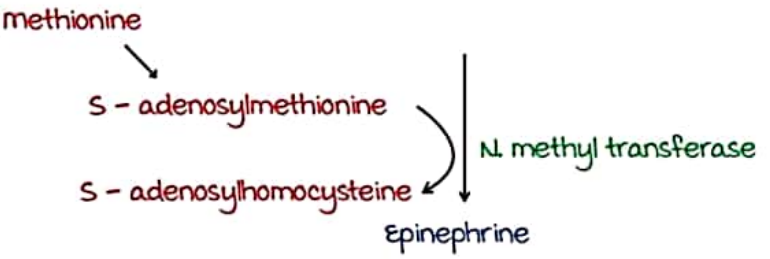
## Anabolic fate of tyrosine - catacholeamines 01:10:41

1) Catecholmines
↓
Synthesis in – chromaffin cells
↓
Adrenal medulla → 80 % adrenaline / epinephrine
Extraadrenal → Sympathetic ganglion, Nerve endings → Nor adrenaline

Pathway of synthesis

Phenylalanine —PAH→ Tyrosine
↓ Tyrosine hydroxylase – Rate limiting enzyme
Dihydroxy phenylalanine (DOPA)
↓ Dopa decarboxylase, PLP
Dopamine – 1st catecholamine sunthesised
↓ Dopamine betaoxidase
Norepinephrine

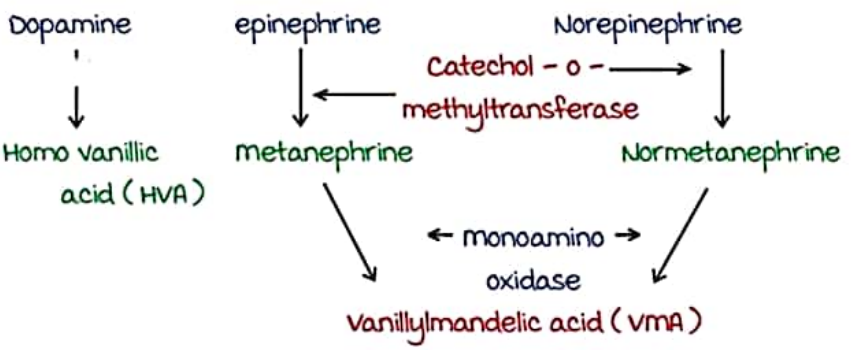
Active space




Three similar aromatic AA hydroxylases.

I) PAH
II) Tyrosine hydroxylase
IIII) Tryptophan hydroxylase

Monooxygenase
Require $BH_4$, NADPH

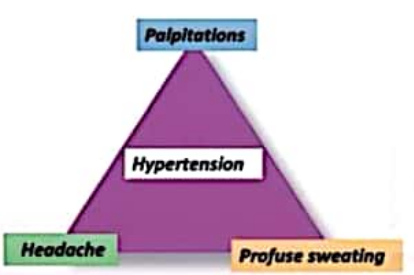



## Pheochromocytoma

1:19:03

- Tumor of adrenal medulla

- **Clinical presentation is highly variable**
- **The classic triad of Pheochromocytoma**




Biochemical diagnosis of pheochromocytoma

24 - hour urinary tests are
1) Vanillylmandelic acid
2) Fractionated metanephrines

Plasma tests are
- Catecholamines
- Free metanephrines

Active space

## Anabolic fate of tyrosine - melanin 01:21:08

Melanin

↓

Synthesised in melanosomes in melanocytes → present in deeper layers gives pigmentation to - skin , hair , iris , retina

Pathway of synthesis

Phenylalanine —PAH→ Tyrosine

↓ Tyrosinase

DOPA

↓ Tyrosinase

Dopaquinone

↓

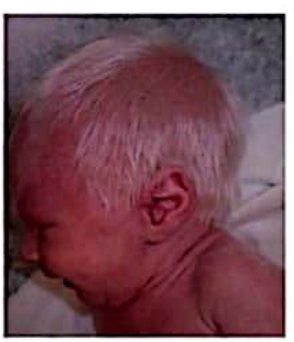
Melanin

Clinical correlation

- Albinisim → defect in tyrosinase enzyme

↓

C / F - milky white skin & hair
Red eye reflex



## Anabolic fate of tyrosine - thyroxin 00:24:23

In thyroid follicles → Thyroglobulin present ( protein )

↓

has 115 Tyrosine residues

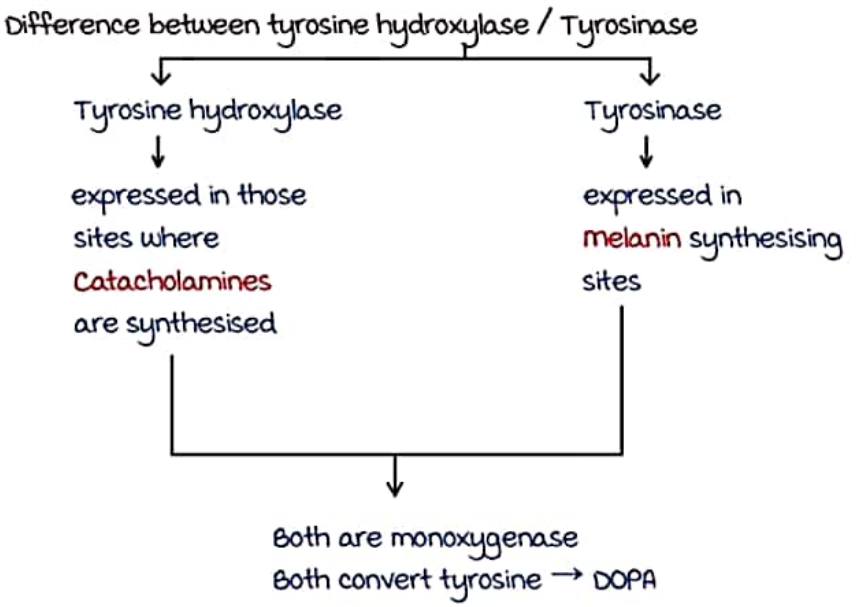
↓

if iodinated → MIT - Monoiodo thyronine
DIT - Diiodo thyronine

↓

MIT + DIT - $T_3$
DIT + DIT - $T_4$

Active space

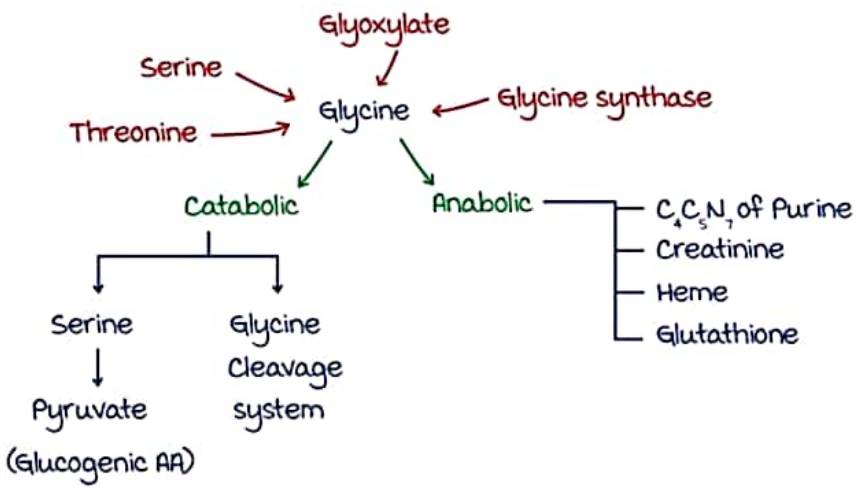



Active space

# GLYCINE & SERINE

## Glycine

00:02:34




Chemistry : $NH_2-\underset{\underset{H}{|}}{\overset{\overset{H}{|}}{C}}-COOH$ } No asymmetric / No optically active carbon atom

- Non essential amino acid
- Purely glucogenic amino acid
- Polar amino acid
- Simple amino acid

## Glycine : pathway

00:07:38

- Serine $\xleftrightarrow{\text{SHMT (PLP, Folic acid)}}$ Glycine .
  methyleneTHFA ($\beta$ Carbon)
  ↓
  Primary donor of 1 Carbon group .

SHMT = Serine hydroxy-methyltransferase

- Glyoxylate $\xleftrightarrow{\text{Glyoxylate alanine aminotransferase(PLP)}}$ Glycine
  Alanine → Pyruvate

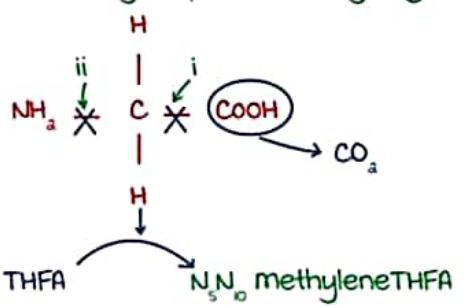
- Glyoxylate ⇄ Glutamate
  Glycine ⇄ $\alpha$ Ketogluterate

Active space

Threonine aldolase

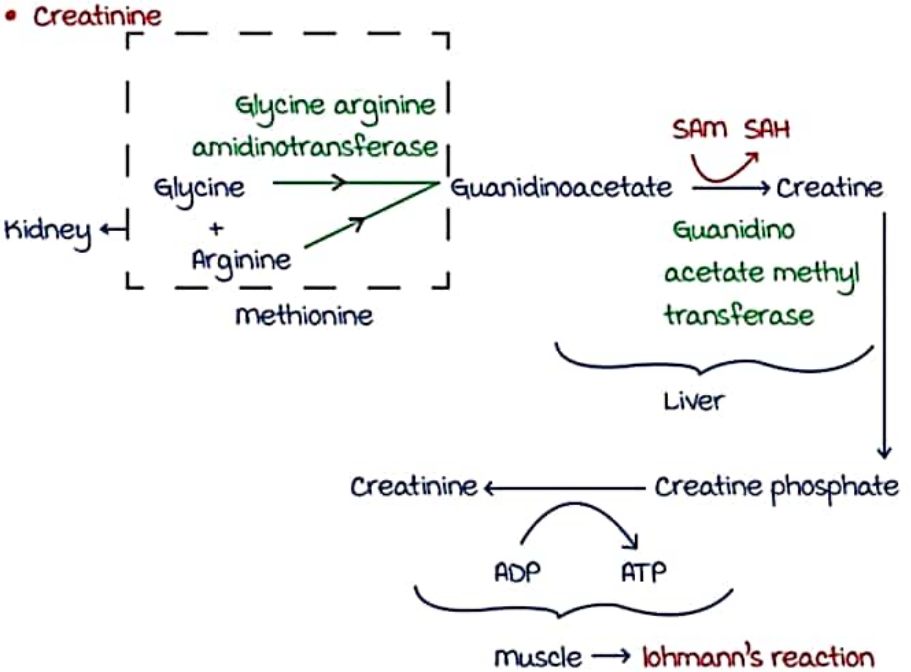
- Threonine ⟶ Glycine

- Glycine Cleavage System (GCS)
  - Multienzyme complex
  - H - Protein [ Covalently linked to 3 enzymes ]
    - i) Glycine dehydrogenase ⟶ cuts the COOH
    - ii) Aminomethyltransferase ⟶ separate methyl group
    - iii) Dihydrolipoamide dehydrogenase




## Glycine : anabolic fate & function 00:16:19

- Creatinine




SAM:-S-adenosylmethionine

SAH:- S-adenosyl homocysteine

"Creatine $PO_4$" ⟶
- Immediate replenisher of ATP in muscle [ first 3 - 4 secs of exercise ]
- High energy compound
- A/K/A Phosphagen

- Heme

  Succinyl CoA + Glycine → → → → Heme

Active space

- Glutathione
- $C_4$, $C_5$, $N_7$ of Purine
- Neurotransmitter
- Conjugating agent – • Bile acids
  • Benzoyl CoA ⟶ Hippuric Acid
- most abundant / recurring amino acid in collagen
- Induces bends in 2° Structure of proteins.

## Glycine : clinical correlation

00:23:09

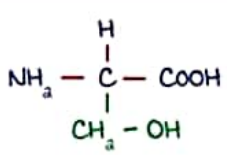
- Hyperoxaluria — 1° — Type I ⟶ defect in Glyoxylate Alanine Aminotransferase
  2° — Type 2 ⟶ defect in Glycerate dehydrogenase / Glyoxylate reductase
  - Vit $B_6$ deficiency
  - Vit C toxicity
  - methoxyflurane
  - Ethylene Glycol poisoning
  - Enteric hyperoxaluria

  Glyoxylate accumulation ↓ Oxaluria

- if defect in GCS ⟶ Non-Ketotic hyperglycinemia

## Serine

00:27:14



- Polar, uncharged amino acid
- Non – essential amino acid
- Purely glucogenic amino acid

– mc site of Phosphorylation

– Can be synthesized from :
- Glycine ⟷ Serine
- 3 Phosphoglycerate ( 3PG )

– metabolic functions :
- Primary donor of 1 Carbon group.
  Serine ⟶ Glycine
  ↘ methylene THFA
- Cysteine synthesis.
- Phosphatidyl serine
- Sphingosine ( Serine + Palmitoyl CoA )
- Selenocysteine precursor

Active space

- Serine $\xrightarrow{PLP}$ Ethanolamine
  - $\searrow CO_2$
- Ethanolamine → Choline (Trimethyl ethanolamine) → Betaine (Trimethylglycine)
- Glycoproteins → O-linked glycoproteins

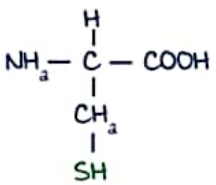
- MC site for phosphorylation (covalent modification of enzymes)

Active space

# SULPHUR CONTAINING AMINO ACIDS

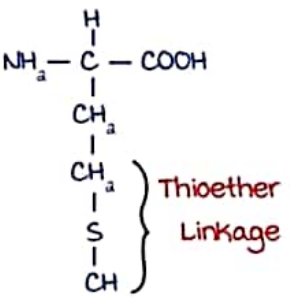
## Sulphur containing amino acids - Introduction

- Cysteine



- Sulfhydryl (-SH) / Thioalcohol / Thiol group
- Non-essential amino acid (AA)
- Purely glucogenic
- Polar amino acid

- methionine

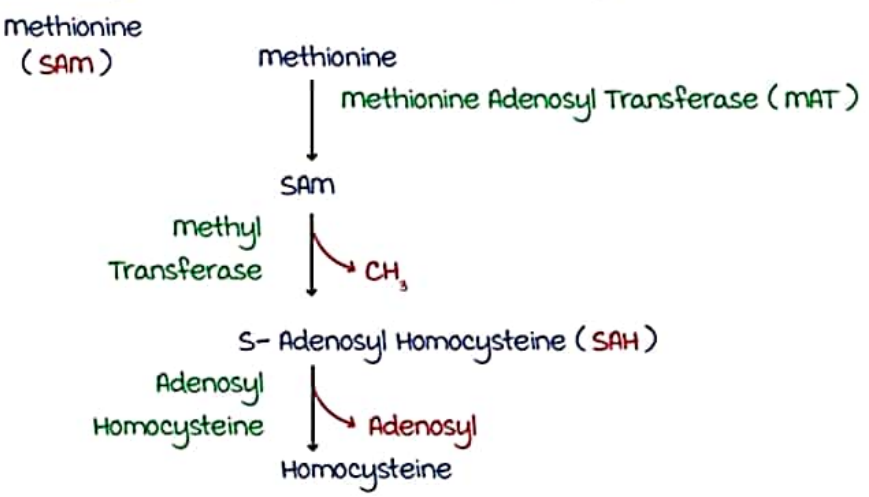



- Non-polar AA
- Does not respond to sulphur test
- Essential amino acid
- Purely glucogenic

## Methionine - Metabolism

00:08:20

- methionine - Not a methyl donor
- S-Adenosyl methionine (SAM) - Principle methyl donor




- Methionine Adenosyl Transferase(MAT)
  - MAT- I → Liver
  - MAT- II → Extra hepatic Tissue
  - MAT- III → Liver

Active space

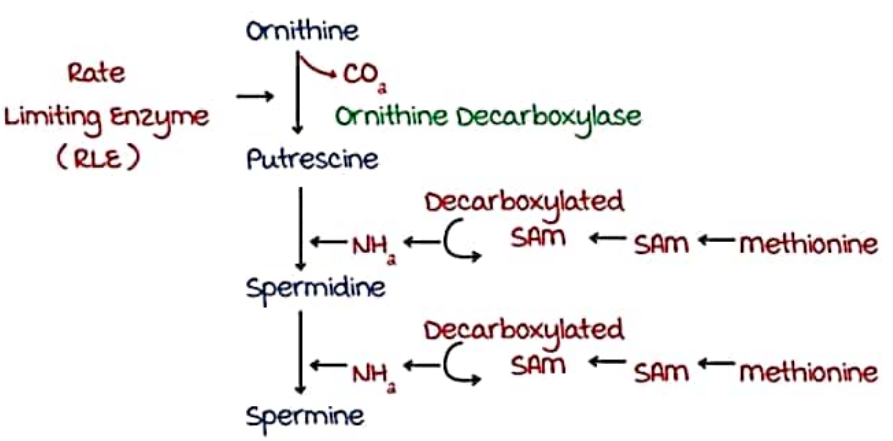
- Significance of S- Adenosyl Methionine (SAM)
  - Transmethylation Reaction
  - Polyamine Synthesis
  - DNA Methylation

## Transmethylation reaction & polyamine synthesis 00:13:52

1. Transmethylation Reaction

| Acceptor | Methylated Product |
|---|---|
| Guanido Acetate | Creatine |
| Nor- Epinephrine | Epinephrine |
| Epinephrine | Metanephrine |
| Ethanolamine | Choline (Trimethylethanolamine) |
| Carnosine | Anserine |
| Acetyl Serotonin | Melatonin |

2. Polyamines
   - Organic compound with > 1 amino group
   - Positively charged
   - Interact with negatively charged DNA
   - Regulates gene expression
   - Synthesis of polyamines :-
     Polyamines are derived from Ornithine and Methionine




   - Polyamine derived from Lysine → Cadaverine
   - Precursor for putrescine → Ornithine
   - Precursor for spermine & Spermidine → Ornithine + Methionine

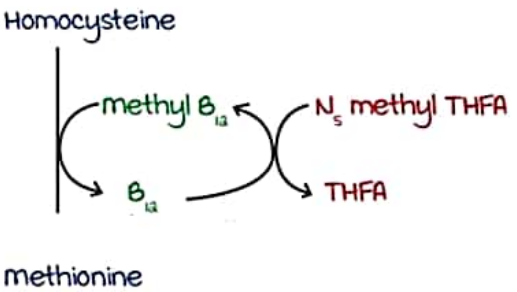
Active space

## Fate of Homocysteine

00:24:17

1. Regeneration of methionine

• THFA – Tetra Hydro Folic Acid




- Deficiency of $B_{12}$
  - ↓ Free THFA
  - A Functional deficiency
  - Known as folate trap / THFA starvation

↓

Defect in DNA synthesis

↓

Megaloblastic anaemia

[i.e ↑ Megaloblast in Bone Marrow
↑ Macrocytes in Peripheral smear]

- Deficiency of $B_{12}$ / Folic Acid

Homocysteine $\xrightarrow{B_{12} / FA}$ Methionine

∴ Deficiency of $B_{12}$ / FA ⟶ ↑ ↑ Homocysteine

↓

Risk factor for Thrombosis

↓

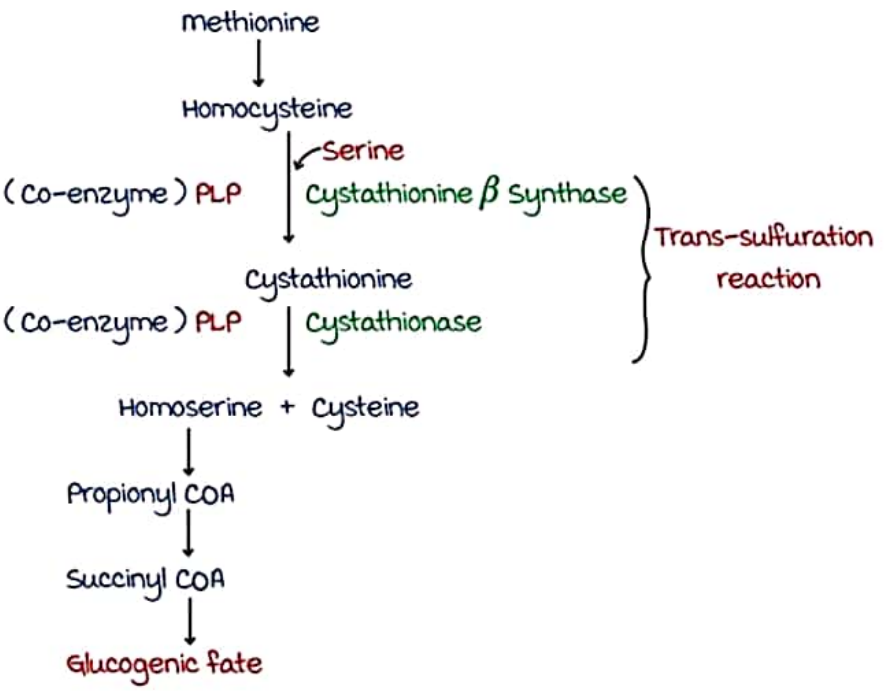
(CAD & CVA)

- Coronary Artery Disease
- Cerebrovascular Accidents

- Deficiency of $B_{12}$ and Folic Acid (FA)
  - ↑ Homocysteine in Blood
  - Homocystine excreted in Urine

Active space

2. Glucogenic fate




- Deficiency of B6
  - ↑ Homocysteine in Blood ⇒ CAD / CVA
  - ↑ Homocystine in Urine

## Biochemical disorders of Sulphur containing aminoacids

00:38:18

1. Oasthouse Syndrome / Smith Strang Disease
   - Defect : Methionine Transporter (intestine)

2. Primary Hypermethioninemia
   - Defect : Methionine Adenosyl Transferase(MAT)
   - Characteristic feature : Boiled cabbage odour

3. Classic Homocystinuria
   - Defect : Cystathionine β synthase

4. Cystathioninuria
   - Defect : Cystathionase

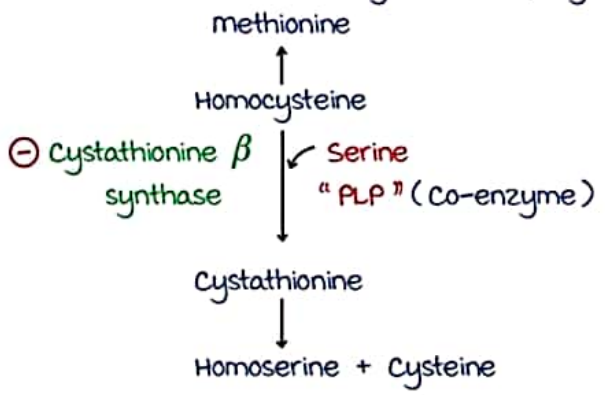
5. Non-classic Homocystinuria
   - Defect : $N_5$ methyl THFA and methyl $B_{12}$

Active space

## Classic homocystinuria - Defect and features

00:42:24

- Autosomal Recessive (AR)
- Biochemical Defect : defective Cystathionine β synthase




- ↑ Homocysteine in Blood
- ↑ Homocystine in urine
- ↓↓ Cysteine synthesis
- Methionine – Normal

- Clinical features

i) Initially – Asymptomatic
⇓
Developmental Delay

ii) At 3 yrs of age :
- ↓ Vision
- Progressive Myopia
- Quivering iris (iridodonesis)

On Examination – Ectopia lentis
(Lens – dislocated medially and downwards)

- Skeletal deformities
- Severe mental retardation
- Thromboembolism

Skeletal Deformities :

Arachnodactyly
Pectus Carinatum
Pectus excavatum

Active space

- Genu Valgum / Varum
- Coxa vara
- Pes cavus
- High arched palate

Homocystinuria resembles Marfan's Syndrome

## Classic Homocystinuria - management 00:52:15

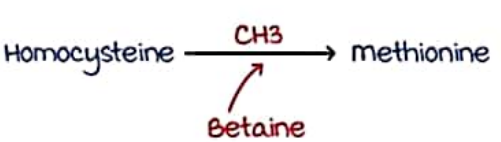
1. Investigations
   - Cyanide Nitroprusside Test → Magenta colour
   - Tandem Mass spectrometry → Best screening method
   - Enzyme Analysis
   - DNA Mutation Studies

Cyanide Nitroprusside test is answered by
- Homocysteine, homocystine
- Cysteine , Cystine

2. Treatment
   - High dose of Vitamin $B_6$
     Reason - Vitamin $B_6$ ( PLP ) → Coenzyme of cystathionine $\beta$ synthase

   - Restriction of Methionine with cysteine supplementation
     Reason - Methionine is Synthesized but cysteine is not

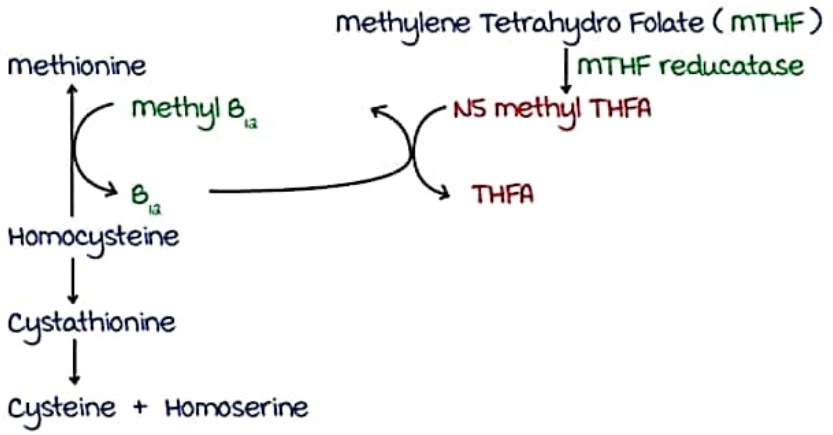
   - Betaine supplementation
     Reason - Trimethyl Glycine ( Betaine ) → Remethylation of Homocysteine




   - Administration of Vitamin C → Improve endothelial function .

## Non - Classic Homocystinuria 00:55:50

- Defect
  - Defect in formation of $N_5$ methyl THFA
  - Defect in formation of Methyl cobalamine




- Level of
  - Methionine ↓ ↓
  - Cysteine – Normal

## Homocystinuria - Comparison

00:58:38

| Feature | Classic Homocystinuria | Methyl Cobalamine defect | MTHFR reductase deficiency |
|---|---|---|---|
| • Homocysteinemia | + | + | + |
| • Methionine level in Blood | Normal | ↓ | ↓ |
| • Cysteine level in Blood | ↓ | Normal | Normal |
| • Megaloblastic Anaemia | Absent | + | Absent |

## Other disorders of Sulphur containing amino acids

01:00:55

1. Cystathioninuria
   - Defect : Cystathionase
   - Cyanide Nitroprusside test : Negative

2. Cystinuria
   - Defect : Dibasic AA transporter in Intestine and Renal tubules
   - A part of Garrod's tetrad : C – Cystinuria
     A – Alkaptonuria
     A – Albinism
     P – Pentosuria

Active space

- Excretion in urine : C - Cystine
  O - Ornithine
  L - Lysine
  A - Arginine

- Cyanide Nitroprusside test : Positive

3. Cystinosis
   - Lysosomal Storage Disorders
   - Defect : Cystine transporter ⟶ Cystonosin [ product of CTNS gene ]
     (Lysosomal $H^+$ driven)
   - Affects :
     Liver → Hepatic failure
     Renal → Renal failure
     Cornea → Corneal opacity
     Bone marrow
   - Treatment : Cysteamine

Cysteamine
↘
Cystine ⟶ Cysteine

## Specialised products from cysteine

01:05:48

1. Cysteine on decarboxylation gives → Betamercaptoethanolamine
2. Co-enzyme A
3. Taurine
   - Conjugates Bile acids
4. Glutathione ( GSH )
5. Cystine
   - 2 cysteine groups joined together by 2 SH groups .

## Glutathione ( GSH )

01:06:53

- It is a tripeptide
  3 AA's - Gamma Glutamic acid + Cysteine + Glycine

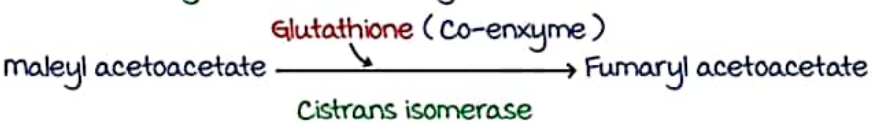
- It is Gamma Glutamyl Cysteinyl glycine

- A pseudopeptide ( Gamma carboxylic acid forms the peptide bond )

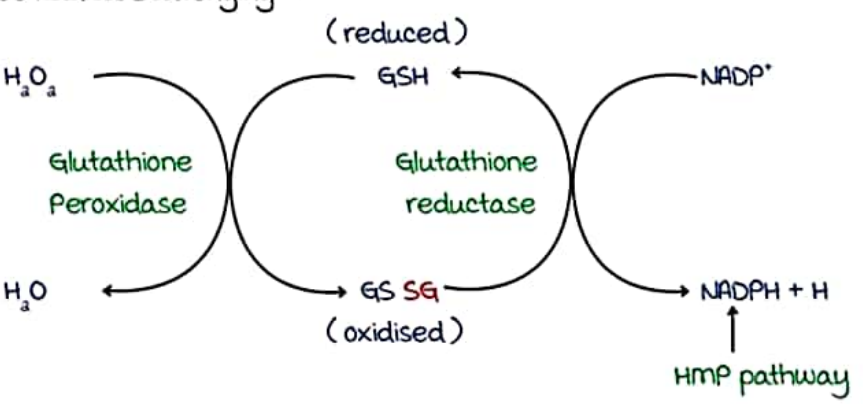
- Active part / Business part / Banking part - is SH group of cysteine

Functions of Glutathione

- Amino Acid Transport
  - Meister's cycle / Gammaglutamyl cycle
- Free Radical scavenging
- Maintains RBC membrane integrity
- Keeps Iron in ferrous state in Hemoglobin
- Antioxidant
- Conjugation
  - In phase-II Xenobiotic reaction
- Acts as co-enzyme for various enzymes




Free Radical Scavenging :-



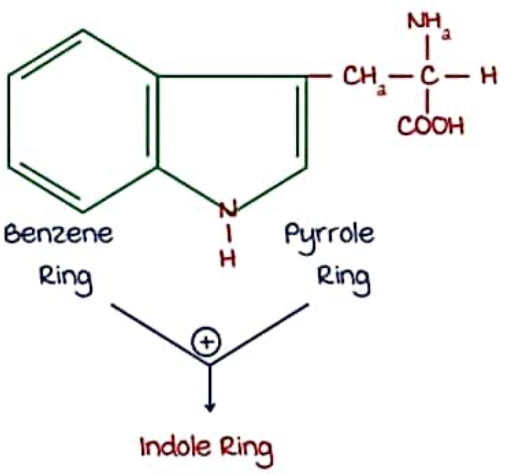

- Glutathione peroxidase
  - A Selenocysteine containing enzyme

- Glutathione reductase
  - Flavin containing enzyme
  - Helps to assess $B_2$ level in Blood.

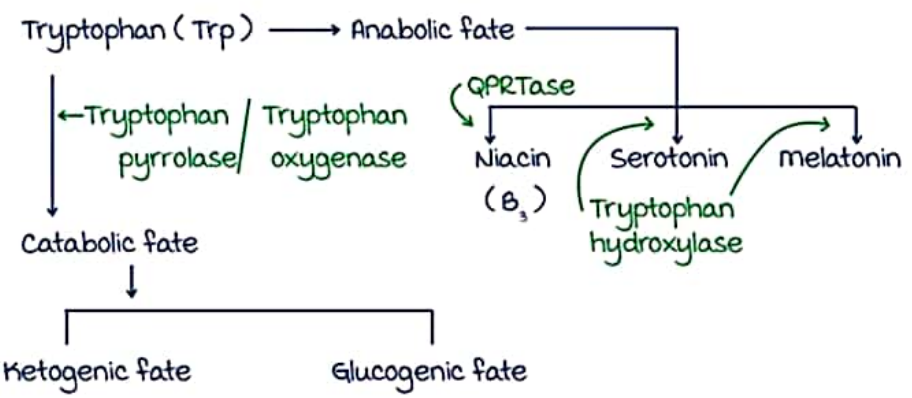
Active space

# TRYPTOPHAN

## Tryptophan : Chemistry 00:02:16

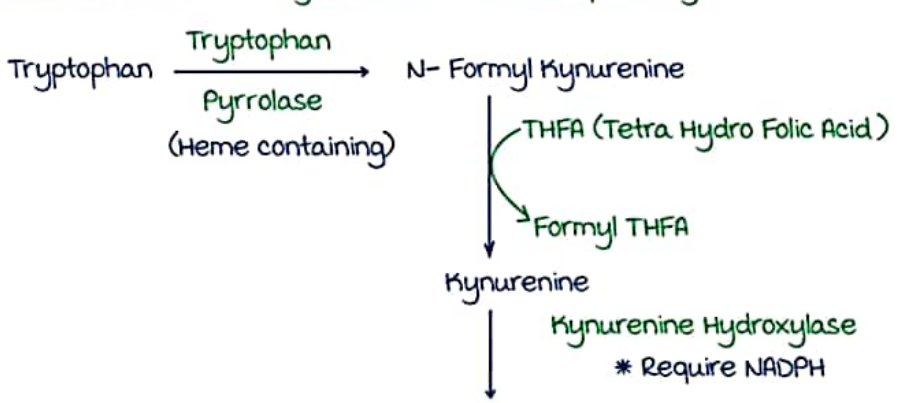



- Aromatic Amino Acid
- Essential Amino acid
- Both Ketogenic & glucogenic

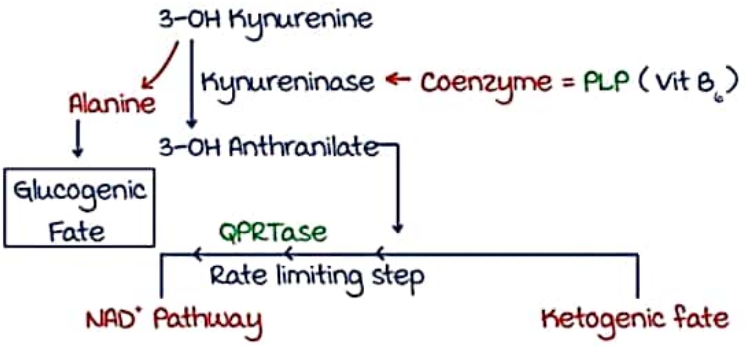



## Tryptophan : Metabolism - catabolic fate 00:06:27

Catabolic fate : A/K/A Kynurenine Anthranilate pathway




Active space




QPRTase – Quinolinate Phospho Ribosyl Transferase

- In $B_6$ deficiency → • 3-OH Kynurenine
  - ↓ Xanthurenic Acid
  - ↓ Excreted in urine
- ↓ Niacin → Pellagra like symptoms.

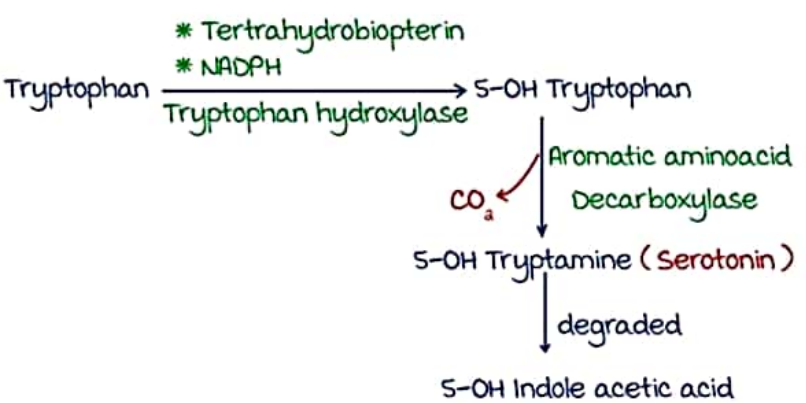
Conversion factor :

- 60 mg of Tryptophan —Converted→ 1 mg of Niacin

## Tryptophan : Metabolism - anabolic fate

00:14:36

- Serotonin :




Functions of serotonin : • Neuro transmitter

- ↓
- Vasoconstriction
- Mood elevator
- Temperature regulation
- Gastro intestinal Tract motility

Active space

Site of synthesis – Argentaffin cells.

↓

- Intestine
- Mast cells
- Platelets
- Brain

- Melatonin:
  - Synthesized in Pineal gland

Serotonin ⟶ Acetyl serotonin

↓ SAM ⟵ methionin
↓ SAH

Methyl Acetyl Serotonin
(Melatonin)

  - Function: • Biological rhythm
    • Neurotransmitter

## Carcinoid tumor / syndrome 00:20:17

- Neuroendocrine tumor
- A/K/A Argentaffinoma.
- Tumor of Argentaffin cells

↓

↑ Synthesis of serotonin
↓ Niacin Synthesis

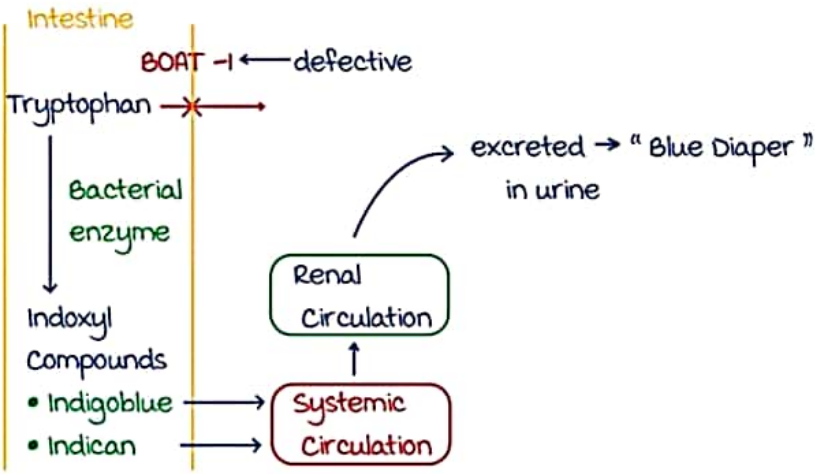
- Clinical features:
  - Intermittent diarrhoea
  - Cutaneous flushing due to ↑ Tachykinins
  - Sweating ↑
  - Fluctuating hypertension
  - ↓ Niacin → Pellagra like symptoms

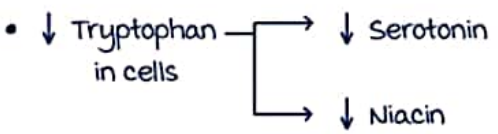
- Diagnosis: – ↑ Serum Serotonin
  – ↑ 5HIAA in 24 hr urine (Ⓝ = < 5mg / day)

## Hartnup's disease 00:25:45

- Defect in absorption of Tryptophan & other neutral amino acids from intestine & renal tubules

Active space




- Mc symptom → Cutaneous photosensitivity
  (Photosensitive dermatitis due to Niacin ↓)
- Neurological manifestation ( ↓ Serotonin ) :
  Wide based gait
  intermittent ataxia

- Diagnosis :
  • Obermeyer Test : Test for indican

Treatment : • Supplement $NAD^+$
• lipid soluble esters of Trytophan

## Drummond syndrome

00:20:17

• BOAT- 1 : Transporter ( of tryptophan) at Intestine
coded by SLC6A19

• Drummond Syndrome :
- BOAT 1 is defective only in intestine
- Blue diaper syndrome

Active space

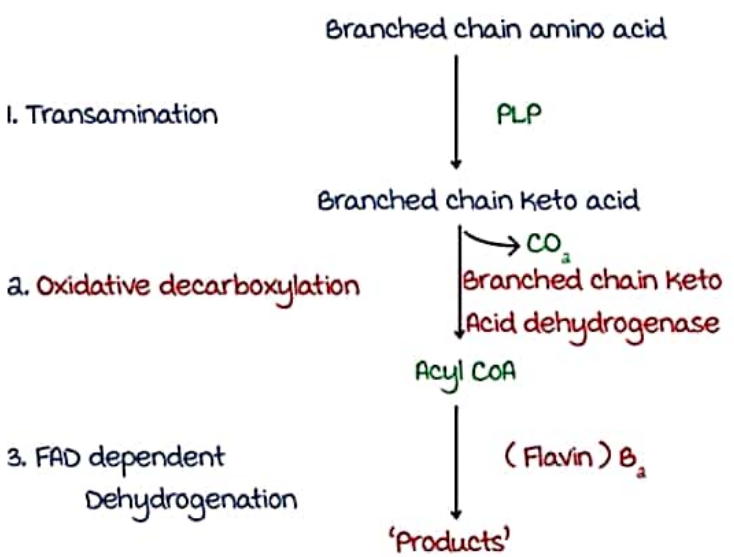
# BRANCHED CHAIN AMINO ACID

## Chemistry of branched chain amino acids 00:01:59

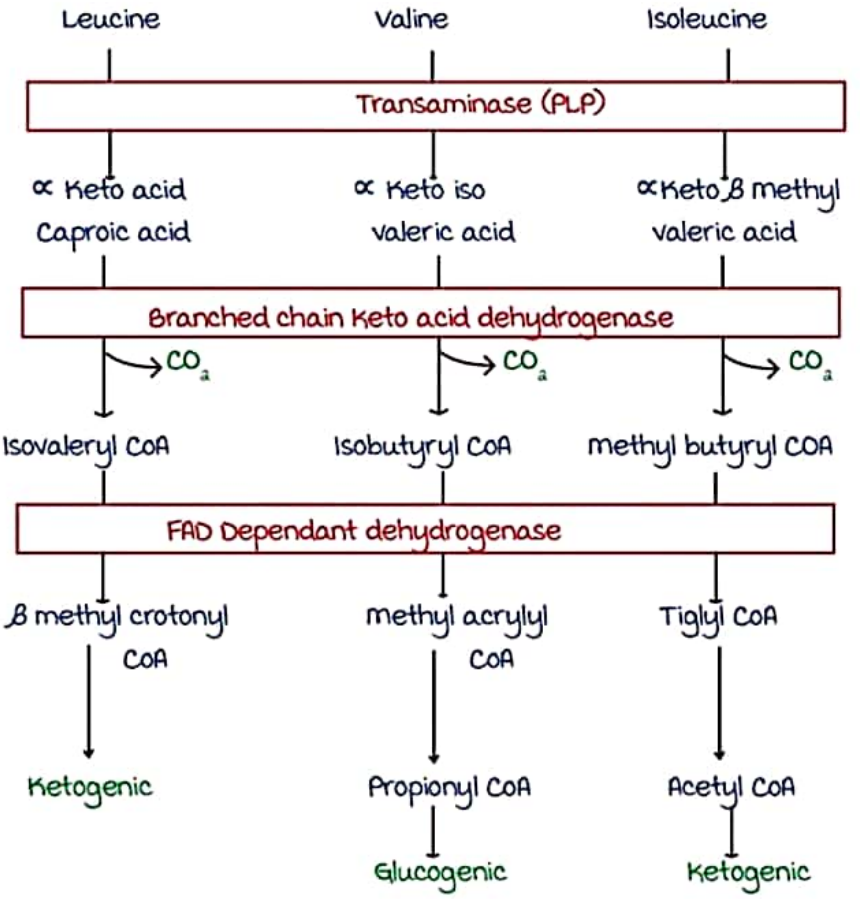
- Branched Chain amino acids are :
  - → Leucine : Ketogenic
  - → Isoleucine : Ketogenic and glucogenic
  - → Valine : Glucogenic

- All are non - polar
- All are essential

## Metabolism of branched chain amino acids 00:04:03




Active space




## Branched chain keto acid dehydrogenase

00:09:06

- Multi enzyme complex
  (Similar to pyruvate dehydrogenase)
- Has 3 enzymes :
  1. Branched chain Ketoacid decarboxylase → Gene $E_1 \alpha$, $E_1 \beta$
  2. Dihydrolipoyl transacylase → $E_2$
  3. Dihydrolipoamide dehydrogenase → $E_3$

- Co enzymes :
  1. Co A
  2. Thiamine pyrophosphate
  3. Lipoamide
  4. FAD
  5. $NAD^+$

Active space

## MSUD ( maple syrup urine disease ) 00:11:40

Defect in :

$E_1\alpha$ ⟶ Type I A (m.c) } Associated with
$E_1\beta$ ⟶ Type I B } Thiamine Pyrophosphate
$E_2$ ⟶ Type II
$E_3$ ⟶ Type III

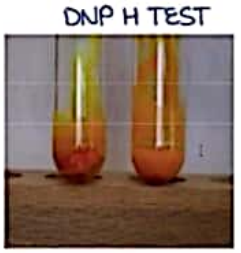
Clinical correlation of MSUD.

- Neonates
- Biochemical defect :→ Branched chain Keto acid Dehydrogenase Decarboxylase enzyme ($E_1$ enzyme component)
  → Defect in oxidative decarboxylation

Branched chain Keto acid ↑ ↑ (Accumulates)
✕ BCKD (defective)
Acyl Co A

- Clinical features :
  → Feeding difficulty
  → Failure to thrive
  → Lethargy
  → Convulsions
  → Hypotonia with bouts of hypertonia
  - boxing
  - bicycling
  → Urine(On refrigeration) -Maple Syrup / Burnt sugar / caramel (smell)

DNP H TEST



- Diagnosis : ↑ Branched chain amino acid } in urine
  ↑ Branched chain Keto acid }
  → Dinitro phenyl hydrazine test : Yellow colour precipitate
  → Rothera's test : purple ring

- Treatment :
  → Restrict branched chain amino acid
  → Supplement thiamine

## Isovaleric aciduria 00:18:00

- Defect in leucine catabolism
- Enzyme defect : Isovaleric acid dehydrogenase
- Smell of sweaty feet

Active space

# ACIDIC AND BASIC AMINO ACID

Acidic amino Acids
- Aspartic Acid → Asparagine
- Glutamic Acid → Glutamine

Basic Amino Acids
- Histidine
- Arginine
- Lysine

## Basic amino acid

00:03:25

| Histidine | Arginine | Lysine |
|---|---|---|
| → Essential | → essential/ Semi Essential | → essential |
| → Polar | → polar (most) | → polar |
| | most Basic | |
| → Imidazole | → Guanidinium | → ε amino group |
| → Glucogenic | → Glucogenic | → Purely Ketogenic |

Histidine
- metabolic function

1. Histidine → Histamine (CO2)
2. Histidine
   ↓ Histidase
   Urocanate
   ↓
   Imidazolone Propionate
   ↓
   Formimino Glutamic Acid (FIGLU)
   ↓ THFA → Formimino Folic acid
   Glutamic Acid

(α Keto glutarate) → Glucogenic fate

Active space

- Deficiency of THFA
  ↓
  ↑ Formimino glutaric Acid
  ↓
  Excreted in urine

- Histidine Load Test
  - If FIGLU is excreted in urine ⇒ folate Deficiency

3. carnosine ( β Alanine + Histidine )
4. Anserine (methyl carnosine)
5. Homocarnosine (GABA + Histidine )

## Arginine and lysine

00:11:34

Arginine
- metabolic functions : synthesis of

1. Agmatine → Antihypertensive
2. Creatine → Glycine + Arginine + methionine
3. Urea ⟶ Arginine —Arginase→ ornithine + Urea
4. Ornithine
5. Nitric oxide

- Nitric oxide
  → Endothelium Derived Relaxing Factor (EDRF)
  → Free radical
  → Gaseous molecule
  → Short half life (0.1s)
  → Arginine —Nitric oxide Synthase + NADPH→ Nitric oxide + citulline
  → Functions : Vasodilator
  Penile erection
  Neurotransmitter

Treatment of :
1. Pulmonary Hypertension
2. Impotence (sildenafil)
   ↓
   (Inhibits cGMP phosphodiesterase)
   ↓
   ↑ cGMP

Active space

3. Angina pectoris
   Glyceryl nitrite → Nitric oxide

- Nitric oxide Synthase
  → mono oxygenase
  → 5 cofactors : 1. Heme
  2. $BH_4$ (Tetra hydro biopterin)
  3. NADPH
  4. FMN
  5. FAD

→ 3 isoforms :
n Nos → neurons
i Nos → macrophages → not activated / Independent of calcium
e Nos → endothelial cells

Lysine

- Metabolic functions :
  1. Histones are rich in Arginine and Lysine
  2. Putrefaction → cadaverine (polyamine)
  3. Carnitine → Lysine + Methionine

## Acidic amino acids

00:21:46


EXTRA – COOH in side chain

→ EXTRA – CONH2
↓ Asparagine
↓ Glutamine

•chemical properties

| Aspartic acid | Glutamic Acid | Asparagine | Glutamine |
|---|---|---|---|
| → Non essential (NE) | NE | NE | NE |
| → Glucogenic | Glucogenic | Glucogenic | Glucogenic |
| → Polar | polar | ← Uncharged | polar → |

Active space

Aspartic acid
- Functions

1. Pyrimidine
2. Purine
3. Urea synthesis

Glutamic acid
- Function

1. N-acetyl Glutamate

Acetyl co A + Glutamic acid
↓
N – Acetyl Glutamate

2. Glutathione
(Gamma glutamyl cysteinyl Glycine)
3. GABA

Glutamate → GABA
$CO_2$

Glutamine
- Functions :

1. N3 N9 of purine
2. N3 of pyramidine
3. carrier of amino group from most organs including Brain
4. source of ammonia (Excretion of Nh3 → Renal regulation of Blood PH)

→ Enzyme required : Glutaminase enzyme

## Synthesis and catabolism of acidic amino acids 00:28:59

1 Aspartic Acid (Transmination)

Oxalo acetate → Aspartate
Glutamate → α Keto Gultarate

2. Glutamic Acid (Reductive Amidation)

Keto gultarate —GDH→ Glutamate

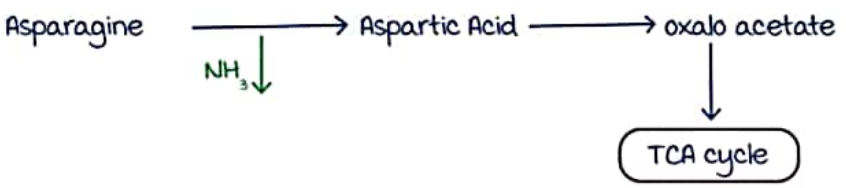
3. Asparate —Asparagine Synthetase→ Asparagine

– COOH → –co Nh2
Glutamine +ATP
Glutamate

Glutamate —Glutamine synthetase→ Glutamine
$NH_3$ + ATP

Active space

Catabolism





## Canavan disease

00:35:10

- Enzyme deficient : Aspartoacylace
  N Acetyl Aspartic ⟶ Aspartic Acid
  Acid
- Clinical features :
  - → Progressive macrocephally
  - → Persistent head lag
  - → Developmental delay
- On examination :
  - → Distorted mitochondria
  - → Severe leukodystrophy
  - → ↑ N-Acetyl Aspartic Acid in Blood , CSF, urine

Active space

# MISCELLANEOUS AMINO ACIDS

## Entry of amino acid to TCA cycle / anaplerotic reaction 00:01:41

- As pyruvate to oxaloacetate :
  - Hydroxyproline .
  - Serine .
  - Cysteine .
  - Threonine .

- As alanine to pyruvate to oxaloacetate :
  - Tryptophan .

- Directly to oxaloacetate :
  - Asparagine → Aspartate → oxaloacetate .

- As glutamate to ∝ Ketoglutarate .
  - Histidine .
  - Proline .
  - Glutamine .
  - Arginine .

- As succinyl COA :
  - Isoleucine .
  - Methionine .
  - Valine .
  - Threonine .

  } These are aminoacids that form propionyl CoA .

- As Fumarate :
  - Phenylalanine .
  - Tyrosine .

## Compounds and their chemical names 00:10:31

- Sarcosine → N methyl glycine .
- Betaine → Trimethyl glycine .(Rx of homocystinuria)
- Choline → Trimethyl ethanolamine .
- Ethanolamine → Serine on decarboxylation .
- Ergothionine → Derivative of histidine .
- $\beta$ mercaptoethanolamine → Cysteine on decarboxylation .
- Carnosine → $\beta$ alanyl Histidine .
- Anserine →Carnosine on methylation .

Active space

- Homocarnosine → GABA + Histidine .
- GABA → Glutamate on decarboxylation .

## Urine odour in various inborn erros of metabolism 00:13:44

| Inborn Errors of Metabolism | Urine Odour |
|---|---|
| Glutaric acidemia ( type II ) | Sweaty feet |
| Hawkinsinuria | Swimming Pool |
| Isovaleric Acidemia | Sweaty Feet |
| 3-Hydroxy 3-methylglutaric aciduria | Cat urine |
| Maple syrup urine disease | Maple syrup / caramel / Burnt Sugar |
| Hypermethioninemia | Boiled cabbage |
| Multiple carboxylase deficiency | Tom cat urine |
| Oasthouse urine disease | Boiled cabbage, Hops like |
| Phenylketonuria | Mousy / Musty |
| Trimethylaminuria ( Fish odour ) | Rotten Fish |
| Tyrosinemia | Boiled cabbage |

## Fish odour syndrome 00:18:01

- Enzyme defect : Trimethylamine oxidase .
  ( Flavin dependent monooxygenase ) .

- Trimethylamine is not metabolised .
  → smell of rotten fish .

- Rx : restrict dietary intake of trimethylamine ( choline ) containing foods . ( eggs, nuts, green leafy vegetables ) .

Active space

# CHEMISTRY OF NUCLEIC ACIDS

- There are two types of nucleic acids → DNA, RNA
- Nucleic acid : made of Nucleotides .
- Nucleotides made of three components :-

Nitrogenous base + pentose sugar + Phosphate

↓

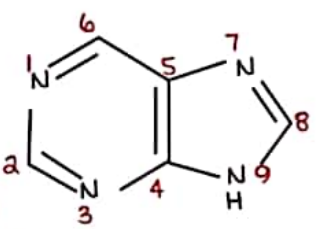
Nucleoside

## Nitrogenous base

00:02:51

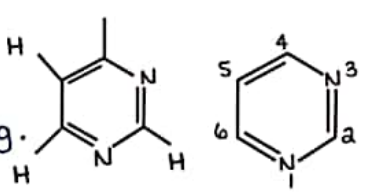
Purines

- They have two rings .
- Heterogenous ring .
- Purines are Adenine, Guanine
- Other minor purines are – Xanthine
  Hypoxanthine
  Uric acid



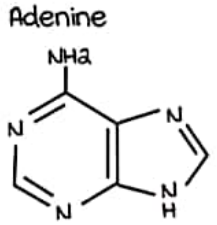
Pyrimidines

- They have a single heterogenous ring .
- Pyrimidines are Cytosine
  Uracil
  Thymine

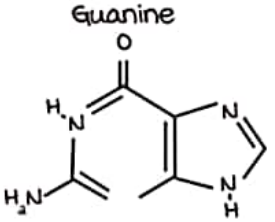


The Purines

Adenine

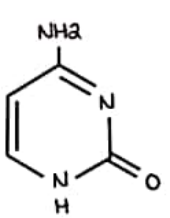


Guanine

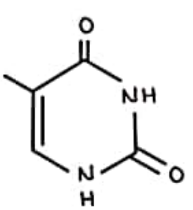


The Pyrimidines

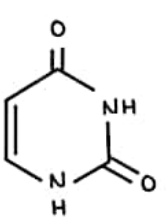
Cytosine



Thymine



Uracil



Active space

## Nucleoside

00:14:03

- Nucleoside = nitrogenous base + pentose sugar
- $N_9$ of purines joins with $C_1'$ of pentose sugar by $\beta$ – N – Glycosidic bond to form nucleoside.
- $N_1$ of pyrimidines joins with $C_1'$ of pentose sugar by $\beta$ – N – Glycosidic bond to form nucleoside.

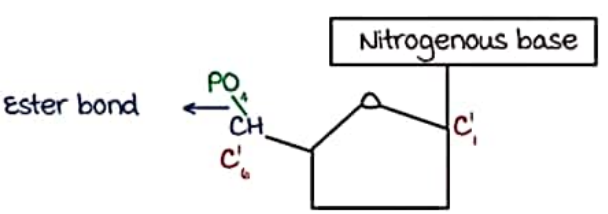
Cytosine < DNA / RNA

Uracil → RNA

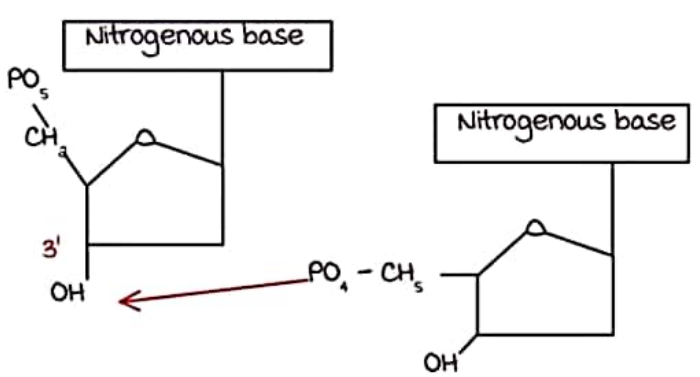
Thymine → DNA

## Nucleotide

00:19:09



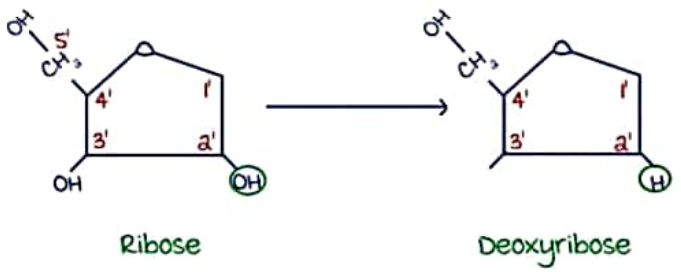

- This $C_5'$ of pentose sugar joins with – $PO_4$ group by Ester bond to form nucleotide.
- Further – $PO_4$ group are attached by Acid anhydride bond
- Dinucleotide is formed by formation of bond between 3' – OH grp and 5' – $PO_4$ group = 3' → 5' Phospho diester bond.




Active space

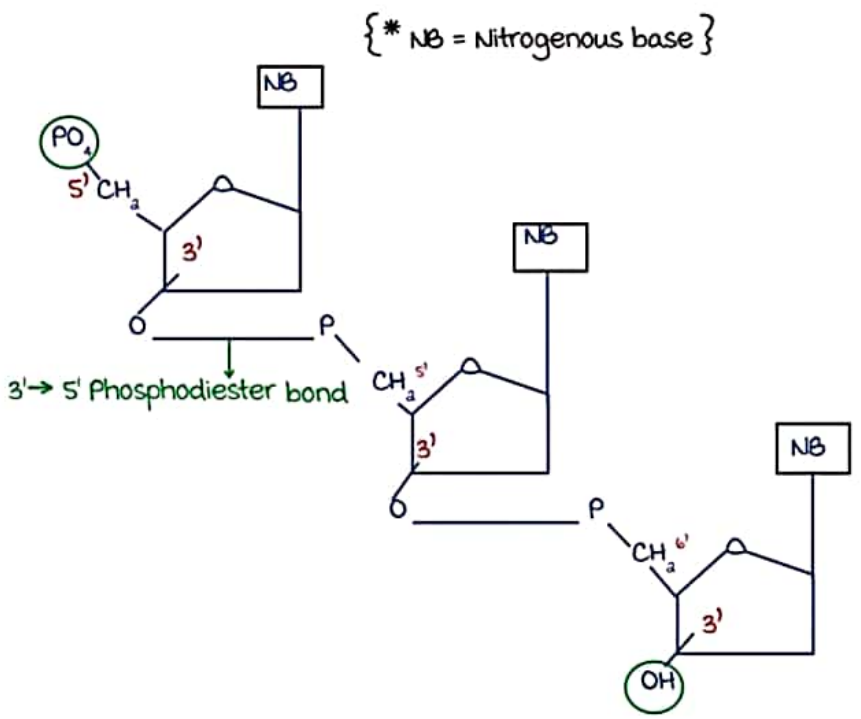
## Ribose and deoxyribose

00:24:34




## Polarity of nucleic acid

00:26:19




* Polarity :- 5' ⟶ 3'
* The nucleotide with the free functional group at 5' position – First nucleotide
* The nucleotide with the freefunctional group at 3' position – Last nucleotide .
* by convention, sequence of the nucleic acid: 5' ⟶ 3'

Active space

## Ribonucleotides

00:34:08

| Nitrogenous base | Nucleoside | Ribonucleotide |
|---|---|---|
| Adenine | Adenosine | Adenosine monophosphate (AMP) |
| Guanine | Guanosine | Guanosine monophosphate (GMP) |
| Xanthine | Xanthosine | Xanthosine monophosphate (XMP) |
| Hypoxanthine | Inosine | Inosine monophosphate ( IMP) |
| Cytosine | Cytidine | Cytidine monophosphate |
| Uracil | Uridine | Uridine monophosphate |

## Deoxyribonucleotides

00:38:03

| Nitrogenous base | Nucleoside | Deoxyribonucleotide |
|---|---|---|
| Adenine | d- Adenosine | d- Adenosine monophosphate |
| Guanine | d- Guanosine | d- Guanosine monophosphate |
| Cytosine | d- Cytidine | d- Cytidine monophosphate |
| Thymine | Thymidine | Thymidine monophosphate |

Active space

# PURINE METABOLISM

## De novo synthesis of purine

00:01:21

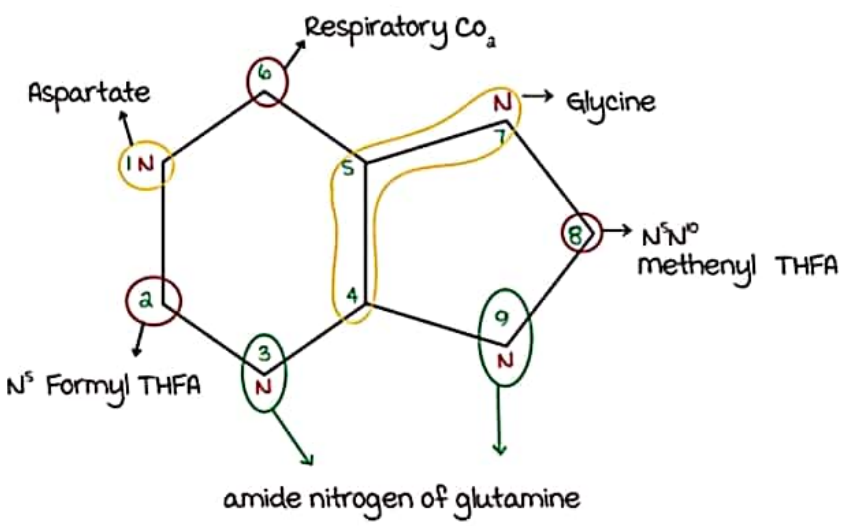
* Synthesis of purine nucleotide from various amphibolic intermediates.
* Site :- All organs especially in liver

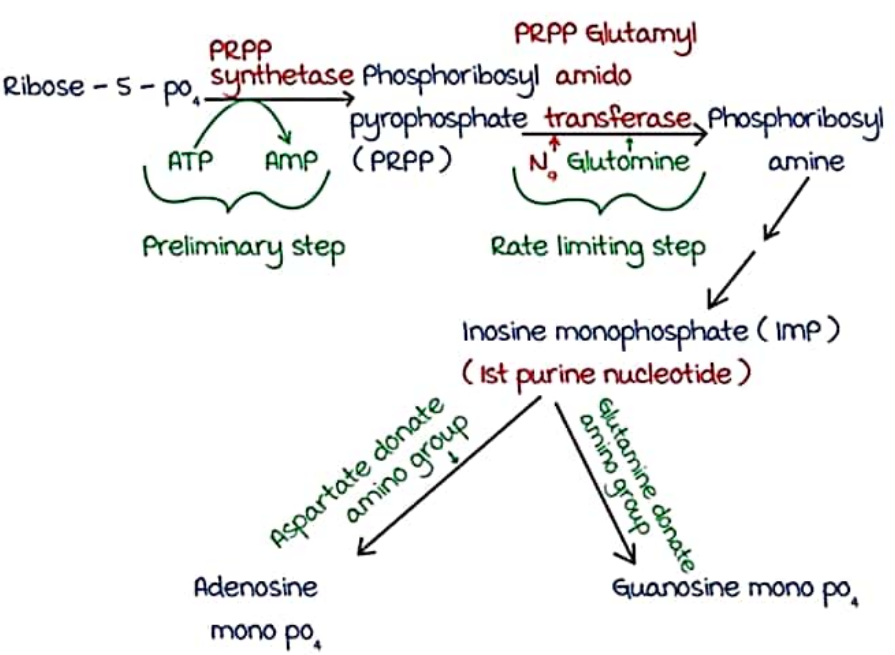
Do not take place in
1. Erythrocytes
2. Leukocytes
3. Brain
4. Bone marrow.

} Solely depend on salvage pathway

* Organelle :- Cytoplasm

Purine -Structure



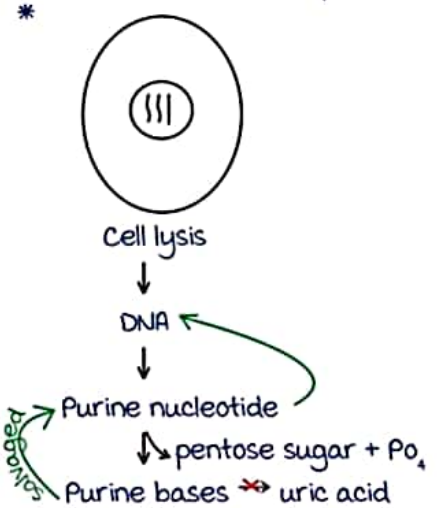

Active space

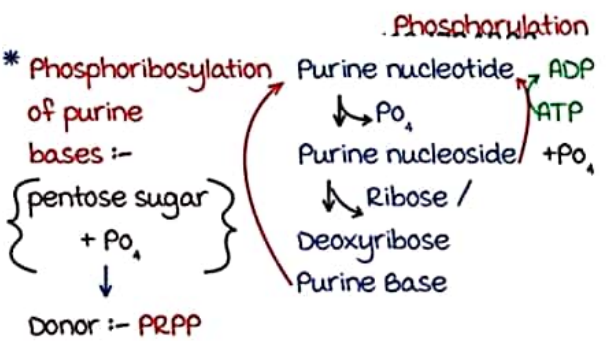
## Salvage pathway

00:17:39

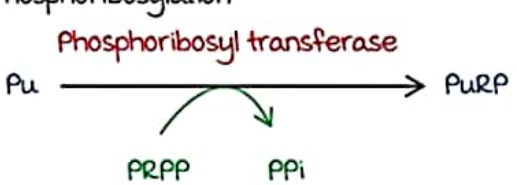
* Recycling of degraded purine nucleotides (from nitrogenous base or nucleoside) back to purine nucleotide.

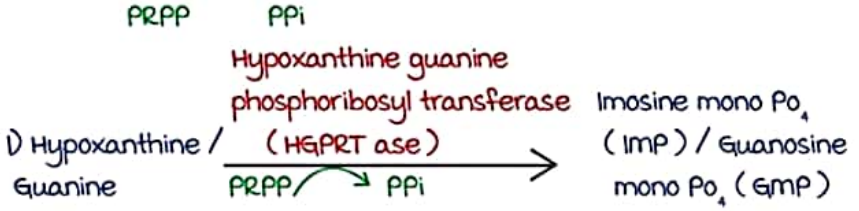
*



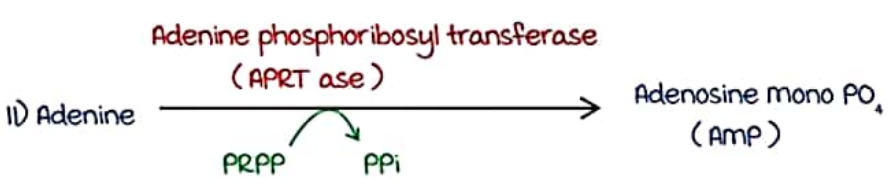




Phosphoribosylation




* Complete deficiency of HGPRTase :- Lesch nyhan syndrome




Active space

Phosphorylation

PuR —Kinase, ATP/ADP→ PuRP

- Adenosine —Kinase, ATP/ADP→ AMP
- Guanosine —Kinase, ATP/ADP→ GMP

Significance of salvage pathway

1. Saves energy.
2. Effective recycling
3. Important in organs with no de Novo purine synthesis especially brain.
   HGPRTase highest concentration in basal ganglia.

## Classic Lesch Nyhan Syndrome 00:34:46

- x-linked recessive disorder
- Complete deficiency of HGPRTase.

Clinical features

- Hyperuricemia
- Intellectual disability
- Compulsive self mutilation.

Diagnosis

- Enzyme studies
- Orange sands (uric acid crystals) in urine
- HGPRTase enzyme activity in erythrocytes.

Treatment

- Allopurinol
- High fluid intake along with alkali (decrease crystallisation of uric acid)

Partial deficiency of HGPRTase :- Kelley-Seegmiller syndrome

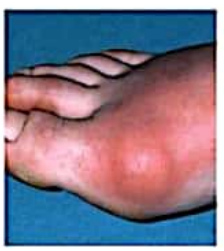
Active space

## Gout

00:39:20

- Group of disorders presented with :- ① Hyperuricemia
  ② Uric acid Nephrolithiasis
  ③ Acute inflammatory arthritis.

- MC is monoarticular
- Typically affects :- 1st metatarso-phalangeal joint.
- Seen in acute Gout



* In chronic gout :- Nodular masses of monosodium urate crystals (Tophi) deposited in soft tissue.

Definitive diagnosis

- Aspiration and examination of synovial fluid.
- Needle shaped negatively birefringent monosodium urate crystals using polarized light microscopy.

Causes of gout

Primary gout :-
- ↑ activity of PRPP synthetase
- ↑ activity of PRPP Glutamyl amidotransferase
- Lesch Nyhan syndrome
- Type I glycogen storage disorder (Von Gierke disease)

Secondary gout :-
- ↑ production of uric acid :- Purine turnover (Malignancy)
- ↓ excretion of uric acid :-
  - Renal failure
  - Lactic acidosis
  - Thiazide diuretics

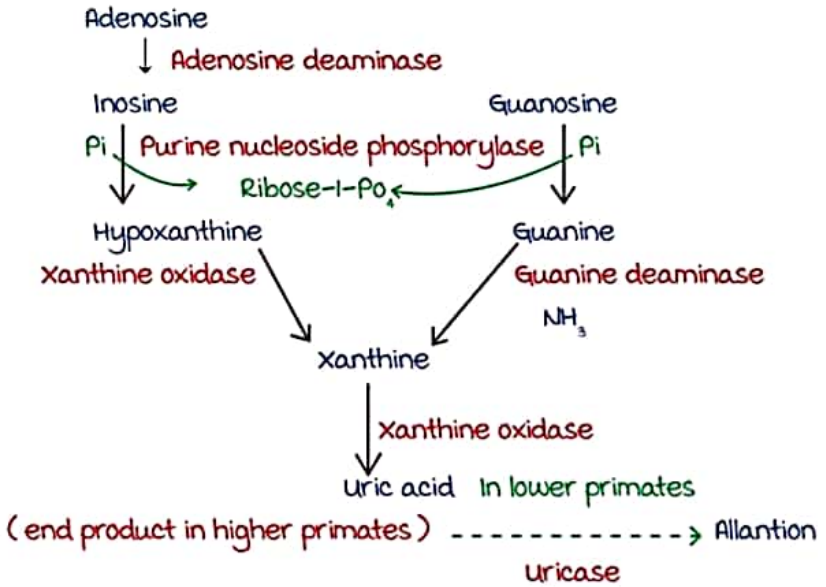
Treatment

* Alkalinization of urine
* High fluid intake
* Allopurinol
* Anti Inflammatory agents → Colchicine
* Uricosuric drugs → Probenecid

Active space

## Purine catabolism 00:47:38

* Site :- liver
* Product is uric acid.




## Severe Combined Immunodeficiency ( SCID ) 00:52:53

* A/K/A :- • Glanzmann Riniker sydrome
  • Bubble Boy disease
* mc cause of SCID :-
  • Defect in Y chain of immunoglobulin.
  • X linked recessive SCID.
* Second MC cause is :-
  • ADA gene defect leading to adenosine deaminase defect
  • Autosomal recessive.
* DNA repair defect causing SCID :- Non-homologous end joining (NHEJ) defect.

Treatment

- Gene therapy (Dr French Anderson-Father of Gene Therapy)
  - Used first for treatment of SCID in a child named Ashanthi de Silva
- Enzyme replacement therapy-Polyethylene glycol modified Haenosine Deaminase( PEG - ADA )

Other disorders of purine catabolic pathway:-

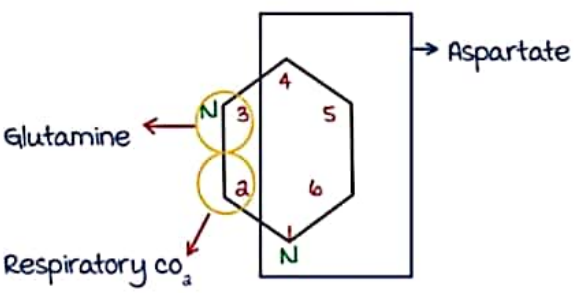
- Defect in purine nucleoside phosphorylase → severe defect in T - cells but B - cells are normal
- Defect in Xanthine oxidase :-
  - Xanthinuria (Xanthine crystals)
  - Hypouricemia

Active space

# PYRIMIDINE METABOLISM

## Pyrimidine synthesis 00:01:12

* Site :- All organs especially Liver .
* Organelle :- Cytoplasm & mitochondria
* Pyramidine ring ← Ribose, $PO_4$ → Pyrimidine nucleotide




$CO_2$ + Glutamine + ATP
↓ CPS II
Carbamoyl $PO_4$ + Aspartate
↓ Aspartate Transcarbamoylase (ATC)
Carbamoyl Aspartate
↓ Dihydro orotase
Dihydro Orotate

"CAD Enzyme" (single polypeptide) — in cytoplasm

NAD → NADH
↓ DihydroOrotate dehydrogenase } only mitochondrial step
Orotate
PRPP → PPI
↓ Orotate phosphoribosyl transferase (OPRT ase)
Orotdylate mono $PO_4$ (OMP)
↓ OMP decarboxylase
UMP (1st Pyrimidine nucleotide)

"UMP synthase" (single polypeptide)

ATP → ADP
UDP —(ATP → ADP)→ UTP —(CTP synthase)→ CTP

Active space

Ribonucleotide reductase  N5N10 methylene THFA ← Dihydrofolate Reductase → Dihydro Folate

dUDP → dUMP → TMP
THIMIDYLATE Synthase

* 5- Fluoro uracil inhibits Thymidylate synthase
  Methotrexate inhibits Dihydro folate reductase.

## Pyrimidine catabolism 00:20:22

* Cytosine, Uracil, Thymine → β - alanine, β- aminoisobutyrate } water soluble
  + $CO_2$ + $NH_3$

## Salvage pathway 00:22:02

Salvaged: Pyrimidine Nucleotide → (PO4) Pyrimidine Nucleoside → Pyrimidine base

## Pseudouridine 00:23:34

* Abnormal pyrimidine nucleoside.
* Uridine :- Uracil + Ribose
  $N_1$ ☒ $C_1'$  C - N Glycosidic bond
* Pseudouridine :- Uracil + Ribose
  $C_5$ ☒ $C_1'$  C - C Glycosidic bond

- Excreted unchanged in urine.
- Found in tRNA

Active space

## Orotic aciduria

00:26:21

TYPE I

* Defective UMP Synthase
  - OPRT ase +
  - OMP decarboxylase

TYPE II

* Defective OMP decarboxylase

Clinical features

* Growth failure
* Developmental delay
* Intellectual deficits
* Megaloblastic anaemia

* ↓ DNA synthesis due to ↓ UMP
  - ↓ CTP → Megaloblast in bone marrow
  - ↓ TMP

Macrocytes in peripheral smear.

* Urea cycle d/o associated with orotic ociduria
  → Type II Hyperammonemia → mitochondria affected, Urea cycle affected

↓

OTC ↓ → ↑ Carbamoyl $PO_4$ in mitochondria

↓

↑ Carbamoyl $po_4$ in cytoplasm

∴ ↑ed Orotic acid.

Treatment

* Feeding with Uridine

↓ Salvage

Pyrimidine Nucleotide

↓

DNA synthesis

Active space

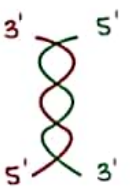
# STRUCTURE OF DNA

* Watson and Crick model of DNA by James Watson and Francis Crick.
* They published the paper in 1953 and Nobel prize in 1962.

## Salient Features of Watson and crick model 00:07:19

1. It has two polydeoxyribonucleotide strand running in a right handed helix.
   It can be compared to a spiral staircase where handrails formed by sugar + $Po_4$ and the steps formed by the non polar bases.

2. Antiparallel nature of the strands.




3. Base pairs joins the two strands horizontally by hydrogen bonds.

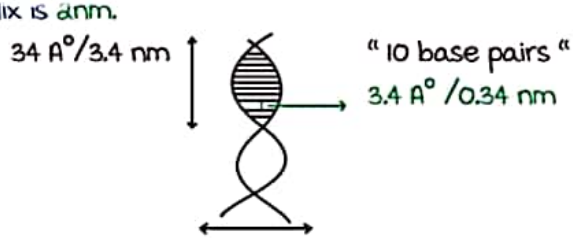
4. Watson crick base pairing rule –
   a) Adenine pair with Thymine by two hydrogen bonds. A = T
   b) Guanine pair with Cytosine by three hydrogen bonds. G ≡ C

5. The no. Of purines = no. of pyrimidines (A + G = C + T)
   This is called the Chargaff's Rule.

6. The major grooves and minor grooves in the DNA are the sites where proteins interact with DNA.

7. Base Stacking – the base pairs stack one above the other by a vertical interaction between the base pairs called the vanderwaal's forces.
   The bond forming distance of a vanderwaal's force is 3.4 A° or 0.34 nm.

* In one turn of the DNA, There are 10 base pairs.
* Height of one turn (PITCH OF THE HELIX) = 34 A° or 3.4 nm
* Diameter of a helix is 2nm.




Active space

## Types of DNA

00:23:22

- There are 6 types of DNA :- A, B, C, D, E and Z
  - A, B, C, D, E → Right handed
  - Z → Left handed

| | A | B | Z |
|---|---|---|---|
| • Direction of turn | Right handed | Right handed | Left handed |
| • No. of base pairs per turn | 11 bp | 10.5 bp | 12bp |
| • Base pair tilt corresponding to axis of helix | 20° | 90° | 9° |
| • Morphology | Broad and short | Elongated and Thinner | Elongated and Thin |

A DNA

* Found in a region where there is –
  - Low humidity
  - Low degree of hydration
  - High salt concentration.

B DNA

* Found in a region where there is –
  - High humidity
  - High degree of hydration.
  - Low salt concentration.
* B DNA is the most common type of DNA and Physiologically most Stable.

Z DNA

* The backbone of Z DNA is " Zig Zag. "

## Non canonical DNA

00:30:48

1. Triple stranded DNA or triplex DNA

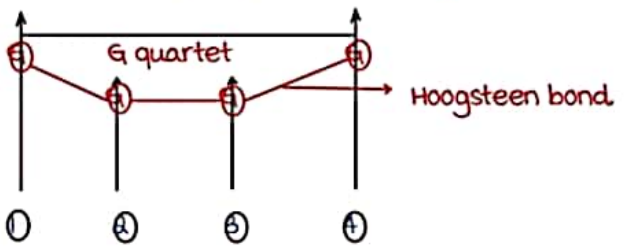
* To the major grooves , if a third strand interact with ds DNA by Hydrogen bond, it in called triple stranded DNA.
* These hydrogen bonds are called Hoogsteen Bond
* Triplex DNA has a Non Watson Crick base pairing

2. Four stranded DNA

* It is seen in G – rich regions which is predominantly seen in Telomeres.

Active space

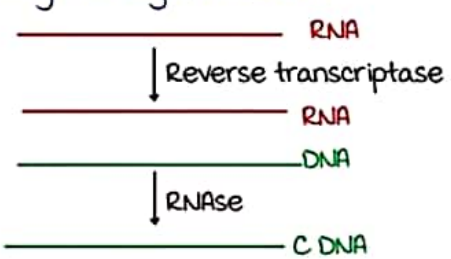
* The G in the four strands interact with each other to form a special arrangement – G quartet via hydrogen bonds – Hoogsteen bond.




## cDNA Or Complementary DNA

00:35:49

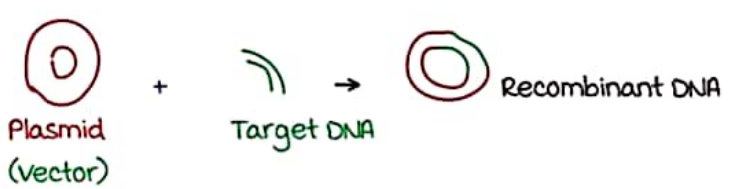
* Complementary to a segment of RNA.




## Chimeric DNA or Recombinant DNA

00:36:50

* When the plasmid combines with a desired DNA or target DNA, this is called Chimeric DNA.




## Mitochondrial DNA

00:37:53

* In a human cell, there are around 2 – 10 copies of mitochondrial DNA.
* It constitutes 1% of cellular DNA.
* It is double stranded, circular and has about 16,569 bps.
* Mt DNA encodes 37 structural genes for –
  - → 2 $rRNA_s$ (16SrRNA and 12 SrRNA)
  - → 22 mitochondrial tRNAs
  - → 13 proteins of ETC
* The 13 proteins in ETC Coded by Mt DNA are :-
  - 7 subunits of complex I
  - Cyt b of complex III
  - 3 Subunits of complex IV
  - 2 subunits of ATP Synthase
* out of 67 subunits in ETC, 13 are by Mt DNA that constitutes 19%.

Active space

Unique features of mitochondrial DNA

* Mitochondria has a unique genetic code.
* Only 22 $tRNA_s$ are involved in translation of mitochondria.
*

| Codons | Nuclear code | Mt. Code |
|---|---|---|
| AUA | Isoleucine | Methionine |
| UGA | Stop codon | Tryptophan |
| AGA, AGG | Arginine | Stop codon |

This is an exception to the universal nature of genetic code

* Mutation rate is very high because :-
  - No introns
  - No Protective histones
  - No effective repair enzymes.
  - It is exposed to oxygen free radicals generated by oxidative Phosphorylation.
* It has non - mendelian type of inheritance ( Cytoplasmic Inheritance )

If mother is affected , all progenies are affected
→ Matrilineal Inheritance.

**Warning** : Not all points are covered in the notes, especially conceptual explanations. Please use the notes in conjunction with Marrow Edition 4 videos.

## Denaturation of DNA

00:44:11

- ds DNA is separated to its component strands

Features of denaturation

1. Hydrogen bonds broken
2. Base stacking lost
3. 3'→5' phosphodiester bond not broken.
   1° structure is not lost
4. 2° and 3° structure are lost.
5. Viscosity decreased.
6. ↑ ↑ Absorption of UV light at 260 nm. ⇒ Hyperchromatism
   UV - C band

Cut off point → > 40% increase in absorbance.

Active space

Factors determining denaturation

* Temperature at which DNA becomes denatured :- melting temp "Tm"

1. Base composition :- IF GC pair is more → ↑ Tm

2. If there is 10 fold rise in monovalent metal ion concentration, $T_m$ ↑↑ (16.6°C)
3. Formamide destabilise hydrogen bond → ↓ $T_m$

Active space

# ORGANIZATION OF DNA

## Levels of organization 00:01:51

I. DNA double helix
II. 10 nm chromatin fibril
III. 30 nm chromatin fibril
IV. Nuclear scaffold formation ( Interphase chromosome )
   a) Condensed loop
   b) Non condensed loop
V. Chromosome

## 10 nm chromatin fibril 00:04:20

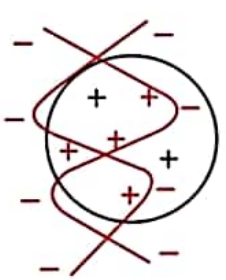
- Made up of nucleosome
- Nucleosome = DNA + Histone

Histones

- Most abundant chromatin protein .
- Small family of basic protein .
- Rich in basic amino acids → lysine & arginine .
- Highly conserved among the species .
- Positively charged .

Nucleosome

- Positively charged histones interact with negatively charged DNA ( due to $PO_4$ group ) by forming Ionic bonds .



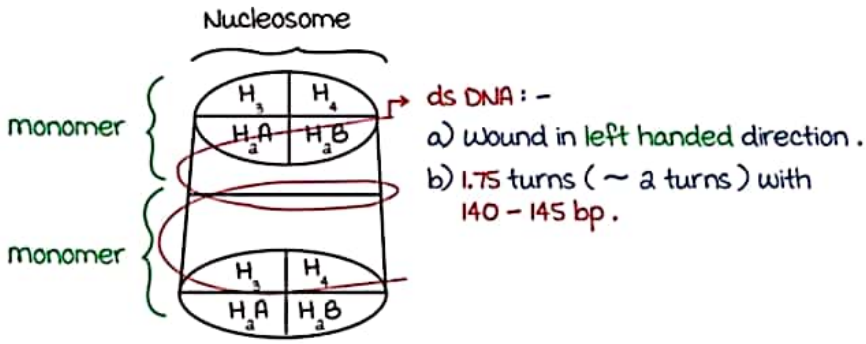
Active space

Histone classes

| Core histones | Linker histones |
|---|---|
| • Seen in histone octamer | • Seen in linker DNA |
| • $H_{2A}$, $H_{2B}$, $H_3$, $H_4$ | • $H_1$ |
| • Histone octamer interacts with ds DNA by ionic bond to form nucleosome | |

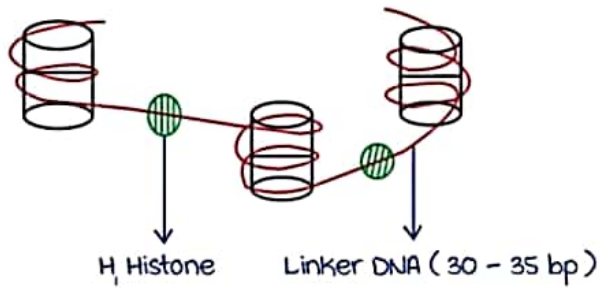
Histone octamer

- When $H_{2A}$, $H_{2B}$, $H_3$, $H_4$ dimerises → Histone octamer




10 nm chromatin fibre

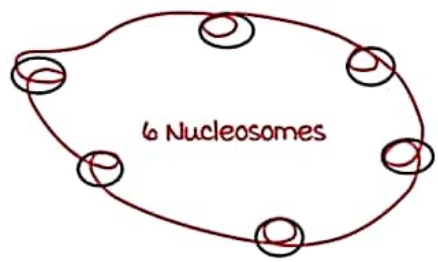
- They have beads on string appearance.




## 30 nm chromatin fibril

00:18:40

- It has 6 nucleosomes coiled to form solenoid with diameter of 30 nm




Active space

Interphase chromosome

- Condensed and non condensed loop .
- 30,000 – 1,00,000 loop seen attached to nuclear scaffold protein .

## Chromosome 00:21:19

- There are 2 regions : –

1. Euchromatin / Permissive chromatin / Active chromatin

- Less condensed / Less organised .
- 10 nm and 30 nm chromatin
- Transcriptionally active .
- Less densely stained

2. Heterochromatin / Repressive chromatin / Inactive chromatin

- Highly organised .
- Transcriptionally inactive .
- Densely stained .

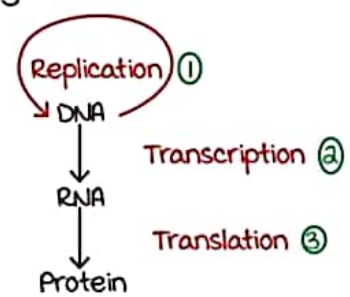
Active space

# STEPS OF DNA REPLICATION

## Concept of dna replication 00:02:12

We get a replica of parent DNA in daughter cell .

Central dogma



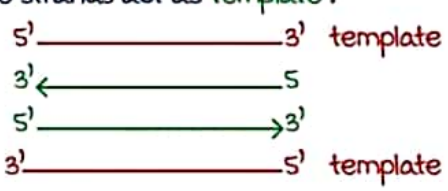

## Definition of replication 00:07:19

The process of formation of two daughter DNA which are each Identical to parent DNA is called replication

## Salient features of dna replication 00:08:31

1 . DNA replication occurs in the S phase ( Synthesis phase ) of cell cycle .

2 . Both the strands act as template .



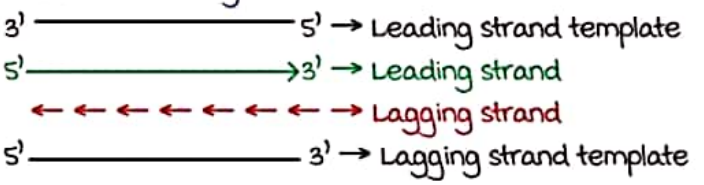

3 . Direction of replication : - New strand formation in 5' → 3' direction and reading of template in 3' → 5' direction .

- Overall , the DNA replication is bidirectional .

4 . Semiconservative model of dna replication . Put forward by meselson & stahl .
Half of the parent strand is conserved in daughter DNA .

Active space

5. Semidiscontinous nature of DNA replication.
Leading strand is synthesised continuously and lagging strand is Synthesised discontinuously.




6. No primer is required.
7. Obey watson crick base pairing rule.

Enzyme dna polymerase
- Reads the frame in 3' → 5' direction.
- Hence synthesis only in 5' → 3' direction.

Steps of dna replication
1. Identification of origin of replication.
2. Unwinding of DNA
3. Formation of replication fork
4. DNA synthesis
5. Termination

## Identification of origin of Replication ( ori )

00:26:46

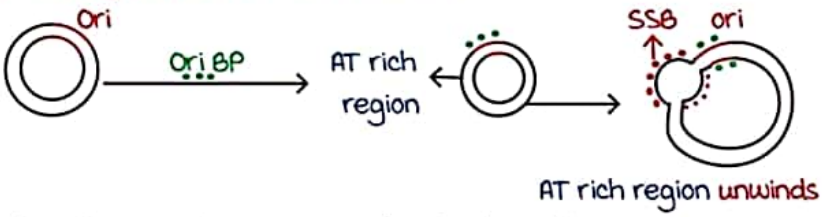
- Fixed points on DNA where replication begins is called origin of replication (ori).
* Ori C → E.oli bacteria
* Ori λ → phage
* ARS → Yeast
↓
(Autonomous replicating sequence)
* In humans, it is similar to ARS.


* In prokaryotes, only single origin of replication.
Multiple ori's in humans (eukaryotes).

* Near to ori, there is an AT rich region.
This AT rich region in eukaryotes is called as
"Dna unwinding element" (DUE).

Active space

* Ori binding proteins ( ori BP ) binds to ori




* When the AT rich region unwinds , it is bound by SSB ( Single strand binding protein ) .

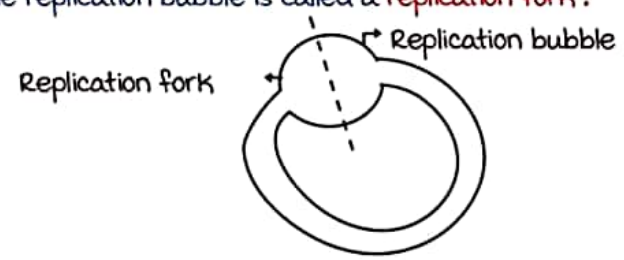
* Function of ssb : - Prevent local reannealing of unwound region .
* Human SSB$_s$ are called RPA → Replication protein A

## Unwinding of DNA 00:38:25

* Further unwinding is done by : -
  - In prokaryotes → helicase ( ATP dependent enzyme )
  - In eukaryotes → MCM ( Mini chromosome maintainance complex )

## Formation of replication fork 00:40:01

* When DNA unwinds , thee is formation of replication bubble half of the replication bubble is called a replication fork .

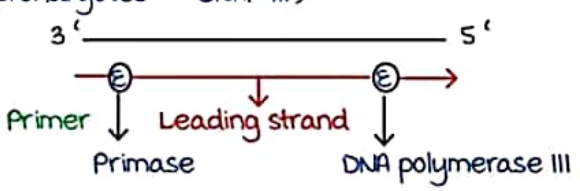



## Dna synthesis 00:41:12

Leading strand synthesis

1. Synthesis of RNA primer by PRIMASE
It is about 10 nucleotides in length and is made up of Ribonucleotides.

2. DNA is synthesised continuously by DNA polymerase
( In prokaryotes → DNAP III )

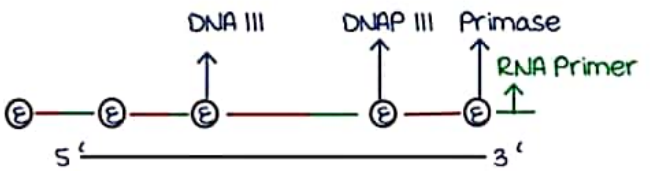



Active space

Lagging strand synthesis

1. Synthesis of RNA primer by primase.

2. Synthesis of okazaki fragments by DNAP III
   - 1000 – 2000 nucleotides in prokaryotes.
   - 100 – 250 nucleotides in eukaryotes




3. Removal of RNA primers and filling of gap by DNA polymerase I.
   - DNAP I has 5' → 3' Exonuclease activity. which removes RNA primer by breaking 3' – 5' phosphodiester bond.

4. Joining of nicks by DNA LIGASE. (ATP requiring).

## Termination

00:58:32

* In a prokaryotic DNA, there is a site called ter in. ter site is a conserved sequence and is bound by "tus" → Termination utilization substance.

Difference in prokaryotes & eukaryotes

| | Prokaryotes | Eukaryotes / Humans |
|---|---|---|
| 1. Ori | Single ori | Multiple ori |
| 2. SSB | SSB | Replication protein A (RPA) |
| 3. Helicase | Helicase | MCM |
| 4. Primase | DNA G | DNAP α |
| 5. DNA synthesis | DNAP III | DNAP, delta, epsilon |
| 6. Removal of primers | DNA P I | RNAse – H & FEN (Flap endo nuclease) |

## One liners

01:04:57

* Replisome :- Multimeric proteins seen in replication fork : -
  1. DNAP
  2. SSB
  3. Helicase
  4. Primase
* Primosome :- Helicase + Primase

Active space

# ENZYMES OF DNA REPLICATION

## Prokaryotic DNA polymerase 00:02:03

I) DNAP I
1. Removal of RNA primer
2. Gap filling in lagging strand
3. Proof reading
4. DNA repair (major)

II) DNAP II
1. Proof reading
2. DNA repair

III) DNAP III
1. Leading strand synthesis
2. Okazaki fragment synthesis
3. Proof reading

## Eukaryotic DNA polymerase 00:05:39

1. DNA – $\alpha$ :– Primase activity
2. DNA – $\beta$ :– Major DNA Repair enzyme
3. DNA – $\lambda$ :– i) Mitochondrial DNA synthesis
   ii) Proof reading
4. DNA – $\delta$ :– i) Lagging strand synthesis
   ii) Proof reading
5. DNA – $\xi$ :– i) Leading strand synthesis
   ii) Proof reading

## One liners 00:12:51

- Most processive DNA polymerase.
- DNAP with maximum chain elongation

} DNAP III

* This property is due to the presence of $\beta$ – subunit. (aka sliding clamp.)

- Kornberg's enzyme

↓

DNAP I discovered by Arthur Kornberg in E_Coli.

Active space

- Klenow polymerase
  ↓
  DNAP I from which 5' to 3' exonuclease activity is removed

- Proof reading :- 3' – 5' exonuclease
  - DNAP I, II, III in prokaryotes
  - DNAP $\gamma$, $\delta$, $\xi$ in eukaryotes.

- Repair :- 5' – > 3' exonuclease
  - DNA I, II in prokaryotes
  - DNA $\beta$ in eukaryotes

- Okazaki fragments :- 1000 – 2000 nucleotides in prokaryotes
  100 – 250 nucleotides in eukaryotes

- Primase :- DNA dependent RNA polymerase
  Reverse transcriptase :- RNA dependent DNA polymerase

Active space

# REPAIR OF DNA

## DNA damages

00:02:50

Causes of DNA damage

1. Imperfect proof reading.
2. Environmental hazards – • Ionizing radiation
   • Chemicals
   • UV rays.

| DNA damaging agents | DNA defect | Repair mechanism |
|---|---|---|
| • Ionizing radiation<br>• X-rays<br>• Anti – cancer drugs | • ds breaks<br>• ss breaks<br>• Intra and inter strand cross links | (1) Homologous recombination<br>(2) Non – homologous and joining |
| • UV light<br>• Chemicals | • Bulky adducts.<br>• Pyrimidine dimers most common is THYMIDINE DIMER | Nucleotide Excision Repair (NER) |
| • Reactive oxygen species<br>• Alkylating agents like Nitrates | • At basic sites<br>• Insertion<br>• Deletion | Base excision repair |
| • Imperfect proof reading | • mismatches | mismatch repair (MMR) |

## Repair mechanisms

00:11:4

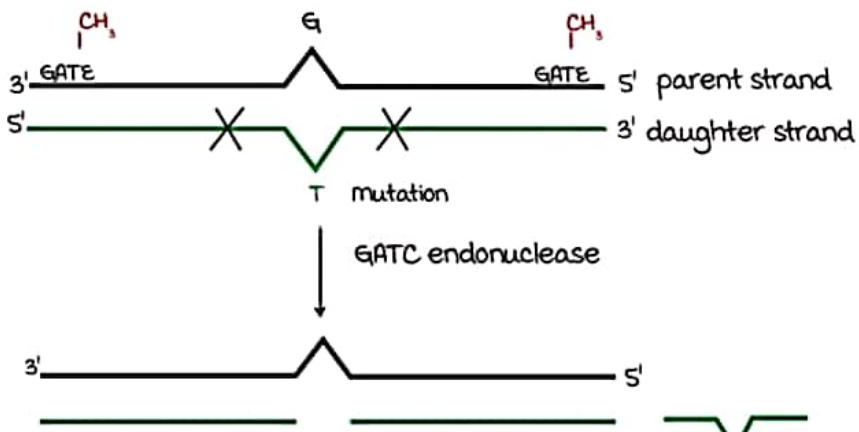
A) Excision Repair :- I) Mismatch repair (MMR)
II) Nucleotide Excision repair (NER)
III) Base Excision repair (BER)

B) Recombination :- I) Homologous recombination
II) Non homologous End joining.

Active space

Mismatch repair

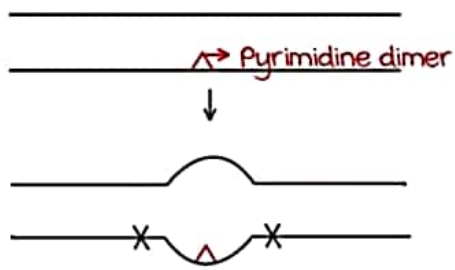
- In parent strand, there is a GATC sequence every 1000 nucleotides and the cytosine residues are usually methylated.
- The mut enzymes (mismatch repair enzymes), scans the newly synthesized DNA from 5' → 3' direction.
- If there is a mismatch, GATC endonuclease will remove that part




- DNAP I fills the gap and the nicks are sealed by DNA ligase.

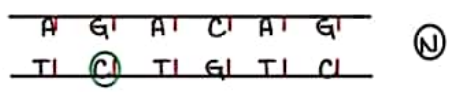
Nucleotide excision repair

- In the newly synthesized DNA, if there is a pyrimidine dimer, the DNA repair enzyme scans the DNA and the mutated region becomes unwound



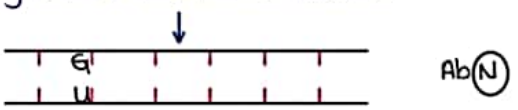

- Certain regions in the mutated DNA gets cut by UV specific endonuclease (UvrABC excinuclease)
- The gap is filled by DNAPI and nicks are sealed by Ligase.

Base excision repair



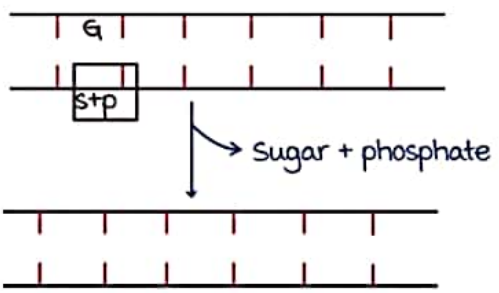

Active space

If cytosine is deaminated to uracil




This mutation is corrected by base excision repair

N. Glycosylase uracil removed.




The gap is filled with cytosine by DNAP I and nick sealed by Ligase.

(1) Scanning
(2) Endonuclease
(3) DNAP I
(4) Ligase

| Defects in DNA | Repair Mechanism | Disorders associated |
|---|---|---|
| Double strand Breaks<br>Single strand Breaks<br>Intrastrand cross links | Nonhomologous End joining (NHEJ) | √ Severe combined Immunodeficiency (SCID)<br>√ Radiosensitive SCID |
| | Homologous Recombination (HR) | √ Ataxia Telengiectasia Like Disorder<br>√ Nijmen Break syndrome<br>√ Blooms syndrome<br>√ Werner syndrome<br>√ Rothmund thomson syndrome<br>√ Breast cancer susceptibility (BRCA 1, BRCA 2) |

Active space

Xeroderma pigmentosa

- Defect in Nucleotide Excision Repair .
- Defective enzyme :- Helicase
- Helicase activity present in TF Roamna in eukaryotes .

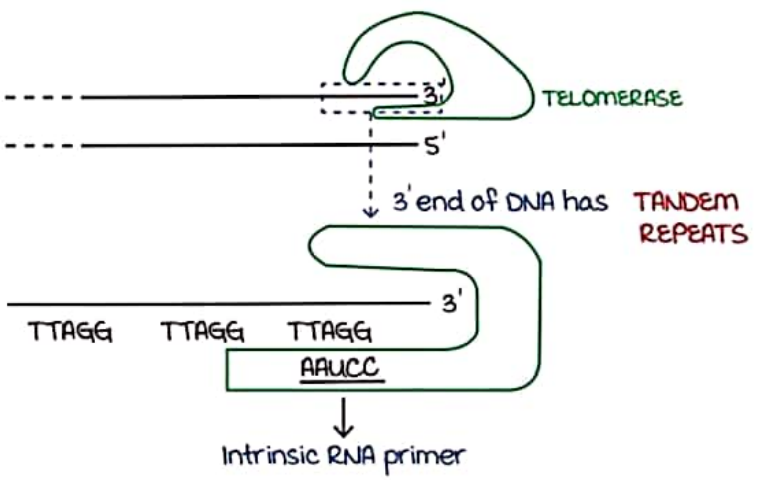
Active space

# TELOMERASE

Telomere :- Ends of chromosome.

Telomerase :- Enzymes seen in telomeres.

- Enzyme + DNA = SHELTERIN




* Telomerase has two peculiarity : -
  i) It has an INTRINSIC RNA PRIMER Complementary to tandem repeat.
  ii) It has REVERSE TRANSCRIPTASE activity.

* Action of telomerase :- It extends the 3' end of chromosome.

End replication error

* After replication, when the end primers are removed -
  i) 3' end of parent strand is not replicated.
  ii) 5' end of daughter strand defective.

* Telomerase won't correct the end replication error but, shortening of DNA is prevented.

Telomerase

* aka Terminal telomere transferase.
* RNA + Protein → Ribonucleo - protein
* Present in : - germline cells.
  - Stem cells.
  - Cancer cells.

Active space

* Absent in somatic cell.
* Clinical correlation :- ↑ Telomerase activity → Cancer
  ↓ Telomerase activity → Premature ageing/ Progeria

Active space

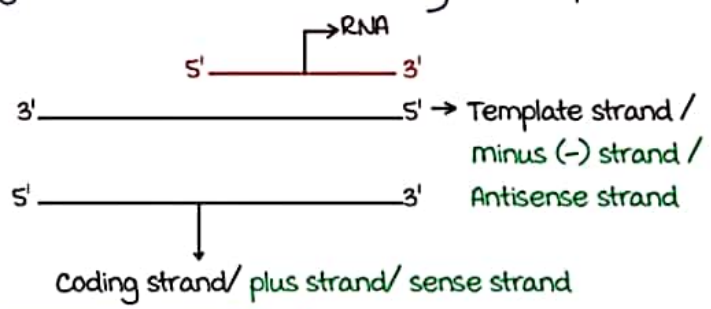
# TRANSCRIPTION

* The process of formation of any kinds of RNA from DNA

## Salient features of transcription

00:05:40

1) Only one strand and only part of it can act as template.
2) RNA is synthesized in 5' → 3' direction. } Polarity of
3) Reading of frame is in 3' → 5' direction } Transcription.
4)

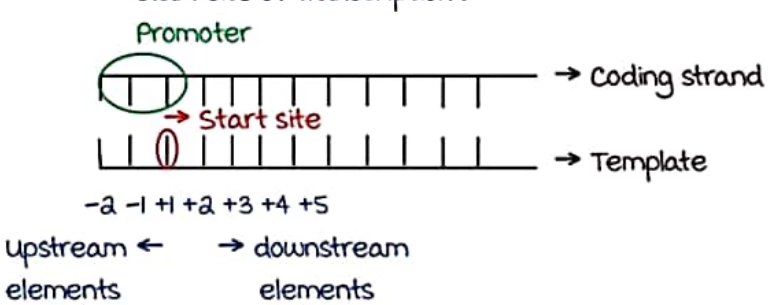



5) Obey base pairing rule → A = U
G = C
6) Base sequence in template strand is complementary to base sequence in RNA
7) Base sequence in RNA is same as that of coding strand
Exception :- T is replaced by U
8) No primer is required.

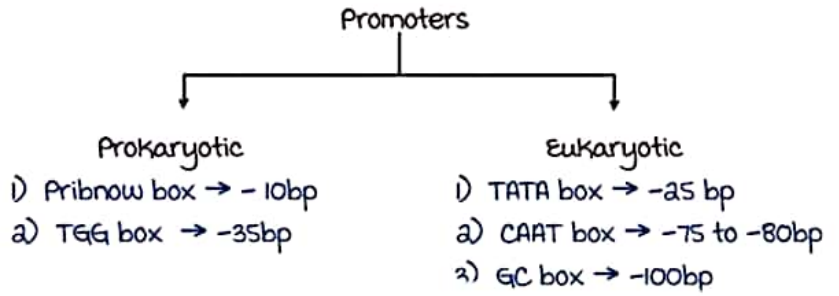
## Signals of transcription

00:22:59

Promoters :- Conserved sequence in the coding strand that specifies start site of transcription.




Active space




* Promoter less sequence → TATA less sequence .
  Then, function of promoter taken up by :-
  1) Inr ( Initiator sequence )
  2) DPE ( Downward Promoter Element) } situated downstream

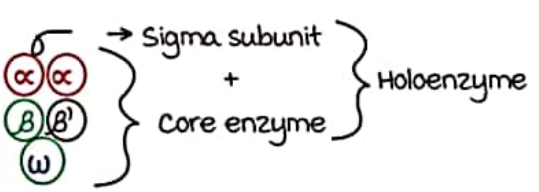
## Enzymes of transcription 00:35:42

RNA Polymerase(RNAP)

* 5' → 3' direction
* Requires $Mg^{2+}$ .
* Only 1 RNAP in prokaryotes and 3 RNAP in eukaryotes

Prokaryotic RNAP

* Multi subunit enzyme .




* β → • Catalytic subunit .
  • $Mg^{2+}$ attached to β subunit .
* Sigma subunit → • Binds to promoter
  • Initiation of transcription

Eukaryotic RNAP

* There are 3 RNAP I, II and III
  All 3 differ in sensitivity towards → α amanitin ( mushroom poison )
* Sensitivity to α amanitin : Maximum :- RNAP II
  Intermediate :- RNAP III
  Least :- RNAP I

Active space

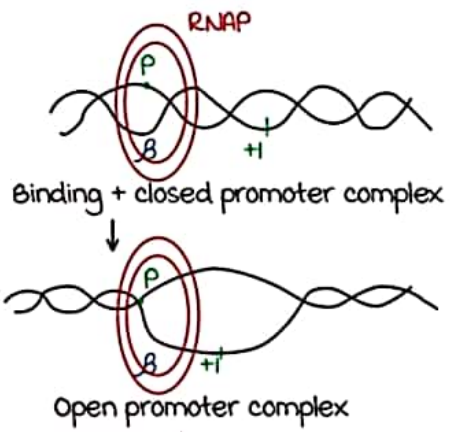
Products of RNAP

- RNAP I → rRNA(most abundant RNA)
- RNAP II → mRNA, Mi RNA, Sn RNA,lnc RNA,circular RNA ( circ RNA )
- RNAP III → t RNA, 5s rRNA, certain snRNA

## Transcription cycle

00:47:48

1) Binding of RNAP to promoter (P)
2) Closed promoter complex
3) Open promoter complex
4) Initiation of RNA synthesis
5) Promoter clearance
6) Chain elongation .
7) Termination .




1st ribonucleotide found attached to β – subunit of RNAP is added .
This is called → Chain initiation

↓

Chain is elongated till it is 10 – 20 nucleotides .

↓

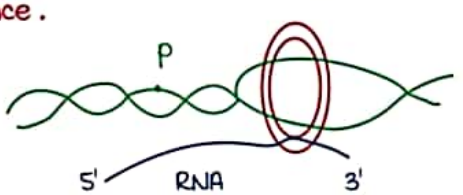
RNAP detaches from the promoter and will move along the template strand → Promoter clearance .

↓

Chain elongation

↓

Termination .




Termination

↓

i) ρ dependent
ii) ρ independent

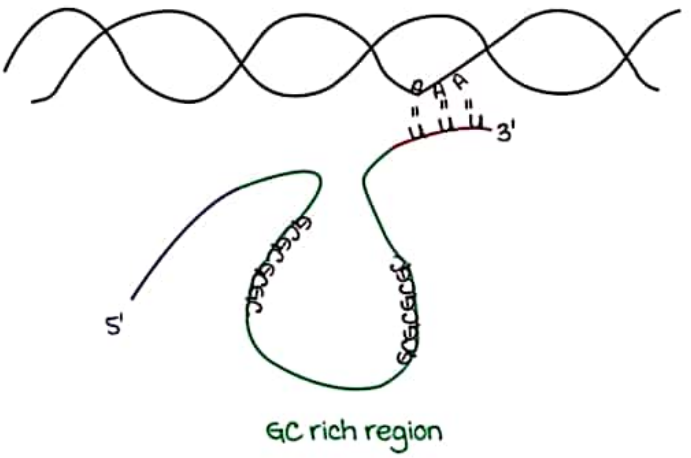
ρ dependent termination

* When the termination signals are met, ρ factor binds to RNA .
* ρ factor has ATP dependent helicase activity . → detaches the RNA from the DNA

Active space

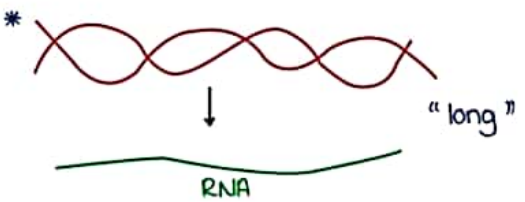
## ρ independent termination

* Series of U in the 3'end of RNA .
* Due to weaker A=U at the end, RNA detach from DNA .




GC rich region

# POST TRANSCRIPTIONAL MODIFICATION




* Newly synthesized RNA aka Primary transcript / heteronuclear RNA ( hn RNA ).
* The modifications happening to primary transcript → POST Transcriptional modifications. ( PTM )
* Site :- Nucleus

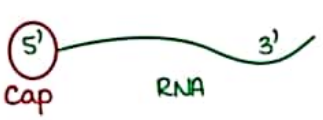
| Prokaryotic ptm | Eukaryotic ptm |
|---|---|
| * mRNA to not undergo PTM. | * All RNA undergo PTM |
| * tRNA & rRNA undergo PTM. | |

## PTM of messenger RNA

00:04:55

① 5' capping
② 3' poly A Tailing
③ Removal of introns and splicing of exons.
④ Alternate RNA splicing / Differential RNA processing.

① 5' Capping
* Cap added to 5' end
↓
7 methyl guanosine cap




* Done in 2 steps :-
① GTP added to 5' end by Guanylyl transferase ( Site :- Nucleus )
② In cytoplasm, methylation at $N_7$ of Guanine.
Methyl donor :- S- Adenosyl methionine
Enzyme :- 7 - methyl transferase

Function of capping
① Prevents the attack of 5' - 3' exonuclease.
② Stabilise the mRNA
③ Initiation of translation :- Cap helps in the attachment of mRNA to 40S subunit of ribosome .

Active space

② 3' Poly a tailing

* Made of 40 – 200 Adenosine residues.
* Present in the untranslated region (UTR)
* The enzyme that add poly A tail :- Poly adenylate polymerase
* Site :- Nucleus

Functions of poly a tail

① Prevents attack of 3' → 5' exonuclease.
② Stabilise mRNA.
③ Initiation of translation.
④ Exit of mRNA from nucleus to cytoplasm

③ Removal of introns & splicing of exons

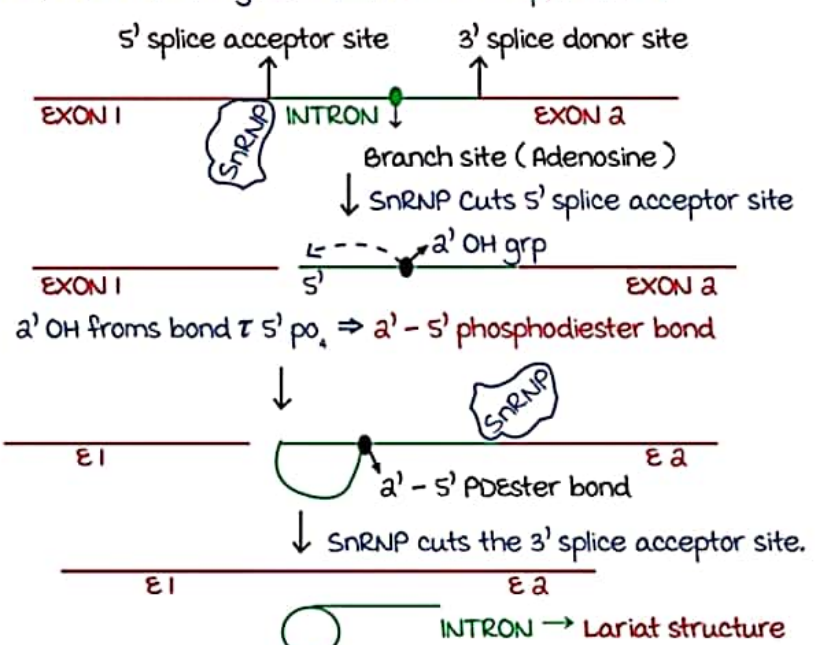
* Exons are coded to the proteins which introns are not.
* Removal of introns and splicing of exons done by a molecular machinery → Spliceosome.
* Component of spliceosome :- i) SnRNA
  ii) Proteins
  iii) Primary transcript / hnRNA

SnRNA

* Product of RNAP II, RNAP III.
* Can be considered as a ribozyme.
* Rich in Uracil Hence they are termed as $U_1$, $U_2$, $U_3$, etc.

SnRNA + Proteins

* Together called small nuclear Ribonucleoproteins. (SnRNP)
* aka snurps.
* Autoimmune disorder associated ↑ snurps → SLE
* SnRNP at exon intron junction of hnRNA → Spliceosome




This intron is degraded.

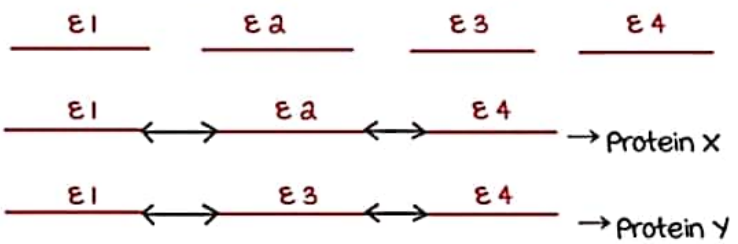
Active space

④ Alternate RNA splicing / differential RNA processing

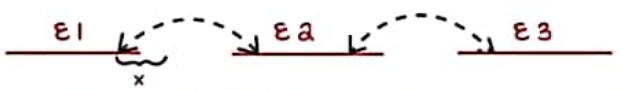
a) Selective splicing
b) Alternate 5' splice acceptor site
c) Alternate 3' splice donor site
d) Alternate polyadenylation site.

Selective splicing

* Different exons join together → Diverse protein product

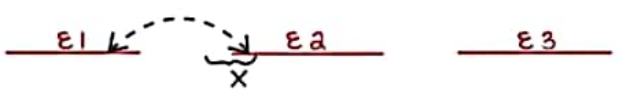



Alternate splice acceptor site



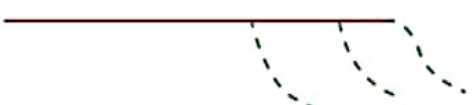

* Splice acceptor site is changing → ∴ different proteins from some hnRNA

Alternate splice donor site




* Splice donor site is changing

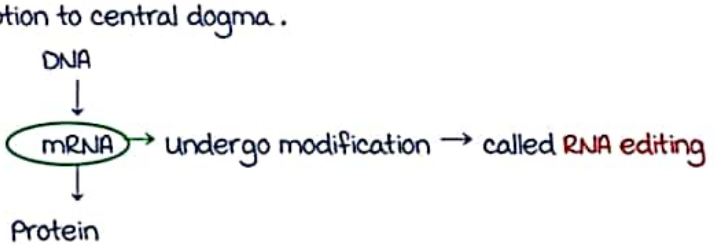
Alternate polyadenylation site



* Polyadenylation site can be changed.
* synthesis of Immunoglobulin → Membrane bound Ig
  → Secretory Ig

## RNA editing

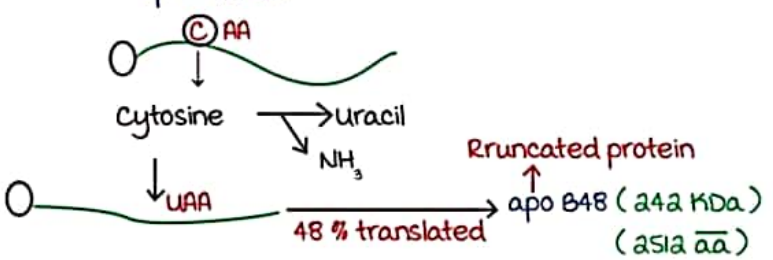
* Exception to central dogma.




Active space

* In liver :- apo B gene
  ↓
  apo B mRNA
  ↓ 100 % Translated
  apo B 100 ( 512 $KD_a$ ) ( 4596 $\overline{aa}$ )

* In intestine :- apo B gene
  ↓
  apo B mRNA




## One liners

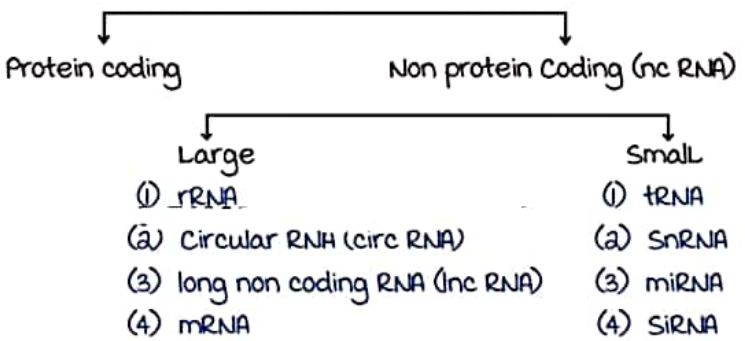
00:47:37

* mRNA without poly A tail :- mRNA for Histones
* Poly A codes for → poly lysine
* Poly G → Arginine
  Poly U → Phenyl alanine
  Poly C → Proline
* Adenosine residues in poly A tail :- 40 – 200
* Eukaryotic hnRNA with no intron is → hnRNA of histone gene .
* Disease caused due to autoimmune response to snRNPs – SLE

# CLASSES OF RNA

## Classification of RNA

00:01:28




## mRNA (messenger RNA)

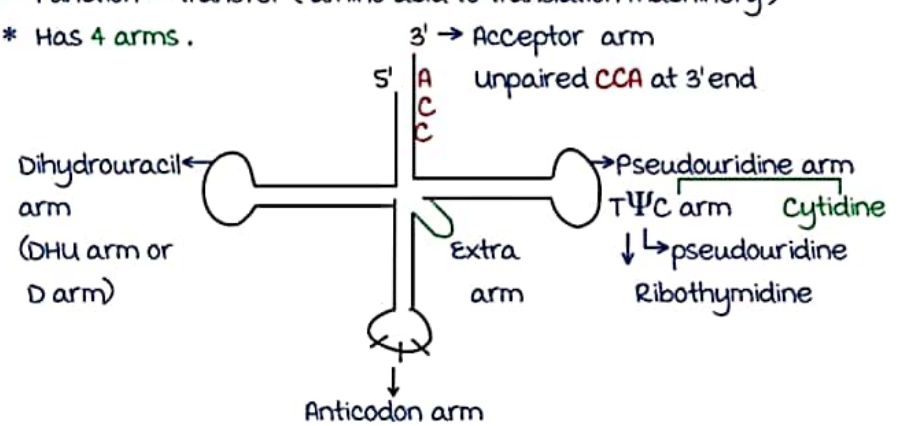
00:04:26

* Constitute 2-5% of RNA
* Code by RNAP II
* Function → Protein coding

## tRNA

00:06:56

* Soluble RNA or sRNA
* Adapter
* Clover leaf shape in 2° structure L- shaped in 3° structure .
* Function :- transfer ( amino acid to translation machinery )
* Has 4 arms .




* Acceptor arm binds to specific amino acid .
* Anticodon arm binds to codon of mRNA
* DHU arm - Fidelity determining arm .
  It binds to specific aminoacyl t-RNA synthetase

Active space

enzyme charge tRNA :- attachment of amino acid with acceptor arm

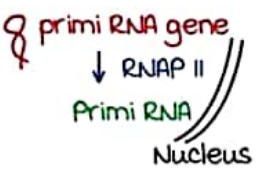
* Pseudouridine arm binds to ribosomal assembly.
* tRNA has max no. of modified/ unusual bases :-
  - DHU
  - Pseudouridine
  - Hypoxanthine → Inosine
* 74 – 90 nucleotide length.
* Only RNA with Thymine.

miRNA / siRNA

* Non coding small RNA.
* Function :- Post transcriptional regulation of gene expression.
* They are small non – coding RNA 21-25 (22) nucleotide length.

Generation of miRNA

* Endogenous origin




* PrimiRNA 5' 3'

↓ Trimmed by DROSHA – DGCR 8

→ Exported out of nucleus with the help of EXPORTIN -5 to cytoplasm

* In cytoplasm :- Trimmed by Dicer → Enters RISC (RNA induced silencing complex) → miRNA

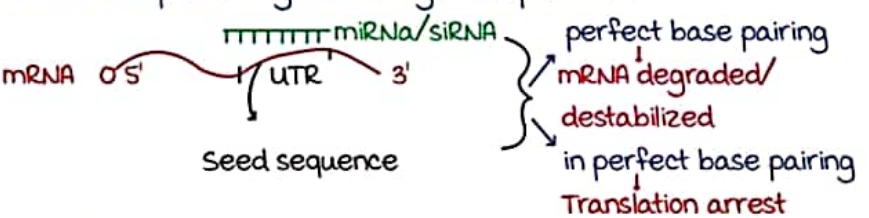
Generation of siRNA

* Exogenous origin

ds RNA → Enter into the cell from outside → Enter RISC → siRNA

FUNCTIONS OF miRNA/siRNA

- Post transcriptional regulation of gene expression.




- Net effect :- Gene silencing
  Gene knockdown
  RNA interference (RNAi)

Active space

* Oncogenic miRNA → OncomiRs
* Gene knockdown technology aka Antisense oligonucleotide technology → SiRNA is introduced.

## SnRNA 00:47:03

* Function :- • rRNA Processing
  • splicing of exons and removal of introns
* Rich in uracil.
* Ribozyme (catalytic activity +)

## Long Non coding RNA 00:48:10

* Functions :- • Gene activation
  • Gene suppression Eg :- Decoy RNA
  • Chromatin modification
  • Assembly of protein complex on DNA regulation of gene expression.
* gRNA :- • guide RNA
  • RNA editing
  • CRISPR cas9
* Sno RNA :- • small nucleolar RNA
  • rRNA processing

Active space

# TRANSLATION

Process by which protein is formed from MRNA : Translation

## Genetic code

00:03:34

* 1 codon is represented by 3 bases
  ↓
  Triplet nucleotide
* Genetic code :- Relationship blw sequence of nucleotide to sequence of amino acid in a polypeptide.
* Consists of 64 codons, out of which 3 are stop codons :-
  - UAA → Ochre
  - UAG → Amber
  - UGA → Opal

Exception :- UGA - Selenocysteine
UAG - Pyrrolysine
UGA - In mitochondrial DNA - Tryptophan

* Start codon :- AUG
* AUG :- Methionine in Eukaryotes
  N - formyl methionine in prokaryotes

* Marshall Nirenberg, Har Gobind Khorana } Cracked genetic code

Salient features

(1) Triplet nucleotide
(2) Start codon
(3) Stop codon
(4) Degenerate (redundant) → 1 Amino acid coded by more than 1 codon
degeneracy lies in 3rd base

* Amino acid represented by
  i) Single codon :- UGG - Tryptophan
  AUG - methionine
  ii) Maximum codon (6 codons) :- a) Serine
  b) Leucine
  c) Arginine

Active space

(5) Unambiguous :- 1 codon can represent only a Specific amino acid
(6) Universal :- 1 codon → Specific amino acid in 1 species
same amino acid in another species
Exception → mitochondrial DNA
(7) Non - overlapping
(8) Non - punctuated

Wobbling

* There are 31 tRNA in cytoplasm
* 61 coding codons → 61 anticodon → 61 tRNA
* Wobbling - Base pairing between 3rd base in codon and anticodon is not stringently following watson Crick base pairing rule .
* This phenomenon explains how 31 tRNA are there for 61 coding codons

## Steps of translation

00:33:36

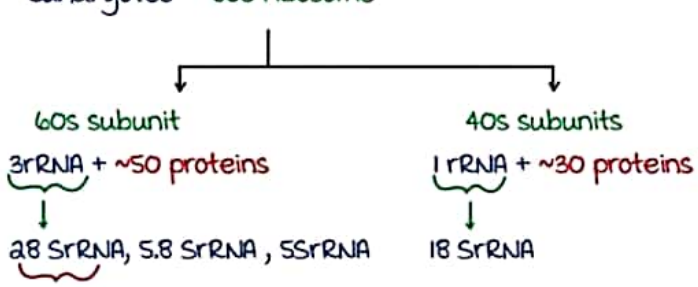
Site :- 1) Free ribosome
2) Rough ER
3) Mitochondria

rRNA

* most abundant RNA
* 80 %
* Enzyme transcribing rRNA :- RNAP - I
- 4 types :- 28s rRNA, 18s rRNA, 58s rRNA } RNAP - I
5s rRNA - RNAP - III

Ribosome

* rRNA + protein
* Eukaryotes :- 80s ribosome

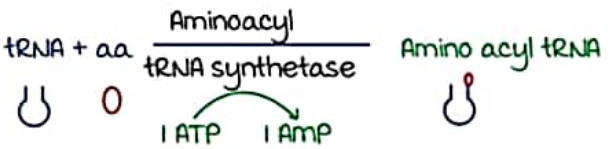



Ribozyme → Peptidyl transferase activity

Active space

Steps
(1) Charging of tRNA
(2) Initiation
(3) Elongation
(4) Termination

## Charging of tRNA 00:42:26




- It need a high energy po4 / 2 ATP equivalents

* DHU arm recognize the aminoacyl tRNA synthetase
  ↓
  Specific amino acid joins with acceptor arm

## Initiation 00:47:00

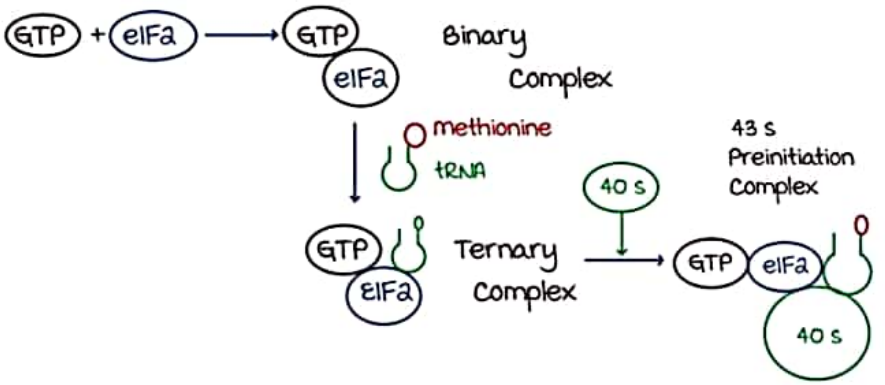
* The first AUG that comes after the marker sequence
  → start codon
* marker sequence :- In prokaryotes :- Shine Dalgarno sequence
  In eukaryotes :- Kozak sequence
* Initiation helped by initiation factor (IF) :- IF in prokaryotes
  eIF in eukaryotes

* Steps of initiation :-
  ① Disassembly of ribosomal unit
  ② Formation of 43s preinitiation complex
  ③ Formation of 48s initiation complex
  ④ Formation of 80s initiation complex

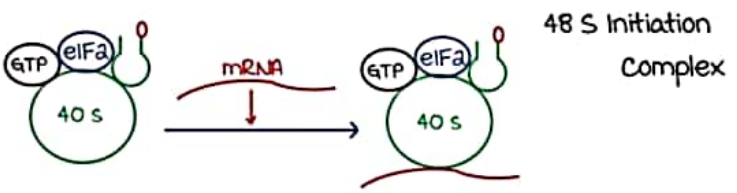
① Disassembly of ribosomal unit


Active space

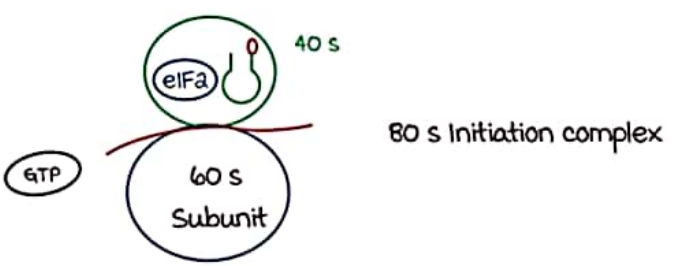
② Formation of 43 s Pre initiation complex



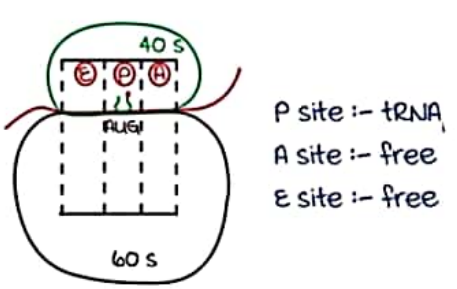

③ Formation of 48 s Initiation Complex




④ Formation of 80 s Initiation Complex




* Sites :-



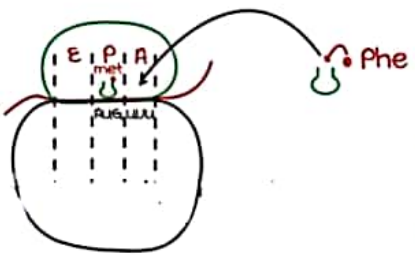

## Elongation

01:02:26

* Helped by Elongation factors
* multistep process :-
  1. Binding of new amino acyl tRNA to A site
  2. Peptide bond formation
  3. Translocation of ribosome on mRNA

Active space

① Binding of new amino acyl tRNA to A site

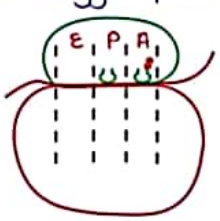



* Helped by EF - 1
* Require hydrolysis of GTP

→ A site :- New tRNA
→ P site :- polypeptide
→ E site :- Free

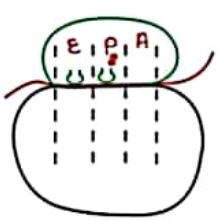
② Peptide bond formation

* amino acid in the p site forms a peptide bond to amino acid in A site
* This requires Peptidyl transferase
* No energy required




A site → polypeptide
p site → Exiting tRNA
E site → free

3 Translocation of ribosome on mRNA




A site → free
P site → polypeptide
E site → Exiting tRNA

* Require EF-2 and hydrolysis of GTP

## Termination 01:14:38

* Termination is specified by Stop codon in A-site
* Releasing factor binds to A site :- RF1 + RF3 + GTP
* Also need peptidyl transferase
* Ribosome separates

## Energetics 01:25:05

* 1 peptide bond synthesis :-
  1) High energy $PO_4$ for charging → 2
  2) Binding to A - site → 1 $PO_4$

Active space

3) Translocation → 1 $PO_4$

Total :- 4$PO_4$

## Post translation modification

01:28:46

1) Cleaving of N - terminal and C terminal amino acid
2) Covalent modification :-
   - Phosphorylation
   - Acetylation
   - Zymogen activation
3) Hydroxylation
4) Glycosylation
5) Gamma carboxylation
6) Histone modification
7) Protein folding

# LAC OPERON

Gene expression :-

DNA
↓ Transcription
RNA
↓ Translation
Protein

| (i) At the level of DNA | (ii) Transcription | (iii) Post transcription |
|---|---|---|
| • Epigenetic modification | • Induction, repression | • RNA editing |
| • Gene amplification | ↓ | • Alternate RNA processing |
| • Gene rearrangement | • Operon concept | • RNA interference (RNAi) |
| • Gene switching | | |
| • Transposons | | |

## Operon concept 00:06:58

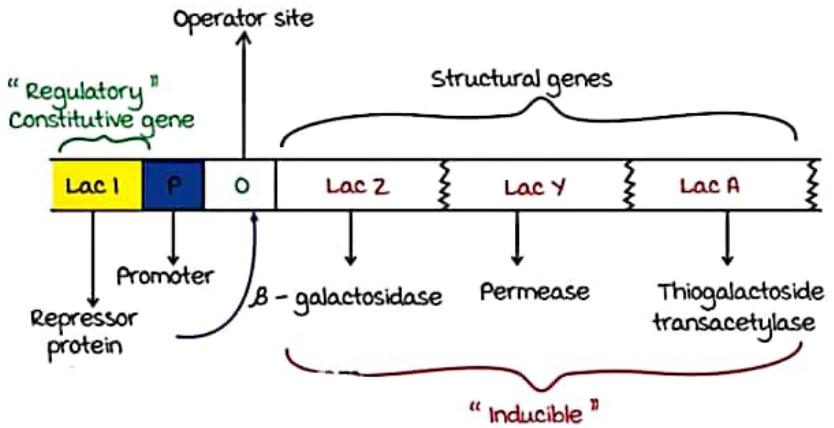
* Put forward by Francois Jacob and Jacques Monod
* Operon :- " array of genes "

| Housekeeping gene | Inducible gene |
|---|---|
| * Constitutive gene | * Expressed ↓ special circumstances |
| * Basal activities | * Eg :- Glucokinase |
| * Expressed at constant rate | |
| * Eg :- Hexokinase | |

**Warning** : Not all points are covered in the notes, especially conceptual explanations. Please use the notes in conjunction with Marrow Edition 4 videos.

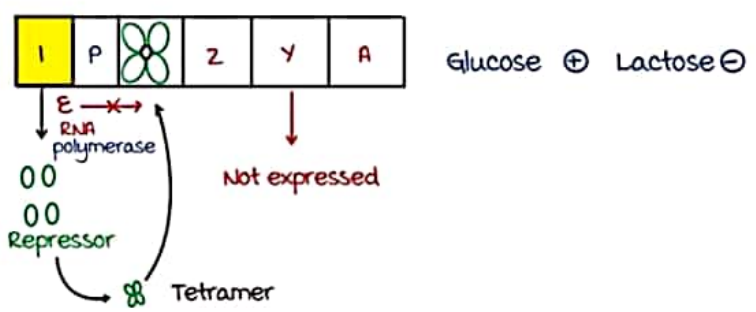
## Lac operon 00:10:03

* Ecoli bacteria :- metabolism of Lactose
* Concept :- Preferred fuel is Glucose
  Glucose absent → Lac operon switched on




* β - galactosidase → metabolism of lactose
* Permease → Allow lactose to enter cell
* Thiogalactoside trans acetylase → Unknown action

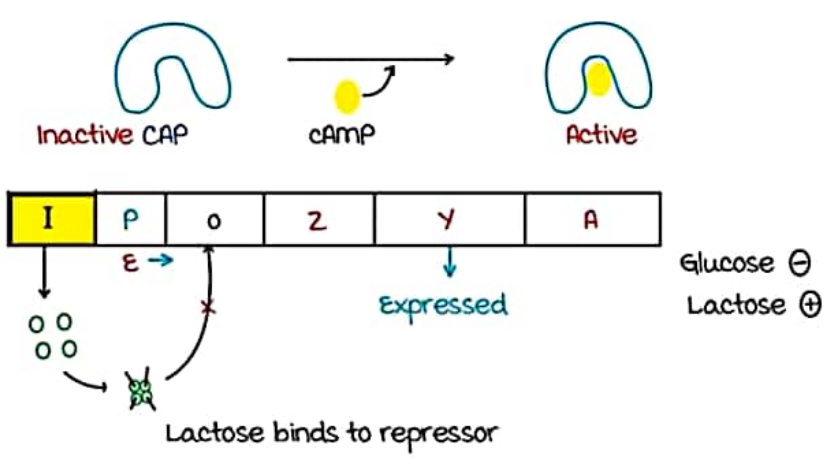
* RNA polymerase binds to promoter site .




## Catabolite repression

00:21:00

* CAP ( Catabolite activator protein ) or CRP ( Catabolite Repressor Protein
* CAP is a Positive regulator of Lac operon
  if CAP active → Lac operon switched ON




Active space

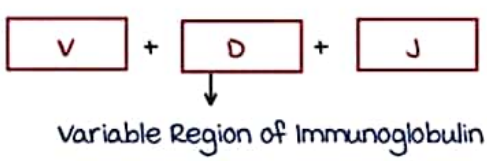
* When Glucose ⊕ Lactose ⊕, cAMP low
  ↓
  CAP Inactive
  ↓
  Lac operon switched off

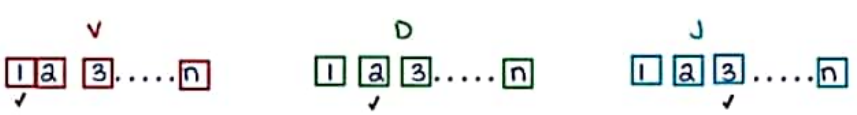
* Gratituous inducer → IPTG ( Iso propyl thio galactose )
  ↓
  ⊕⊕ Lac operon

## Gene amplification 00:46:51

* The no. of genes available for expression is increased.
* Eg :- Patient on Methotrexate develop resistance ;
  ↑ DHF Reductase

## Gene rearrangement 00:50:55



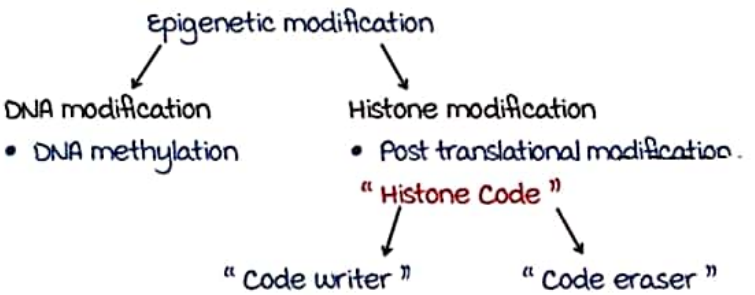

## Transposons 00:54:46

* " Jumping genes "
* Certain genes move from one location to another genome
* >50% of human genome
* Require Transposase enzyme
* Retroposons :- DNA moves with help of an RNA intermediate

Active space

# EPIGENETICS

* Reversible heritable chemical modification of DNA or chromatin without altering the nucleotide sequence.




## DNA Methylation

00:08:00

* Enzyme :- DNA methyl transferase (DNMT)
* On cytosine residues
* CpG islands.
  ↓
  " Promoter region "
* Effect :- ↓ Transcription of gene
  ↓
  Gene silencing

## Histone modification

00:12:08

Histone acetylation

Acetylated ($CH_3COO^-$)

↓ positive charges → in histones → Chromatin less condensed

↑ Euchromatin → ↑ed expression of genes

" Permissive chromatin "

Histone deacetylation

* ↑ Positive changes in histone → Chromatin Condensed → Heterochromatin
  ↓
  ↓ expression of genes
  " Repressive chromatin "

Active space

| Histone acetylation | Histone deacetylation |
|---|---|
| • Enzyme :- Histone Acetyl Transferase (HAT) | • Histone deacetylase (HDAC) |
| • Euchromatin | • Heterochromatin |
| • Less condensed | • Highly condensed |
| • Permissive chromatin | • Repressive chromatin |

| Histone modification | Functional consequence |
|---|---|
| * Acetylation | Activation of gene expression |
| * Deacetylation | Inactivation of gene expression |
| * Methylation | Activation / Inactivation of gene expression |
| * Phosphorylation | Chromatin open/closed |
| * H1 phosphorylation | Chromatin condensation |
| * ADP Ribosylation | DNA Repair |
| * Monoubiquitination | Activation / Inactivation of gene expression |
| * Small ubiquitin related modifier (SUMOylation) | Chromatin condensation(repression of transcription) |

## Physiological application of epigenetic modification 00:24:03

- Regulation of gene expression
- X chromosome inactivation.
- Genomic imprinting
- Ageing
- Embryogenesis

Pathological applications

- Fragile X syndrome :- FMR - 1 gene is silenced
- Cancer :-

  Methylation of Tumour suppressor → ↓ expression of TSG → Causes cancer

  Methylation of oncogene → Prevent cancer.

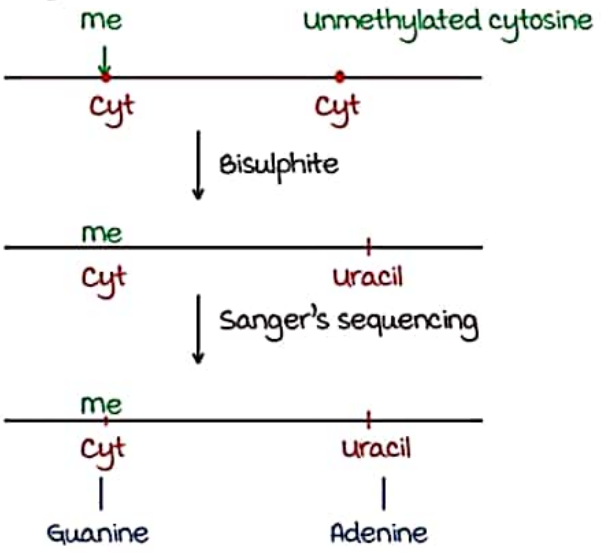
Therapeutic Applications

- DNMT inhibitors → 5 Azadeoxy cytidine, Decitabine
- HDAC inhibitors → Vorinostat, Valproic Acid

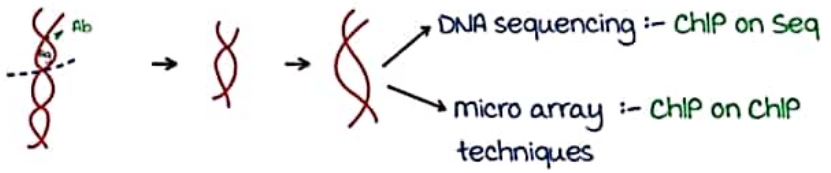
Active space

## Methods to study epigenetic modification 00:34:03

① Bisulphite sequencing




②methylation specific PCR
③methylation sensitive restriction endonuclease digestion
④Chromatin immuno precipitation (ChIP):




Active space

# BLOTTING TECHNIQUES

Blot techniques

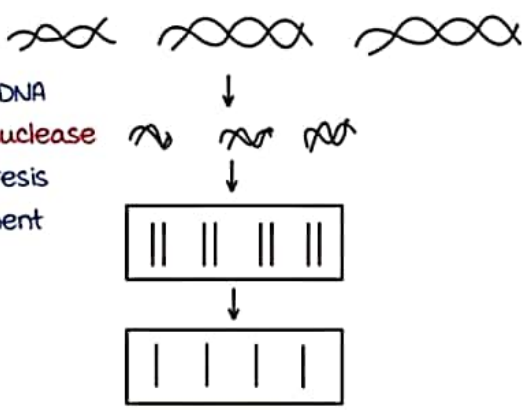
- Southern Blot
- Northern Blot
- Western Blot
- Other blot techniques
  - South Western Blot
  - Zoo Blot
  - Dot Blot.

## Southern blot

00:02:42

* By Edward Southern (1975)
* Technique to detect a specific DNA fragment
* Principle :- DNA – DNA hybridisation

Technique / procedure



1. Isolate all DNA
2. Fragment the isolated DNA using Restriction endonuclease
3. Agarose gel electrophoresis to separate DNA fragment
4. Denaturing the DNA
5. Blotting to nitrocellulose membrane
6. Add radio labelled / fluorescent labelled probe

Uses

- Detect bacterial / viral DNA
- Mutation studies
  - → Large gene detection
  - → Large gene insertion
  - → Point mutation.
- Screening of inborn errors of metabolism
- Conventional PCR → At end point, detect amplicon

↓

Southern blot

Active space

## Northern blot

00:14:32

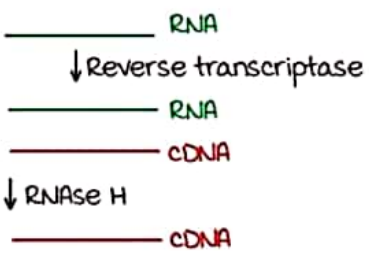
* Technique to detect a specific RNA.
* Principle :- RNA – DNA hybridisation.

Procedure

1. Isolate all RNA.
2. separate it using electrophoresis
3. Blot to nitrocellulose membrane.
4. Radiolabelled cDNA or fluorescently labelled cDNA added

↓

cDNA or Complementary DNA




Uses

- Detect RNA
- Study gene expression

## Western blot

00:23:10

* Principle :- Antigen – Antibody interaction
* Aka Immunoblot

Procedure

1. Isolate antigens from sample
2. Protein electrophoresis
3. Add radiolabelled / fluorescent labelled antibody . after blotting on nitrocellulose membrane .

* South western blot
  - DNA
  - Protein

  ∴ , Detect DNA – Protein interaction
* Slot blot / Dot blot technique
  - Commonly used to detect protein
  - No blotting to nitrocellulose membrane .
* Zoo blot

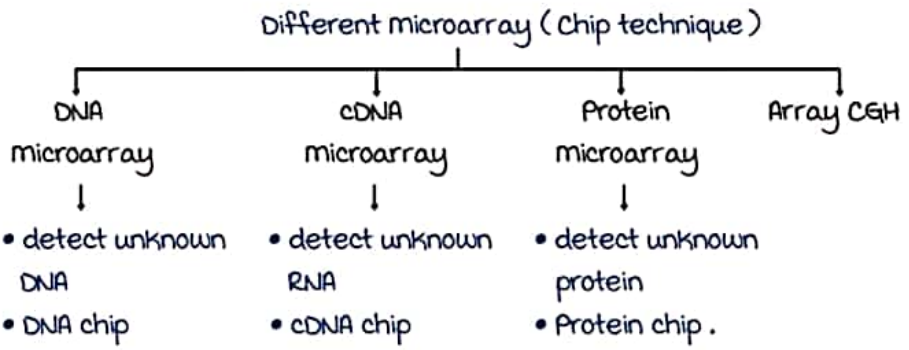
  ↓

  Study of evolution.

Active space

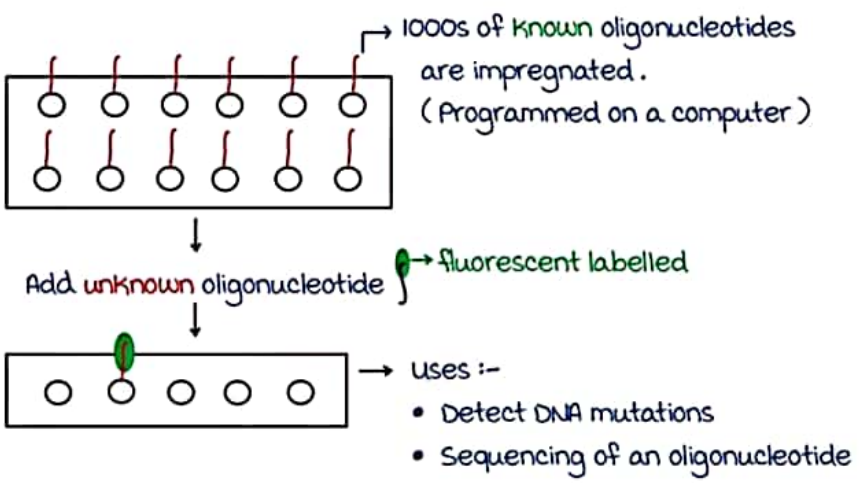
# MICROARRAY TECHNIQUES

- We use glass microscopic slide .
- A/K/A Chip technique



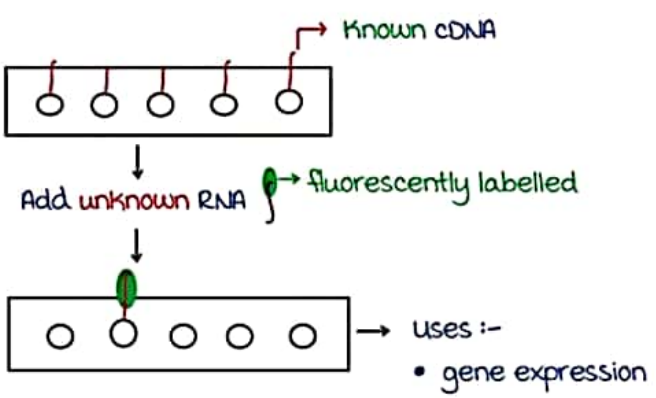

## DNA microarray

00:08:54




## cDNA microarray

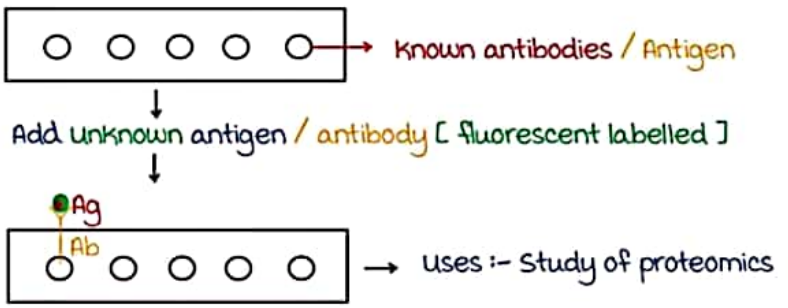
00:16:21




Active space

## Protein microarray

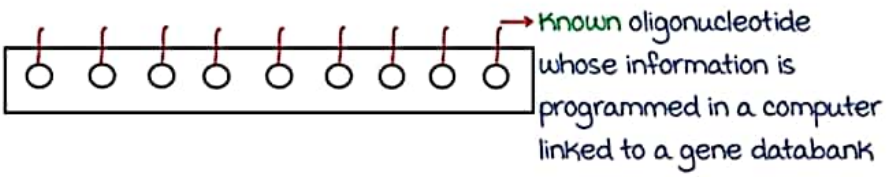
00:20:15

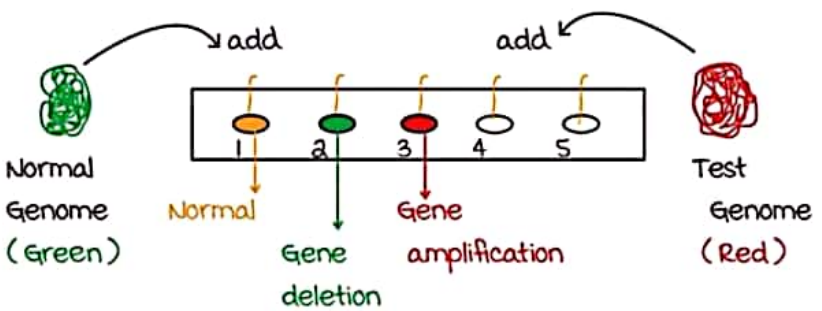



## Array comparative genomic hybridisation

00:25:33

- Genome chip → Entire genome of an organism is impregnated on a chip.




Uses :-

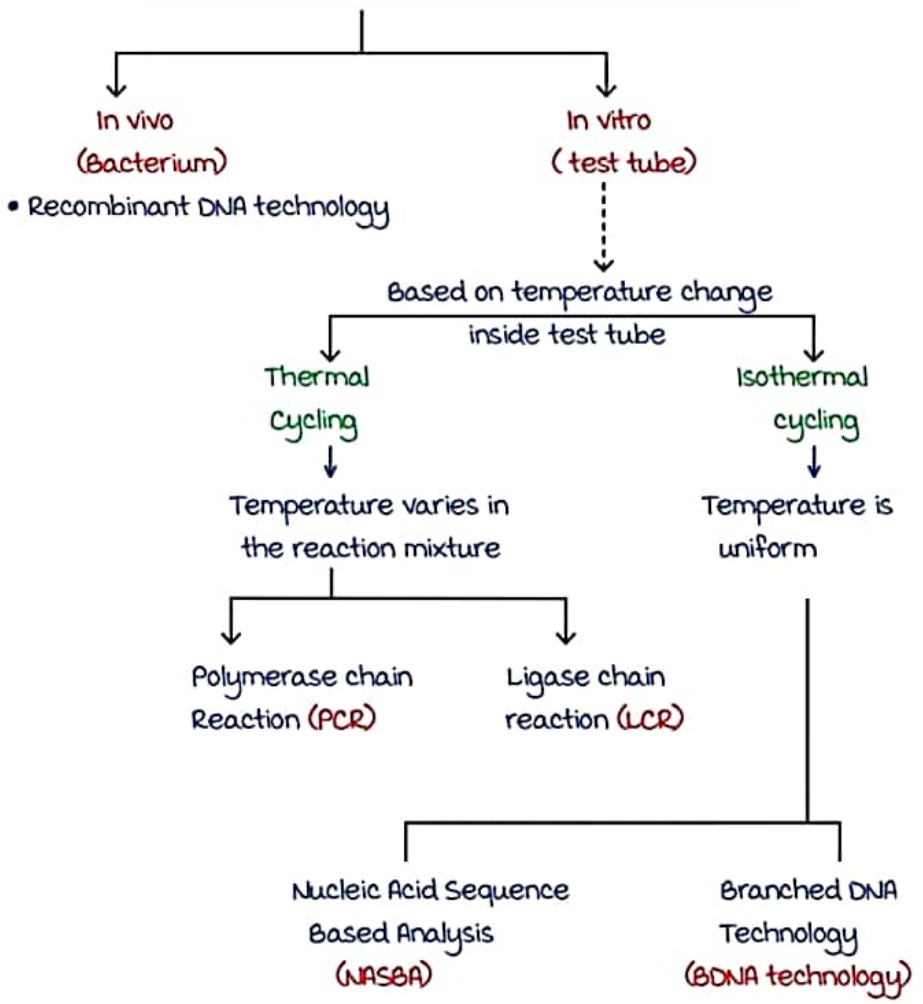
(1) Gene deletion
(2) Gene amplificaiton
(3) Copy number variation
(4) Aneuploidy
(5) Compare genomes
(6) Study of disorders with unknown etiology
(7) Structural abnormalities { balanced translocation cannot be detected }

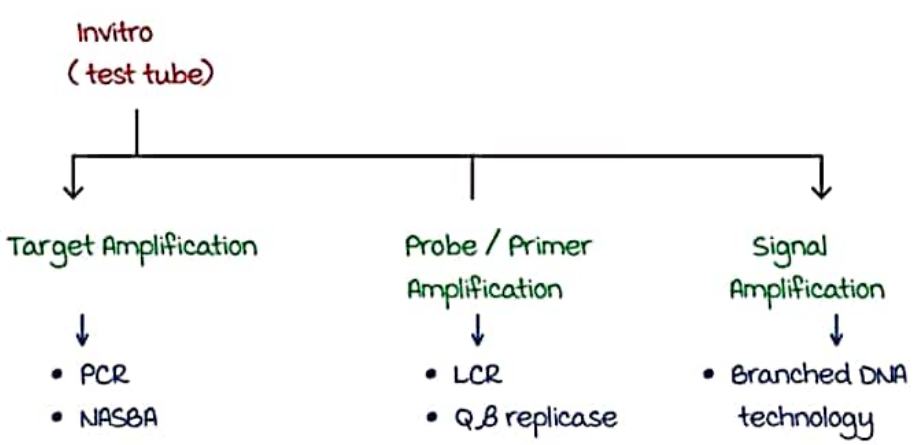
Active space

# RECOMBINANT DNA (rDNA)

## Amplification Techniques

00:00:32




Active space

## Recombinant DNA technology 00:09:39

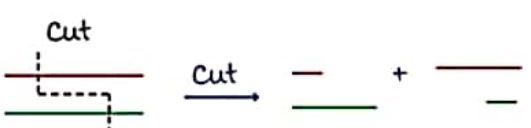
- Employing recombinant DNA / Chimeric DNA
- In vivo amplification of a desired DNA fragment inside a living cell. ( Bacterial cell)

- Restriction Endo nuclease :

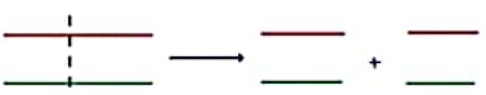
- a/k/a molecular scissors
- Aim : cuts a double stranded DNA
  ↓
- Breaks 3'-5' phosphodiester bond
- it is a hydrolase
- Discovered by Werner Arber
- Types : • type I : Cuts the dsDNA at random site
  • type II : cuts the dsDNA at palindromic site
    • used in molecular biology technique
    • Discovered by Hamilton Smith, Daniel Nathans.
- Isolated from bacteria
- Function : Restrict the entry of virus / Phages

## Restriction endonuclease : Action & Use 00:17:47

- Cuts the dsDNA at palindromic site
- ECoRI :
  - Obtained from E.coli
  - Palindromic site – G AATTC → Cut → G + AATTC / CTTAA G → CTTAA + G
  - Sticky end-has over hanging sequence — Sticky end / Staggered end / Cohesive end




- Hpa I :
  - palindromic site – GTT AAC / CAA TTG → GTT + AAC / CAA TTG
  - Blunt end – No overhanging Sequences — Blunt end




Active space

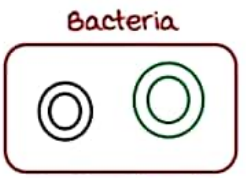
- Restriction endonuclease is specific for bacterial palindromic site
- cannot cut its own DNA (due to site specific methylates)

## Vectors 00:25:29

- They are the carriers of desired DNA to the host cell (bacterial cell)
- Types : Plasmid, Phages, Cosmids, Artificial Chromosomes

**Plasmids:**

- These are circular dsDNA outside bacterial DNA.
- Function : • confer antibiotic Resistance
- they can replicate on its own
  ↓
  has its on origin of replication
  K/a "ori"




- extrachromosomal
- Episomes

- Plasmid can carry 0.01 to 10 Kbp of foreign DNA

**Phages :**

- A/K/A Bacterial viruses
- Phage DNA → linear DNA
- can carry 10 – 20 Kbp of foreign DNA (DNA insert Size)

**Cosmids :**

- plasmids with cos site (cos gene)
- cos gene → helps in packing of λ DNA to the phage
- has feature of plasmids + phages
- DNA insert size : 30 – 50 Kbp

**Artificial Chromosomes :**

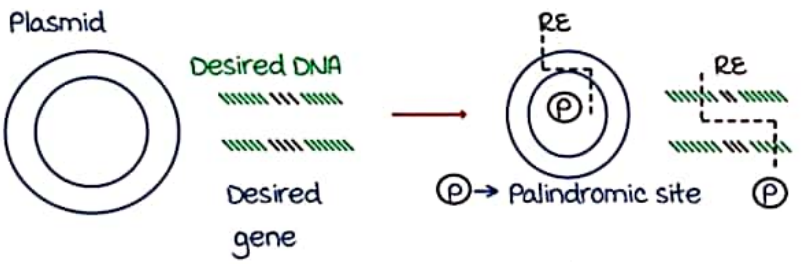
- Artificial created plasmids
- if based on ; Bacterial chromosome → BAC
  - Phage chromosome → PAC (P1 Phage of E.coli) } 50 – 250 Kbp
  - Yeast Chromosome → YAC (500 – 3000 Kbp)

## Steps of r-DNA 00:36:39

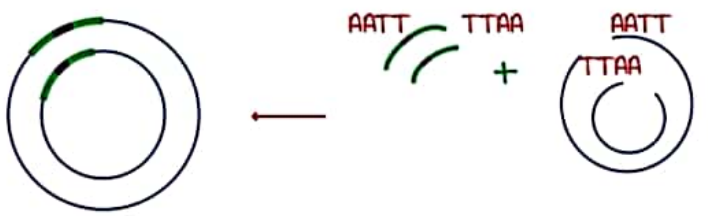
1) Isolate the desired DNA Segment :
   - Isolate MRNA from desired cell [Eg: Pancreas → Insulin gene]
     ↓
     MRNA for insulin $\xrightarrow[\text{Transcriptase}]{\text{Reverse}}$ c DNA
2) Select a vector : Based on size of the DNA to be amplified
3) Synthesis of recombinant vector / Chimeric DNA / recombinant DNA
4) Introduce recombinant vector to host cell
5) select successfully ligated plasmid

Active space

Synthesis of recombinant vector :




Restriction endonuclease (RE)
(Same on both)




Recombinant plasmid / Chimeric DNA

↓

multiplied as the bacteria replicates

## Gene Library

00:51:11

- Collection of recombinant clones

Genomic Gene library

- Represent the entire genome of an organism

↓

each fragment of the DNA will have recombinant clones

cDNA library

↓

Collect mRNA

↓

cDNA

↓

RDNA technology

- Introns ⊖
- more informative
- Easy to replicate

Active space

## Expression vector & recombinases 00:56:23

Insulin gene
↓
Recombinant Vector ⟶ MRNA ⟶ Protein produced
↓
insulin

- Helps in study of gene expression

Recombinases :
- Enzymes with site specific incorporation by Homologus recombination.
- Used as an adjuvant to restriction endo nuclease.

| Recombinase | | recognition site | |
|---|---|---|---|
| • CRE recombinase | ⟶ | Bacterial lox P site | } (Cre - lox system) |
| • λ Phages → INT Protein | ⟶ | λ att site | |
| • Yeast → Flp Recombinase | ⟶ | FRT site | |

Active space

# POLYMERASE CHAIN REACTION ( PCR )

* In vitro technique to amplify a desired DNA using enzymes .
* Invented by Dr Kary B Mullis in 1989 and he got Nobel prize for Chemistry in 1993 .
* Exponential amplification .

Prerequisites

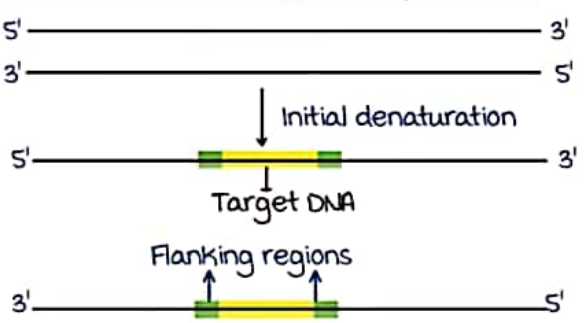
1) Sample DNA (pure)
2) Primers →
   - Excess
   - Length : 18 – 25 nucleotide length .
3) dNTP
4) cation :- $Mg^{2+}$, $K^{+}$
5) Taq polymerase

## Technique of PCR

00:10:11

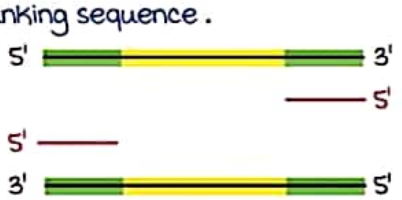
I) Denaturation

* Thermocycler- rapidly changes the temperature .
* Initial denaturation :- 90 – 96° C ( 94° C ) → 3 min




II) Annealing of primers

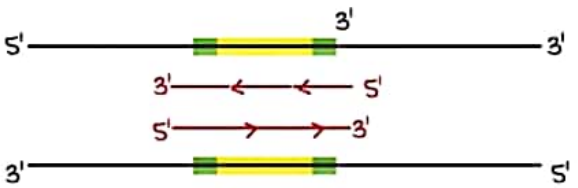
* Primer attached to 3' end of flanking sequence .
* Temp :- 50 – 70° C ( 60°C )

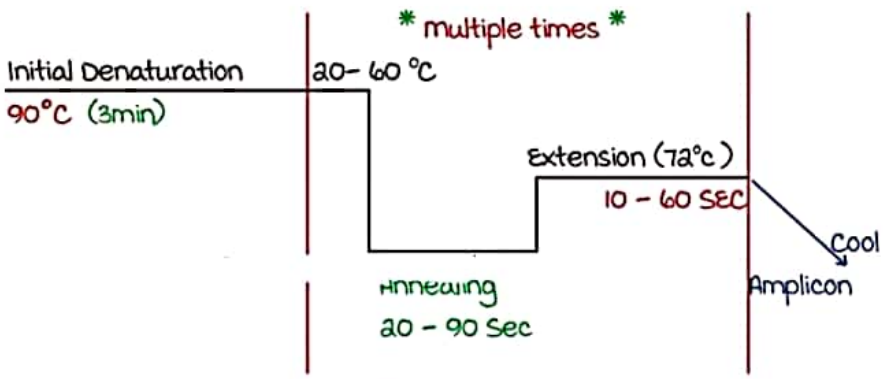



Active space

III) Extension

* We need :-
  - Taq polymerase ( present in Thermus aquaticus )
  - d NTP
  - $mg^{2+}$
* Temp :- 68 - 75° C ( 72° C ) optimum temp of Taq polymerase




* Exponential amplification = $2^{n}$ → no of cycles

## Variants of PCR

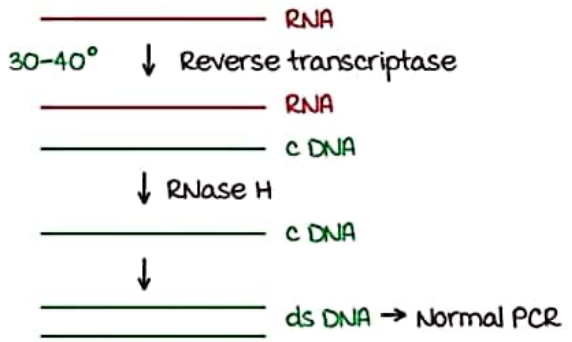
00:31:57

- Real Time PCR

* A/K/A Quantitative PCR [ "q PCR" ]
* Simultaneous amplification + detection / quantification of amplicon .
* Techniques :-
  - Ethidium bromide
  - SYBR Green (dye)
  - Sequence specific probes :-
    - molecular beacon
    - Taq man probe
    - FRET probe(Fluorescence Resonance Energy Transfer)

- RT PCR

* Reverse Transcriptase PCR
* Detect / quantify any kinds of RNA
* Use :- study of gene expression .




* In RT PCR we use Tth polymerase derived from Thermus thermophilus
  ↓
  a enzyme activity :- Reverse transcriptase + DNA Polymerase
* Used to estimate viral load.

| Simplex PCR | Multiplex PCR |
|---|---|
| * In a single reaction mixture, only one target DNA amplified<br>* Time consuming<br>* Specific | * In a single reaction mixture multiple targets are amplified.<br>* Less time consuming<br>* Non specific |

- AP PCR (RAPD)

* Arbitrarily Primed PCR (Randomly Amplified Polymorphic DNA).
* Multiplex PCR
* Small multiple primer "10 – 15 nucleotide length"

**Warning** : Not all points are covered in the notes, especially conceptual explanations. Please use the notes in conjunction with Marrow Edition 4 videos.

## Applications of PCR

00:48:27

1) In Forensic Medicine
2) Microbiology → Viral load
   Bacterial load
3) Study of mutation
4) Study of repeat length polymorphism
5) Preliminary Technique for many other molecular biology techniques.

Active space

# FLUORESCENCE IN SITU HYBRIDIZATION ( FISH )

- Simple detection of a specific genetic information on a morphologically intact tissue using fluorescent probes
- Cell division arrested at metaphase → fluorescent probes added

## Types of FISH

00:05:58

Chromosome painting

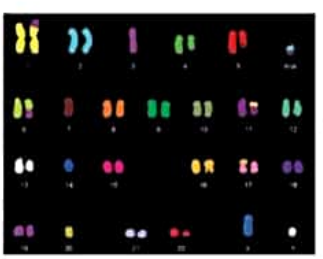
- Using fluorescent probes, each chromosome is identified by different colour .
- Not unique .
- Fluorescent dyes are limited .

Multicolour FISH

- 23 distinct fluorescent colours made by mixing 5 fluorophores .
- Each chromosome is identified by a unique colour .

Uses

① Detect numeric abnormalities .
② Detect Subtle microdeletions .
③ Detect gene amplification .
④ Detect Structural abnormalities .
⑤ Map a newly isolated gene to its correct chromosomal loci .



Disadvantages :- • Prior knowledge is needed .

Interphase FISH / nuclear FISH

- Very rapid .
- Growing cells are not needed
- Uses :- • Prenatal diagnosis
  • Tumours

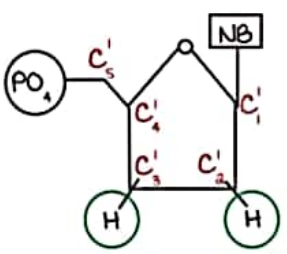
Active space

# DNA SEQUENCING

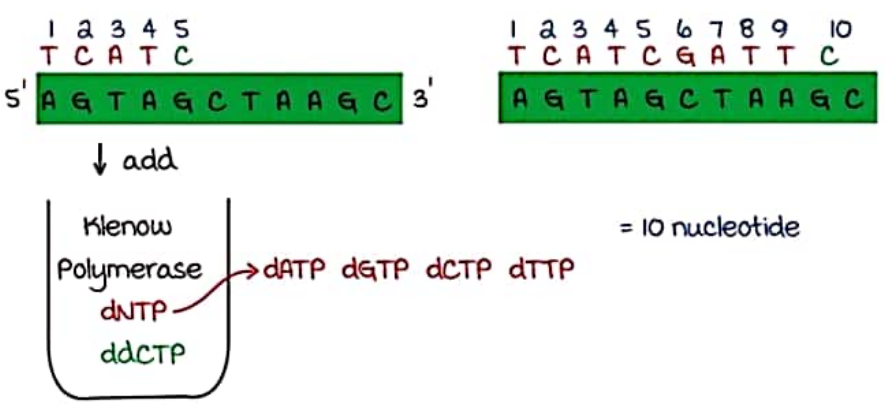
## Sanger's sequencing

00:02:23

* Invented by Frederick Sanger .
* A/K/A Controlled chain termination method .
* Most popular and most widely used method .
* Automated .
* Gold standard mutation detection technique .
* Requirements : -
  - Sample DNA ( Single Stranded )
  - ∝ dNTP
  - Klenow polymerase → DNA polymerase from which 5' – 3' exonuclease activity removed .
  - dideoxy nucleotides ( chain terminators )
* Dideoxy ribonucleotide → Terminate the chain growth




Technique :




Active space

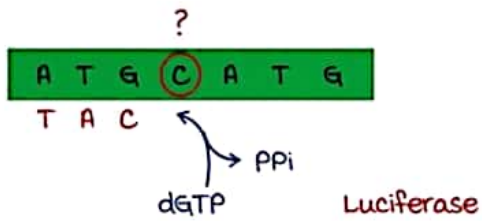
## Maxam Gilbert technique 00:28:56

* Chemical cleavage method.
* Only for small fragments of DNA.

## Pyrosequencing 00:29:36

* dNMP + dNTP → dNMP – dNMP
  PPi
  Secondary reaction.
  Luciferase
  "Bioluminensence"

* Eg:-




* more sensitive than Sanger's sequencing.

## Next Generation Sequencing(NGS platform) 00:35:48

* For population study. (Sequencing in a massive parallel manner).
* Steps :- ① Spatial separation.
  ② Amplify all DNA simultaneously.
  ③ Parallel Sequencing

* WES : Whole Exome Sequencing
* WGS : Whole Genome Sequencing
* CAGE : Cap analysis of Gene Expression

Active space

# MUTATION

* Any permanent change in the nucleotide sequence irrespective of its functional consequences.
* Epigenetic modifications :-
  - Nucleotide sequence is not altered.
  - Reversible
* Polymorphism :-
  - Normal changes in nucleotide sequence.

## Types of mutations

00:04:07

Point mutation

* Change in single base
* most common mutation.

Class I :- Base substitution

* m/c point mutation
* One base replaced by another

| | Group | Type |
|---|---|---|
| Polypeptide not altered ☒ | Synonymous | Silent mutation |
| | Nonsynonymous | Missense mutation<br>Nonsense mutation |

Silent mutation

Base substitution → codon replaced ↕ by Another codon } coding for same amino acid (Degeneracy of codon)

Missense mutation

Base substitution → codon I → codes for an amino acid

↓

codon II → codes for a different amino acid

Active space

a) Conservative missense mutation
↓
- code for different Amino acid of similar characteristics
- Eg :- Valine ⊠ Leucine

b) Nonconservative missense mutation
↓
- code for different Amino acid with different characteristics
- Eg :-In HbS
  $\beta$ Chain:- Glutamic acid → Valine
  (Polar) (Non polar)

Missense mutation

Acceptable
- No clinical symptoms
- Eg :- Hb Hikari
↓
Change in electrophoretic mobility

Partially acceptable
- Function of protein not affected
- Eg :- Hb S

Unacceptable
- Function is Lost
- Eg :- Meth Hb

Transition
A ⊠ G
C ⊠ T
purine ⊠ purine
pyrimidine ⊠ pyrimidine

Transversion
A ⇄ G
C ⇄ T
purine ⊠ pyrimidine
pyrimidine ⊠ purine

Nonsense mutation
Base substitution :- coding codon replaced by stop codon causing a "Translation arrest"

* Insertion : Addition of single nucleotide
* Deletion : Deletion of single nucleotide
  Effect of indels → most harmful mutation
↓
"Frame shift mutation"
↓
reading frame is garbled
* If insertion & deletion is in multiple of 3 → No frame shift mutation
* Null mutation :- Mutation results in no gene product.
* Constitutive mutation :-Inducible gene
↓
Constitutive gene

Active space

Run on polypeptide

Eg :- Hb Constant spring

originally a stop codon

Codon 1 - 2 - 3 - (4) - 5 - 6 - - - ⇒ Polypeptide more than 3 Amino acid

↓

mutated to a non-stop codon

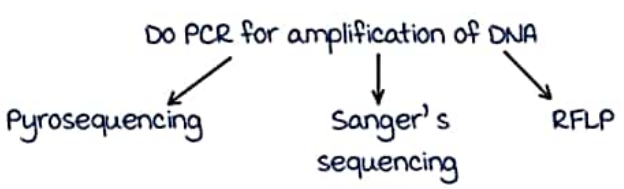
## Mutation detection techniques 00:32:43

Ames test

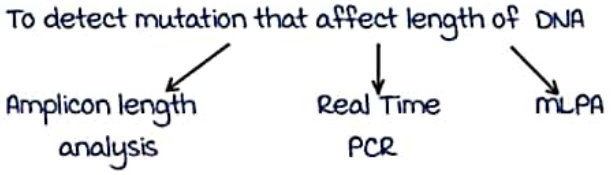
* Test to detect mutation .
* Salmonella typhimurium .

* Numerical or structural abnormalities in chromosome detected by
  - cytogenetic analysis
  - Fluorescent In Situ Hybridization( FISH)
* Techniques that detect point mutation - small insertion or deletion
  - DNA sequencing
  - Restriction Fragment Length Polymorphism ( RFLP )
  - SSCP ( Single Strand Conformational Polymorphism )
  - DGGE ( Denaturing Gradient Gel Electrophoresis )
  - OSH ( Oligonucleotide specific hybridization )
  - RNAse cleavage
  - Microarray ( DNA chip )

* To detect mutation with DNA sequence alterations :




* To detect mutation that affect length of DNA




MLPA-Multiplex Ligation-dependent Probe Amplification

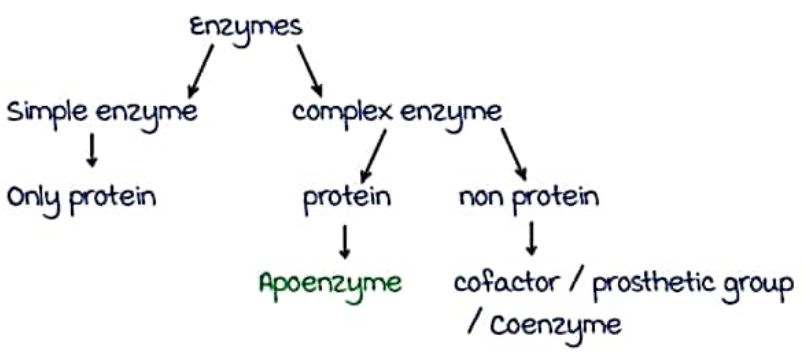
Active space

# GENERAL ENZYMOLOGY

- Enzymes are highly specialised proteins which act as catalyst in biological system.
- Enzyme term coined by Friedrich Kuhne which means " in yeast ".
- Substrate : Substance on which the enzyme acts.
- Product: Substance produced by enzyme action.

## Categories of enzymes

00:03:43




- Apoenzyme + Cofactor = Holoenzyme

| Apoenzymes | Coenzymes |
|---|---|
| • Proteins<br>• Heat Labile.<br>• 16 % by weight $N_2$<br>• Precipitated by protein precipitating agents. | • Low mol. wt organic molecule.<br>• Heat stable.<br>• Considered as second Substrates / cosubstrates<br>• m/c coenzymes → B - Complex vitamins |

## Non proteinaceous enzymes - Ribozymes

00:09:07

- Ribozymes : RNA that act as catalyst.

  Eg:- * Sn RNA in spliceosome → Endonuclease action
  * 28 SrRNA → Peptidyl transferase activity.
  * Group II introns → Self splicing introns → transesterification.
  * RNAse H → lyse the RNA

Active space

Abzyme

- Antibodies with catalytic activity.

## Cofactors & Prosthetic Group 00:16:17

Cofactors

- Non protein part of complex enzymes .
- m/c are metals

**metalloenzyme**

- Metal is tightly bound to enzyme .
- Eg:- Zn in carboxypeptidase
  Cu in tyrosinase

⇓

**Prosthetic group :**
- Non protein part
- Metals
- Tightly integrated to enzyme .

**metal activated enzyme**

- $Ca^{2+}$ in lipase activity
- $Mg^{2+}$ in Kinase activity .

## Coenzymes V/s Cofactors V/s Prosthetic Group 00:20:53

| Coenzyme | Cofactor | Prosthetic group |
|---|---|---|
| • Organic compounds | • Metals | • Metals |
| • Reversibly associated with enzymes | • Not organic compound | • Tightly integrated to enzyme |
| | • metal activated enzyme | • metalloenzyme |

## Metals as cofactors - Zn, Mg & Fe 00:25:10

* Zn :
- Alkaline phosphatase
- Carbonic anhydrase
- Carboxy peptidase
- Alcohol dehydrogenase
- ALA dehydratase
- LDH
- Adenosine deaminase
- Cytosolic SOD

* Mg :
- Phosphotransferase
- Phosphohydrolase
- Mutase
- Enolase

Active space

* Fe : • Heme Iron → 1. Tryptophan Pyrrolase
  2. Peroxidase
  3. Nitric oxide synthase
  4. Catalase
  5. Cytochromes

  • Non heme Iron → 1. Succinate Dehydrogenase
  2. NADH Dehydrogenase
  3. Cytochrome Oxidase

* Mo : • Xanthine Oxidase
  • Sulfite Oxidase
  • Aldehyde Oxidase

* $Mn^{a+}$ : • Phosphotransferase
  • Arginase
  • Mitochondrial sod
  • Enolase

* $K^{+}$ : • Pyruvate Kinase
  • $Na^{+}$ $K^{+}$ ATPase

* $Cu^{a+}$ : • Tyrosinase
  • Super oxide dismutase
  • Lysyl Oxidase
  • Peptidyl Glycine hydroxylase
  • Amino acid Oxidase
  • Cytochrome c Oxidise
  • Dopamine B hydroxylase

* Ni : • Urease

* $Ca^{a+}$ : • Lipase
  • Lecithinase

## Coenzymes

00:35:42

| Enzymes | Coenzymes |
|---|---|
| • Kinases | • GTP/ATP |
| • Dehydrogenases | • NAD/FAD |
| • Reductase | • NADPH |
| • Transketolase | • TPP |
| • Transaminase | • PLP |
| • Decarboxylase | • PLP |
| • Carboxylase | • Biotin/ATP |

Active space

# FACTORS AFFECTING ENZYMES

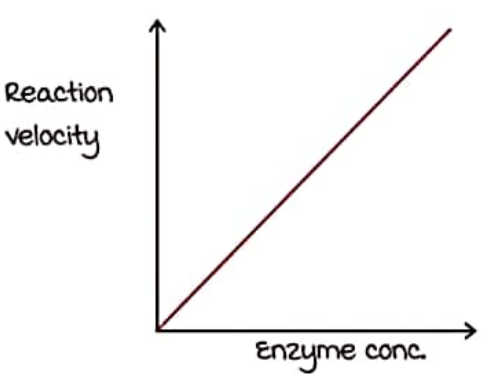
## Classification of enzymes - Recent update 00:00:38

I. Oxidoreductase
II. Transferase
III. Hydrolases
IV. Lyases
V. Isomerases
VI. Ligase
VII. Translocase :
- Transfer $H^+$
- Transfer ions – $Ca^{2+}$ channels
  $K^+$ Channels
- Transport Compounds

## Factors affecting enzyme activity 00:03:37

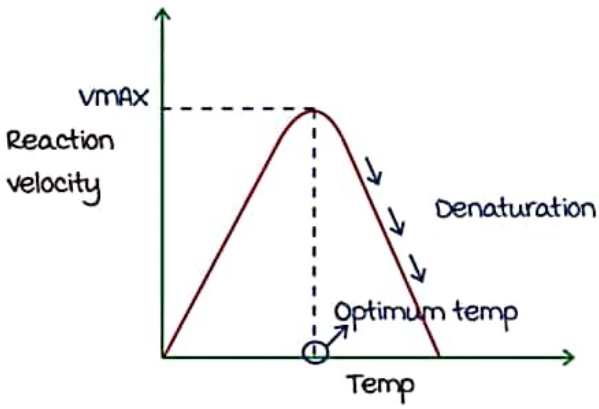
1) Enzyme concentration
2) Temperature
3) pH
4) Substrate concentration

## Enzyme concentration & temperature 00:05:27




$v \propto$ Enzyme concentration

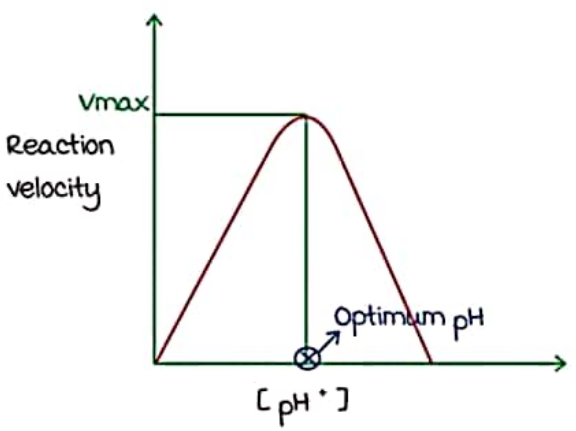
Active space




- Optimum temp for enzymes :- 35 - 40°c .
- Q 10 → For every 10° rise in temp, velocity doubled .

## Hydrogen ion concentration - pH

00:11:27

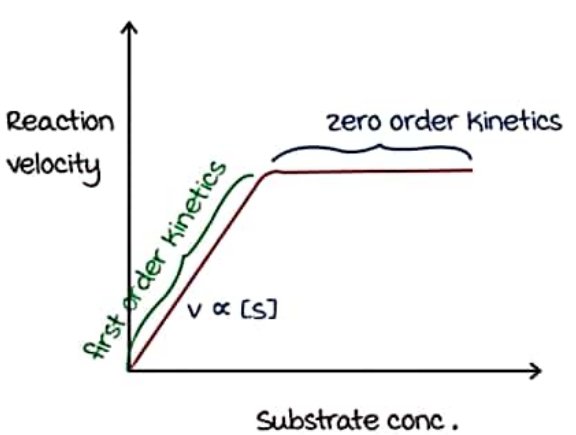



- Optimum pH :- 5 - 9

## Substrate concentration

00:14:56

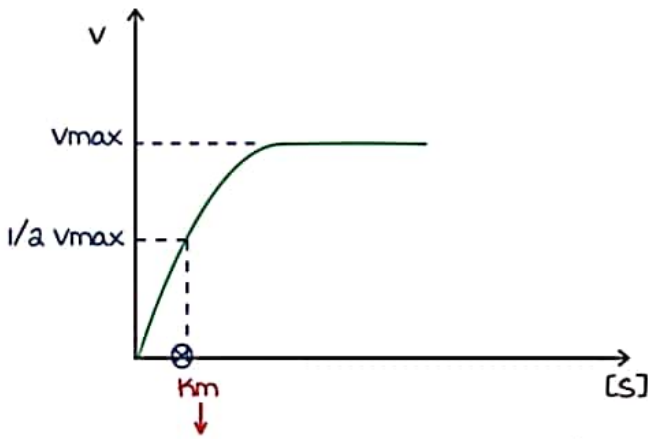
- Enzyme concentration → constant




Active space

## Landmarks of velocity V/s Substrate concentration curve

00:19:43




Michaelis constant : Substrate conc. at 1/2 Vmax

## Significance of Michaelis constant

00:26:55

- Signature of an E-S pair .
- Constant for an E-S pair .
- Higher the Km, Lower is its affinity to enzyme .
- Lower the Km, Higher is its affinity to enzyme .

## Michealis Menten Equation

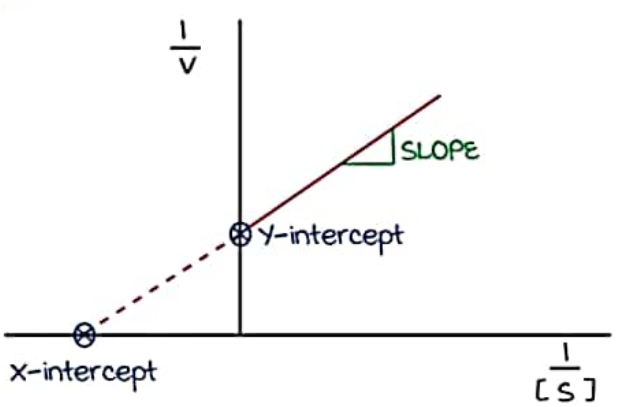
$$Vi = \frac{Vmax \times [S]}{Km + [S]}$$

Vi = Initial Velocity
Vmax = Maximum Velocity
[S] = Substrate Conc .
Km = Michaelis Constant .

## Lineweaver Burk plot

00:32:43

- Double reciprocal plot .




Active space

- $\frac{1}{V} = \frac{Km}{Vmax} \times \frac{1}{[S]} + \frac{1}{Vmax}$ $(y = ax + b)$

- x - intercept $\Rightarrow (y = 0)$
  $\therefore$, x - intercept $= \frac{-1}{Km}$

- y - intercept $\Rightarrow (x = 0)$
  $\therefore$ y - intercept $= \frac{1}{Vmax}$

- Slope $= \frac{Km}{Vmax}$

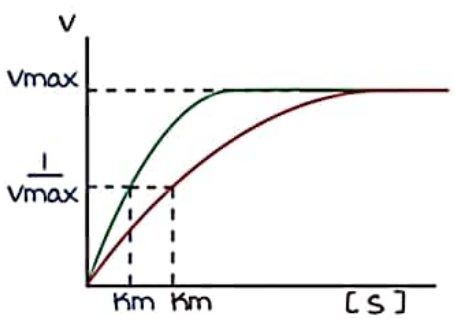
Active space

# ENZYME INHIBITORS

## Enzyme inhibition - Classification 00:00:40

(1) Competitive inhibition
(2) Non- competitive inhibition
(3) Uncompetitive inhibition
(4) Feedback inhibition
(5) Allosteric inhibition
(6) Suicide inhibition

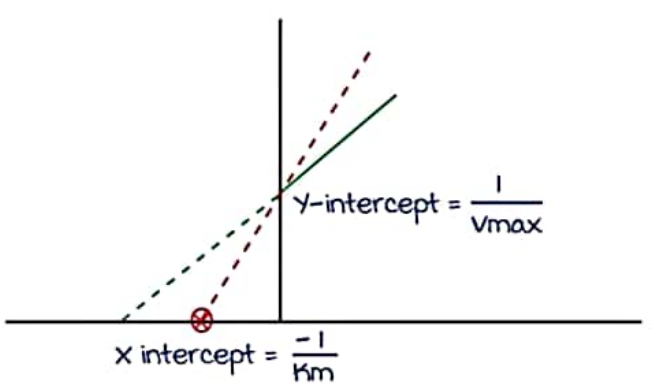
## Competitive inhibition 00:02:04




- Km ↑ ↑
- Vmax remains constant.

Features :

(1) Inhibitor and substrate are structural analogs.
(2) Inhibitor binds to same site where the substrate binds.
(3) Reversible




(4) X-intercept move towards zero
y-intercept remain the same

Active space

## Examples of competitive inhibition

00:12:38

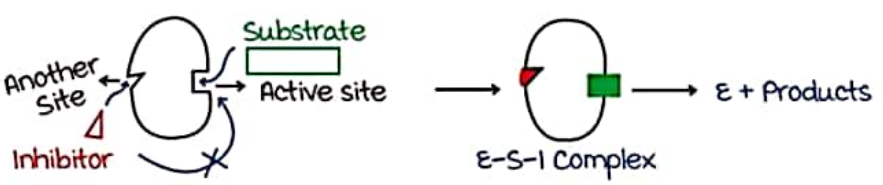
- Drugs :
  (1) Statins $\xrightarrow{\ominus}$ HMG CoA Reductase
  (2) Dicoumarol $\xrightarrow{\ominus}$ Vit K epoxide reductase
  (3) Methotrexate $\xrightarrow{\ominus}$ DHF reductase
  (4) Succinyl CoA $\xrightarrow{\ominus}$ Acetyl choline esterase

- Other than drugs

| | Substrate | Inhibitor |
|---|---|---|
| LDH | Lactate | Oxamate |
| SDH | Succinate | malonate |
| HMG CoA Reductase | HMG CoA | HMG |

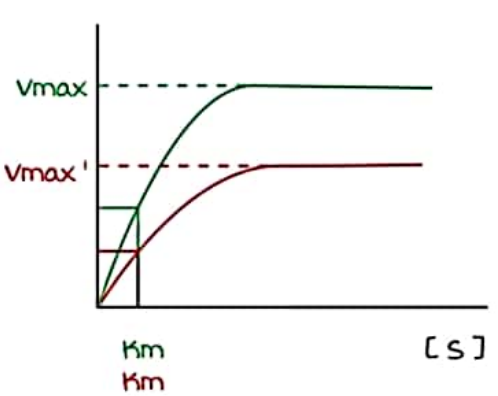
## Non competitive inhibition

00:15:20




Features:

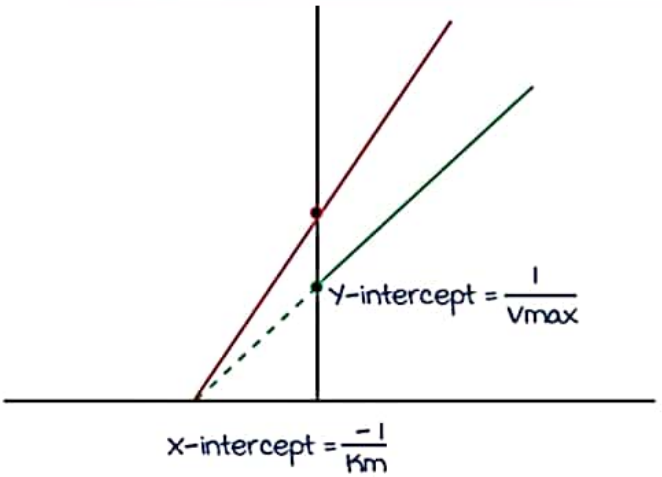
(1) Substrate and inhibitors are not structural analogues
(2) Inhibitors binds to seperate site




(3) Vmax ↓ , Km remains constant
(4) Most non- competitive inhibitions -reversible
Except : Trypsin inhibitor on Trypsin

Active space

## Lineweaver Burk plot of non competitive inhibitor 00:22:10

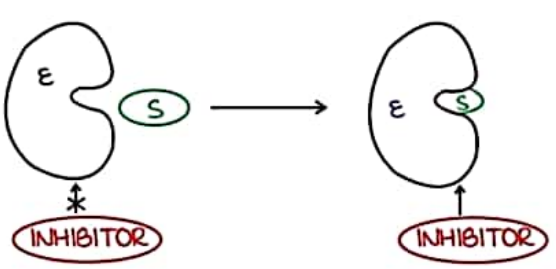



## Examples of non competitive inhibition 00:24:28

- Non competitive inhibitors : Poisonous agents

| | Inhibitor |
|---|---|
| (1) Cyt C oxidase | Cyanide |
| (2) Glyceraldehyde 3 – $PO_4$ dehydrogenase | Iodoacetate |
| (3) Aldehyde dehydrogenase | Disulfiram |
| (4) Enolase | Fluoride |
| (5) ∝ KGDH | Arsenite |

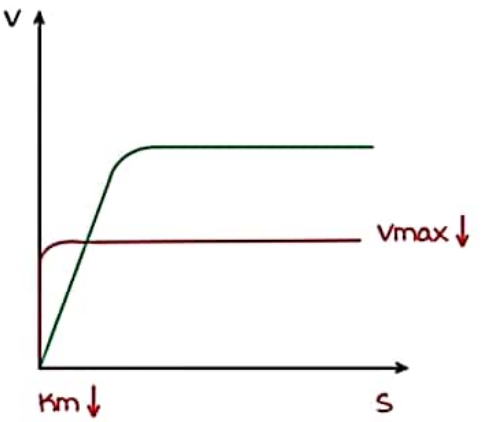
## Uncompetitive inhibition 00:25:47

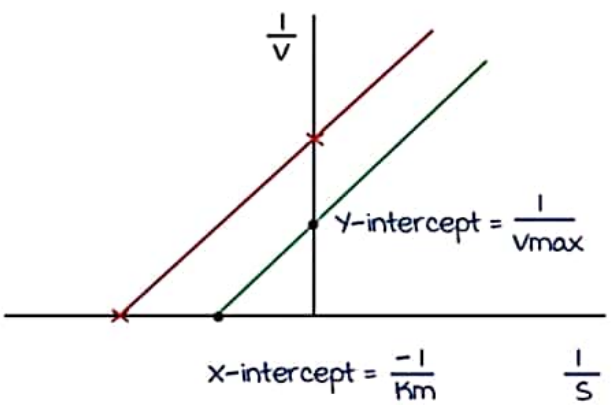



Inhibitor cannot bind with free enzym

Inhibitor binds to E-S complex

Active space



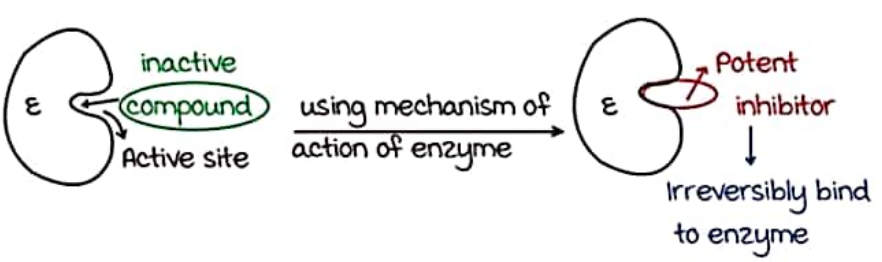

- X-intercept moves away from zero
- Y-intercept increases

Eg :- Placental ALP ⊖ phenylalanine

## Suicide inhibition

00:32:03

- Mechanism based inhibition

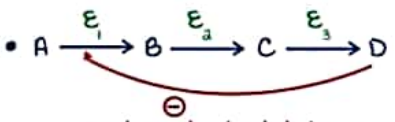



- Eg :-
  - Allopurinol ⊖ Xanthine oxidase
  - Aspirin ⊖ Cyclo oxygenase
  - Difluoro methyl ornithine ⊖ Ornithine decarboxylase

Active space

## Feedback inhibition

00:36:06




- Eg :- AMP ⊖ Purine synthesis

⊖

End product inhibition : End product inhibits the first enzyme

- End product inhibit Gene that transcribe & translate → $E_1$

↓

Feed back regulation

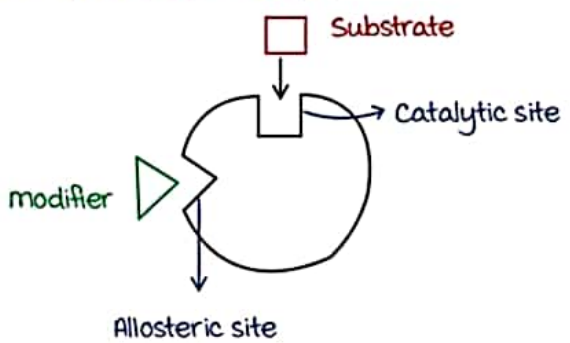
# REGULATION OF ENZYME ACTIVITY

Regulation of enzyme activity

| Quantity | Quantity / Catalytic activity |
|---|---|
| • Control of enzyme synthesis | ① Allosteric regulation |
| ↓ | |
| Induction & Repression | ② Covalent modification |

## Allosteric enzyme 00:02:55

- Enzyme whose activity in the catalytic site is modified by the presence / absence of a modifier .
- Modifier bind to allosteric site .




Allosteric activation

- If modifier = Activator and binds to allosteric site , it converts unfavourable catalytic site to favourable catalytic site , so that the substrate can bind .

Allosteric inhibition

- If modifier = Inhibitor and bind to allosteric site , it converts favourable catalytic site to unfavourable catalytic site , so that the substrate can't bind to the catalytic site.

## Features of allosteric regulation 00:07:06

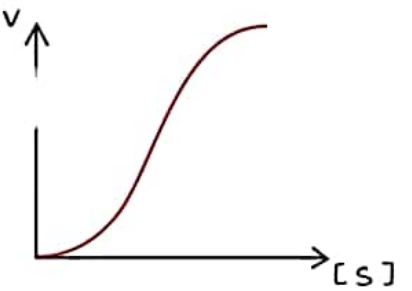
1) The modifier need not be a structural analogue of the substrate .
2) • most allosteric enzyme are multi subunit enzyme .
   They possess " quarternary structure ".
   • This result in a process called Cooperative binding i.e. binding of one substrate favour binding of other substrate to the same enzyme .

Active space

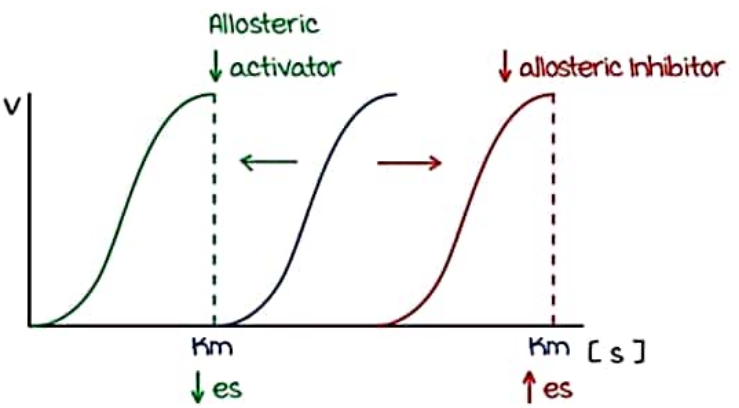
- This is the reason for sigmoidal shape.

3) Multiunit enzymes show positive or negative cooperativity.

4) Does not follow Michaelis – menten hyperbolic kinetics, instead gives sigmoid curve.




5) Allosteric enzyme occupy key regulatory positions in metabolic pathway called key enzymes or rate limiting enzymes




| Enzyme | Allosteric Inhibitor | Allosteric Activator |
|---|---|---|
| I. ALA synthase | Heme | |
| II. Aspartate Transcarbomylase | CTP | ATP |
| III. HMG CoA Reductase | Cholesterol | |
| IV. Phospho FructoKinase | Citrate, ATP | AMP, F2, 6P |
| V. Acetyl CoA carboxylase | Acyl CoA | Citrate |
| VI. Citrate Synthase | ATP | |
| VII. Carbamoyl Phosphate Synthetase – I | | NAG |
| VIII. Carbamoyl phosphate Synthetase – II | ATP | |

Active space

## Covalent modification of enzyme action 00:21:47

- 2 types :-
  - Irreversible : Partial proteolysis / zymogen activation
  - Reversible : Addition / removal of a particular group
    - mlc :- Phosphorylation and dephosphorylation
    - Others :-
      - Acetylation
      - ADP ribosylation
      - SUMOylation

## Hormonal regulation of enzyme action 00:24:25

| Insulin | Glucagon |
|---|---|
| • Generally dephosphorylate RLE | • Phosphorylate RLE |
| • Enzyme active under the influence of insulin is active in dephosphorylated state . | • Enzyme active under the influence of glucagon is active in phosphorylated state . |

## Serine proteases & serpins 00:26:27

- In the active site of serine protease , there is :
  - serine
  - Aspartic acid
  - Histidine
- Eg of serine protease :
  - Chymotrypsin
  - Trypsin
  - Elastase
  - Thrombin
  - Plasmin
  - Factor X
  - Factor Xi

Substrate specificity of serine proteases

- Trypsin breaks basic amino acids
- Chymotrypsin breaks bulky amino acids- phenyl alanine, Tryptophan
- Elastase breaks small amino acids ( Alanine )

Serpins

- serine protease inhibitor

  Eg :- Alpha 1 Antitrypsin

## Bi - Bi reaction 00:30:48

- Involves two substrate two product reactions .
- An ordered Bi – Bi reaction .

  Eg :- NAD ( P ) H – dependent Oxidoreductases
- A random Bi – Bi reaction .

Active space

Eg :- Kinases , Dehydrogenases

- Ping - pong mechanism :
  - Transaminase
  - Serine protease
  - GLUT

## Marker enzyme of cell organelle

00:34:38

- Plasma membrane :
  1) 5' Nucleotidase
  2) Adenylyl cyclase
  3) $Na^+K^+$ ATP ase
- Endoplasmic reticulum : Glucose - 6 - phosphatase
- Golgi apparatus : Galactosyl transferase
- Mitochondria : ATP Synthase .
- Lysosomes : Acid phosphatase .

# CLINICAL ENZYMOLOGY

## Functional & non functional enzymes 00:01:23

- Functional Enzyme
  ↓
  has function in the blood
  - Lipoprotein lipase
  - Clotting factors

- Non Functional Enzyme → No function in blood

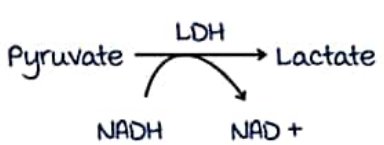
## Isoenzymes 00:04:23

- Physically distinct forms of the same enzyme .
- Catalyse the same reaction .

Properties of isoenzymes

- May be product of different gene .
- May be made up of different subunits . Eg :- LDH, CK
- Different electrophoretic mobility .
- Differ in head stability .
- Km or substrate specificity differ .
- Cofactor requirement varies .
- Different tissue localisation .

## Lactate dehydrogenase 00:09:19




- LDH is tetramer with 2 types of subunits : - H & M

Active space

Isoforms :

Lactate dehydrogenase

| Iso enzyme | Sub-units | Mobility at pH 8.6 | Tissues of origin | % in serum |
|---|---|---|---|---|
| LDH - 1 | $H_4$ | Fastest | Heart muscle | 30 |
| LDH - 2 | $H_3M_1$ | Faster | RBC, Kidney | 35 |
| LDH - 3 | $H_2M_2$ | Fast | Brain, Spleen, Lungs, Lymph node, leukocyte, platelets | 20 |
| LDH - 4 | $H_1M_3$ | Slow | | 10 |
| LDH - 5 | $M_4$ | Slowest | Liver & SK. muscle | 5 |

## Creatine Kinase

00:15:41

- Dimer with two monomer M and B .

Creatine Kinase

| Isoenzyme | Electrophoretic mobility | Tissue of origin | Percentage in blood |
|---|---|---|---|
| CK-1 (BB) | Maximum | Brain | 1 % |
| CK-2 (MB) | Intermediate | Heart | 5 % |
| CK-3 (MM) | Least | SK muscle | 80 % |

## Alkaline Phosphatase - Isoenzymes

00:18:38

1) $\alpha$1 - ALP
2) $\alpha$2 - Heat labile ALP
3) $\alpha$-2 Heat stebl ALP
4) Pre- beta ALP
5) Gamma ALP
6) Leucocyte ALP

1) $\alpha$ - 1 ALP

- Present in biliary canaliculi .
- Elevated in Obstructive jaundice and metastatic Ca liver .

Active space

2) ∝ - 2 Heat labile ALP

- By hepatic cells, ↑ed in hepatitis

3) ∝ - 2 heat stable ALP (most heat stable)

- By placenta, inhibited by phenyl alanine
- Considered as Regan isoenzyme (tumor marker.)

4) Pre-beta ALP

- Present in Bones
- ↑ed in disorder associated with bones

5) Gamma ALP

- Present in intestines.
- ↑ed in ulcerative colitis

6) Leucocyte ALP

- Present in WBC

## Cardiac Biomarkers 00:22:56

1. Creatine Kinase [ CKMB ] → 1st enzyme to rise
2. Cardiac Troponin T [ CTnT ]
3. Cardiac Troponin I [ CTnI ]
4. Brain Natriuretic Peptide [ BNP ]
5. Myoglobin → 1st biomarker to rise (Least specific)
6. Ischaemia modified albumin
7. LDH
8. AST

} less significant cardiac biomarker

| | Rise | peaks | normalise |
|---|---|---|---|
| CKMB | 4-8 hrs | 24 hrs | 48-72 hrs |
| CTn | 4-6 hrs | 24-36 hrs | 3-10 days |

## BNP & Flipped pattern of LDH 00:26:55

BNP : Reliable marker of ventricular volume overload and not MI

Flipped pattern of LDH

(N)
↓
LDH 1 – Heart 30 %
LDH-2 – RBC 35 %
LDH 2 > LDH 1

MI
↓
LDH 1 > LDH 2

## Liver Biomarkers 00:28:35

I) Enzymes whose elevation in serum reflects damage to hepatocyte
- Aminotransferases ( transaminaes )
  ↓
  - ALT → more Specific
  - AST

II) Enzyme whose elevation in serum reflects cholestasis
- $\alpha_1$ ALP
- 5' – nucleotidase – most Specific
- $\gamma$ glutamyl transpeptidase (GGT) – marker of alcoholism

## Viral hepatitis Vs Alcoholic liver disease 00:31:45

- AST : ALT < 1 → VIRAL HEPATITIS
- AST : ALT > 2 → ALCOHOLIC LIVER DISEASE
- GGT is easily inducible by alcohol, elevated in all forms of FATTY LIVER .

## Biomarkers in pancreas, prostate, bone & kidney 00:33:04

Pancreatitis

1) Amylase → Not Specific
2) Lipase → more Specific

Prostate cancer

1) Tartarate Labile Acid phosphatase
2) Prostate specific antigen ( serine protease )

Active space

# UPDATES IN ENZYMES

## Allosteric enzyme v/s michaelis enzyme

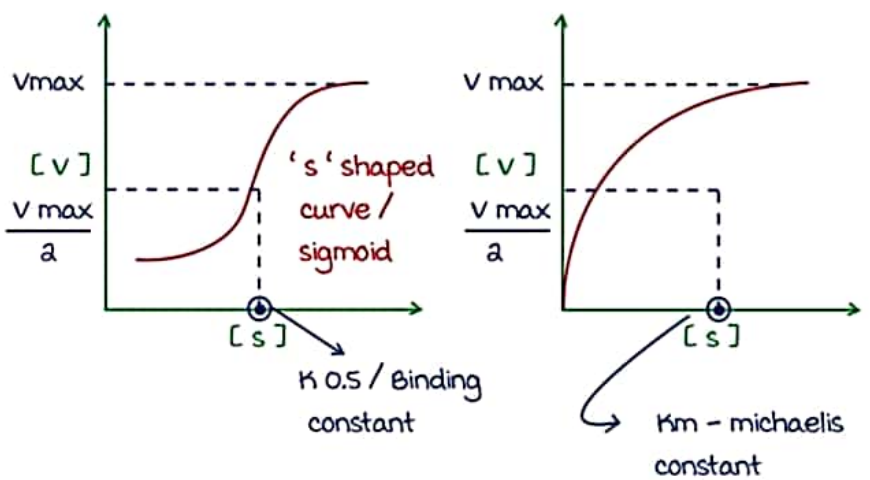
00:00:34

**Allosteric enzyme**

- Whose activity depends upon the presence / absence of an activator / inhibitor
- Velocity v/s substrate concentration

**Michaelis enzyme**

- Usally acts without presence of an activator / inhibitor
- [ v ] v/s [ s ]




- 2 types :

**K . series**

- K 0.5 ↑
- Vmax constant

↓

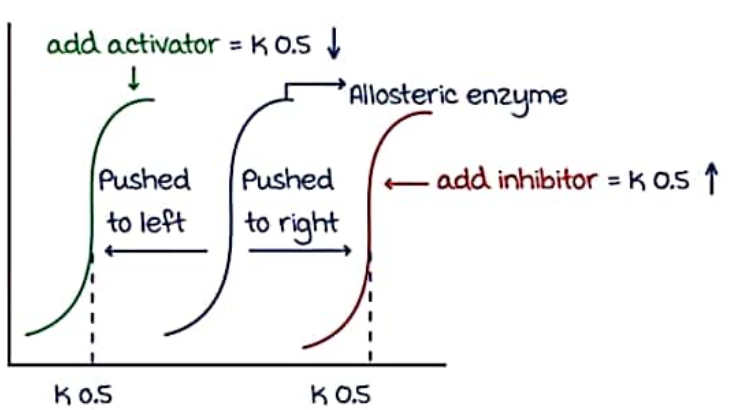
Similar to competitive inhibition

**V . series**

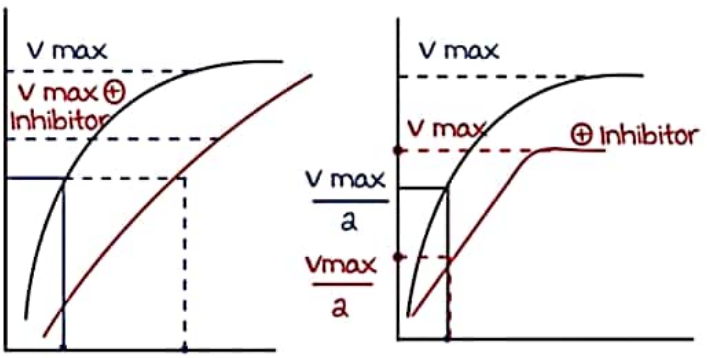
- Vmax ↓
- K 0.5 constant

↓

Similar to non competitive inhibition




Active space

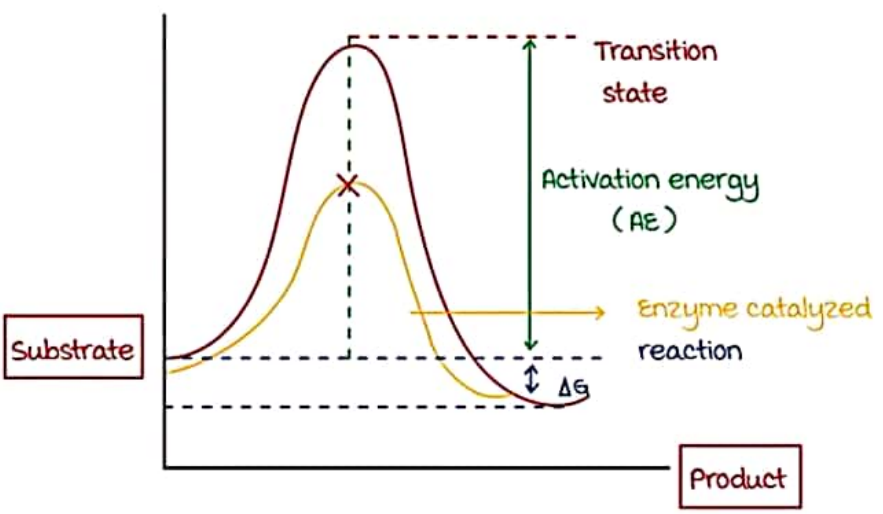



- Km' is ↑
- Km is unchanged
- Vmax ↓

## Lowering of activation energy

00:10:50

- Activation energy → Difference between energy of substrate & transition state




- ΔG → difference between free energy of substrate & product (free energy change)
- In an Enzyme catalized reaction → AE ↓ so that the product is formed. ΔG is unchanged
- If ΔG is ⊖ → substrate is converted to product

Active space

- Energy Barriers tackled by the enzyme to lower activation energy :
  - Entropy → ↓
  - Desolvation of active site & substrate
  - Proper alignment of substrate with active site
    * Emil Fischer's theory
    * Koshlands induced fit theory : Well accepted

substrate + Active site → enzyme undergo conformational change → snug fit the energy to substrate

    * William Jenk & Linus pauling :

(N) → Stickase (enzyme) + stick (substrate) → less products formed

complimentory to

- Here – The Active site of enzyme is not complementary to stick but complementary to transition state of stick

## Lineweaver burk plot 00:23:20

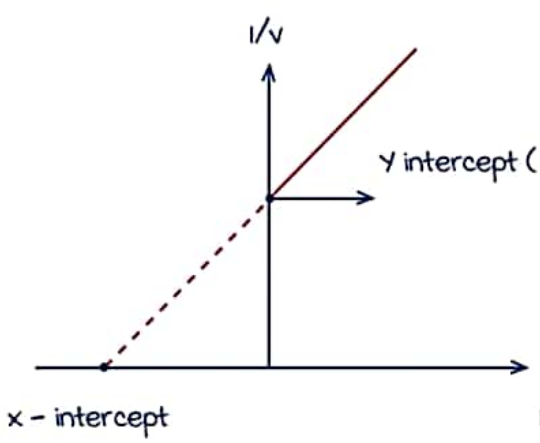
- For enzymes that follow Michaelis Menten Kinetics ,

$$V_i = \frac{V_{max} \times S}{K_m + S} \quad \text{If,} \quad \frac{1}{V_i} = \frac{K_m + S}{V_{max} \times S}$$

$$\frac{1}{V_i} = \frac{K_m}{V_{mx}} \times \frac{1}{S} + \frac{1}{V_{max}}$$

(1/Vi: Variable; Km/Vmx: Constant; 1/S: variable; 1/Vmax: constant)

$y = ax + b$ → Equation of line




* for y intercept
∴ if x = 0
y = b

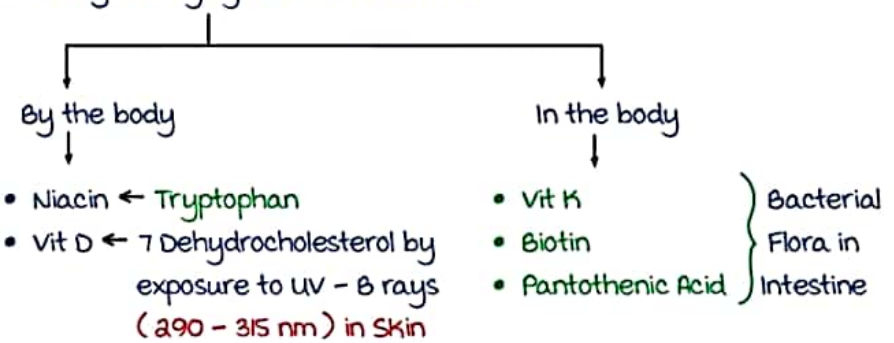
Active space

# VITAMINS : INTRODUCTION

## Definition of vitamins

00:03:16

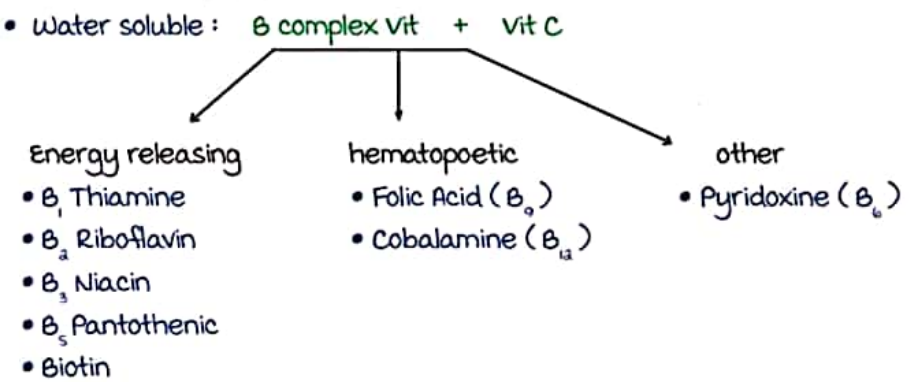
- Organic compounds present in small amounts in various food substances, needed for growth & maintenance of the body.
- dietary essential (not synthesised in the body)
- Endogenously synthesized vitamins




## Classification of vitamins

00:09:34

- Fat soluble : A, D, E, K
- Water soluble : B complex Vit + Vit C




## Fat soluble vs water soluble vitamins

00:12:34

| | Fat soluble | Water soluble |
|---|---|---|
| Absorption | Chylomicrons | Do not need chylomicrons |
| Storage | Stored in liver, adipose tissue | "Not stored" |
| Excretion | not excreted | excreted in urine |
| Toxicity | Toxic | not toxic<br>Exception : B6 & Niacin |
| Function | Varied<br>(Vit K has Coenzyme role) | Coenzyme function |

Active space

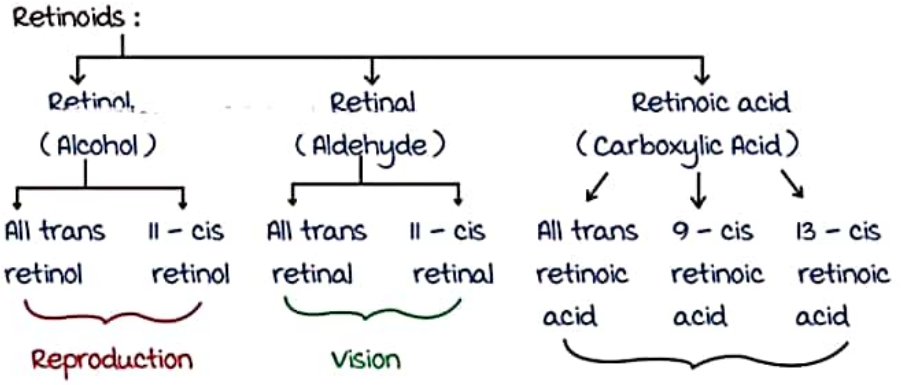
# VITAMIN - A

## Retinoids & Carotenoids : Introduction 00:06:11

| Retinoids | Carotenoids |
|---|---|
| • Active vit. A | • Provitamin A |
| • Animal sources | • Plant sources |
| • Compounds which are chemically related to Retinol | • Most prevalent carotenoid is β carotene |
| | • Richest source : carrot |

## Different retinoids & carotenoids 00:10:01

- Carotenoids :
  - β Carotene :- Antioxidant
  - Lutein, Zeaxanthin :- Rx of macular degeneration
  - Lycopene :- Rx of prostate cancer
- Retinoids :




Regulation of gene expression
- Growth & development
- Tissue differentiation

## Structure of Vitamin A 00:15:56

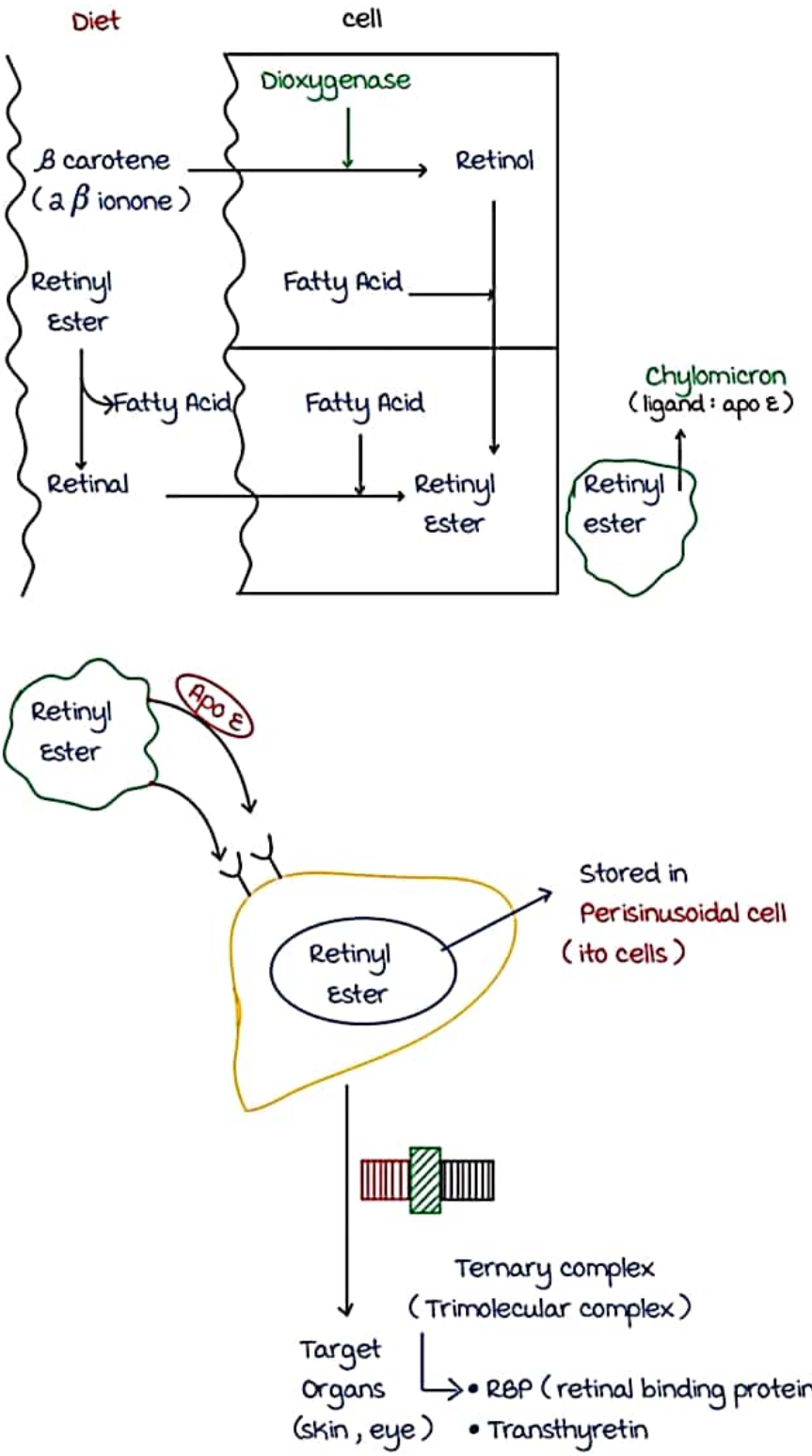
β – ionone ring " single " + isoprenoid chain

( β – carotene → a β ionone ring )

Active space

## Metabolism of Vitamin A - absorption & transport 00:17:32

Absorption & transport



## Function of Vitamin A

00:30:30

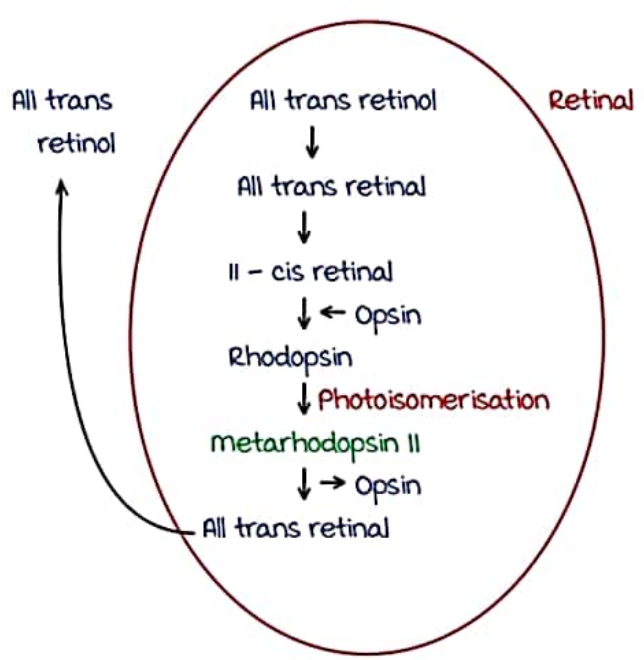
① Vision → 11 cis retinal
② Skin of mucosa → Maintenance of epithelium
③ Reproduction → Retinol
④ Regulation of gene expression → Retinoic acid
⑤ Growth & development
⑥ Tissue differentiation
⑦ Antioxidant vitamin → β . carotene
↓
Photosensitive

## Vitamin A & vision

00:33:47

11 cis retinal + opsin
⇓
Rhodopsin ( Visual purple )

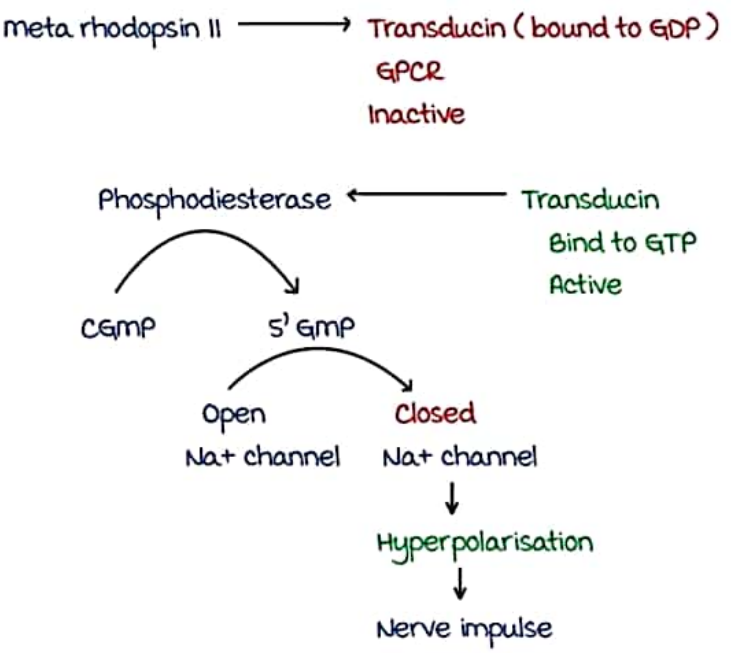
Wald's visual cycle :-




Active space

## Action of Metarhodopsin II

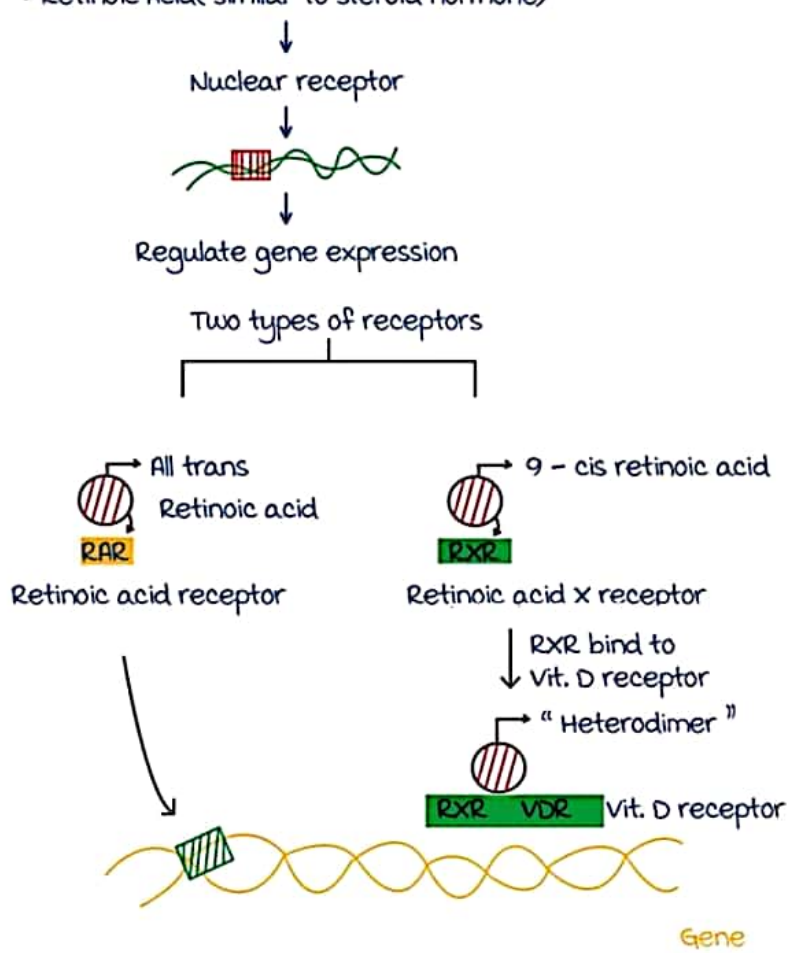
00:39:37




## Regulation of gene expression & Vitamin A

00:43:30

- Helps in growth & development, tissue differentiation
- Retinoic Acid (similar to steroid hormone)




Active space

Vit. D deficiency / Thyroid hormone deficiency
↓
Vitamin A deficiency

## Deficiency of Vitamin A 00:52:45

Eye :
- Loss of vision to green light – Earliest sign
- Earliest symptom – Nyctalopia ( Night Blindness )
- Dryness in conjunctiva & cornea ( Dry eyes – Xerophthalmia )
- Keratitis
- Bitot's spots
- Corneal ulcers ( Keratomalacia )
- MC vit. deficiency
  MCC of preventable blindness

Skin :
- Follicular hyperkeratosis / papular dermatosis / Toad skin ( Phrynoderma ) due to blockage of adnexal glands
- Squamous metaplasia in mucus secreting epithelium → urinary tract, Respiratory tract

## Toxicity of Vitamin A 01:01:45

- Acute :- * Pseudotumor cerebri → headache → Vomiting → dizziness → blurring of vision
  * Exfoliative dermatitis
- Chronic :- * weight loss
  * Nausea, vomiting
  * Bony exostoses
  * Joint pain
  * ↑ Retinoic acid → ↑ Osteoclast → Bone resorption ↓ # of bone ( especially hip bone )

## Therapeutic application of Vitamin A 01:05:39

- β – Carotene → cutaneous photosensitivity

Active space

- All trans retinoic acid (Tretinoin) →
  - Skin ageing
  - mild acne
  - Acute promyelocytic leukemia (Differentiation therapy)

- 13 cis retinoic acid (Isotretinoin) →
  - Cystic acne
  - Childhood neuroblastoma

↓ Teratogenic

## Source & RDA of Vitamin A

01:09:04

- Animal source → Retinoids
  Liver, Egg, Butter, Cheese, milk, Fish, meat

- Richest plant source → carrot
  (β - carotene)
- Richest source → halibut liver oil (fish oil)

RDA

Children → 400mcg / day
men, Women } 600 mcg / day
Pregnancy → 800 mcg / day
Lactation → 950 mcg / day

Active space

# FAT SOLUBLE VITAMINS : D , E & K

## Vitamin D

00:00:43

- Group of sterols which has hormone like action .
- Vit $D_2$ → Ergocalciferol
- Vit $D_3$ → Cholecalciferol
- a/k/a Sunshine Vitamins .
- Except for fish , food unless fortified is a poor source of vit. D .
- Endogenously synthesised vitamin .

## Synthesis of vitamin D

00:04:16

- sunlight
  UVB rays ( 290 nm – 315 nm )

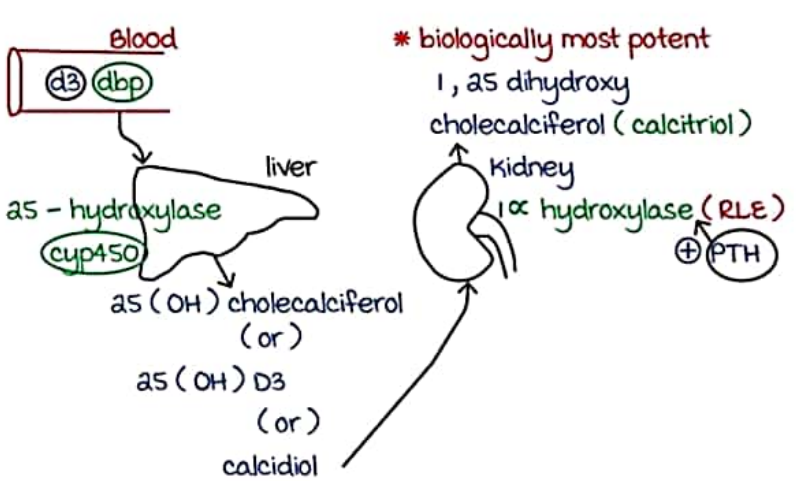
  SKIN

  7 dehydrocholesterol → Cholecalciferol ( 1 – cm gap ) ( Calciol )
  ( Vit $D_3$ )

- D, I, E, T
  Ergocalciferol ( Plant Source ) ( $D_2$ ) → Cholecalciferol ( $D_3$ )
  Cholecalciferol ( fish ) ( $D_3$ )

## Metabolism of vitamin D

00:08:10




Active space

- If the body does not require vit D :


## Functions of vitamin D

00:18:41

1. Regulation of $Ca^{2+}$ and $Po_4^{2-}$
2. Immunomodulatory
3. Antiproliferative
4. Bone development

## Regulation of $Ca^{2+}$ & $Po_4^{2-}$

00:20:11

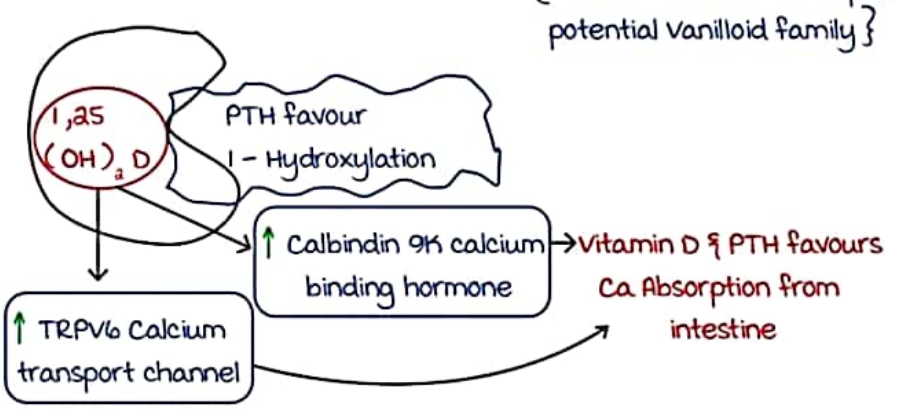
Regulation OF $Ca^{2+}$

1. Vit D
2. PTH ← Parathyroid gland
3. Calcitonin

} Factors regulating $Ca^{2+}$ level in the body → 3 sites : – Intestine, Kidney, Bone

- 1, 25 (OH) 2 D → ↑ serum $Ca^{2+}$ & $Po^{2-}$
- PTH → ↑ Serum $Ca^{2+}$ & ↓ Serum $Po_4^{2-}$
- Calcitonin → ↓ Serum $Ca^{2+}$

{TRPV : – Transient receptor potential Vanilloid family}




Active space

In Kidney :-

- In distal tubules , [ 1, 25 $(OH)_2$ D ], ↑es the level of • Calbindin 28 K
  • TRPV 5
- It favour $Ca^{2+}$ reabsorption of $PO_4^{2-}$ reabsorption .
- ∴ , ↑ed Serum $Ca^{2+}$ & Serum $PO_4^{2-}$

- PTH Kidney :- $Ca^{2+}$ reabsorption
  $PO_4^{2-}$ excretion
  Hence ↑ Serum $Ca^{2+}$ & ↓ Serum $PO_4^{2-}$

In Bones :-

- Osteoblast has RANK ligand .
- PTH
  1,25 $(OH)_2$ D } ⊕⊕ Rank ligand
- Pro osteoclast has receptor for RANK ligand

↓

Mature osteoclast

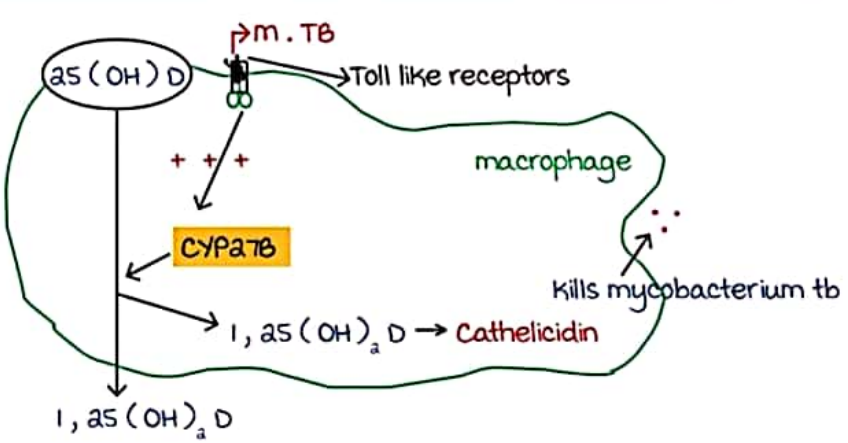
↓

Bone resorption ( ↑ Serum $Ca^{2+}$)

- Calcitonin → ↑ osteoblast activity ( ↓ Serum $Ca^{2+}$)

## Immunomodulatory action of vitamin D

00:34:38




## Anti proliferative function of vitamin D

00:38:30

- Ideal level of 1 , 25 $(OH)_2$ D : 20 – 100 ng/ml
- 1 , 25 $(OH)_2$ D level less than 20 ng/ml is associated with ↑ed incidence of : • Colorectal cancer
  • Breast cancer
  • Prostate cancer
- Vit D is protective against Pre diabetes & metabolic syndrome .

Active space

## Bone development & vitamin D

00:39:29

- During bone development, it : ↑ Osteoblastic activity
  ↑ Mineralisation of bone
  ↑ Osteocalcin

## Deficiency of vitamin D - Rickets

00:41:26

↓ minerilastion of osteoid

- Before closure of epiphysis → Rickets
  - Skeletal deformity
  - Windswept deformity
  - Rachitic rosary
- After closure of epiphysis → Osteomalacia
  - Craniotabes
  - Harrison groove

Nutritional rickets

Nutritional deficiency of vit D
↓
1, 25 (OH)$_2$ CC ↓↓
Sr. $Ca^{2+}$ ↓↓ → Secondary hyperparathyroidism → ↑ PTH
↑ PTH → ↑ 1 ∝ Hydroxylase
↓
25 (OH) CC → 1, 25 (OH)2 D3 (Normalised)
↓
Normalised Serum $Ca^{2+}$

↑ excretion of $PO_4^{2-}$
↓
Serum PO42- ↓↓

## Sources & RDA of vitamin D

00:52:51

RDA :

- Children : 10 mg / day (400 IU)
- Adults : 5 mg / day (200 IU)
- Pregnancy : 10 mg / day (400 IU)

## Toxicity, Assay & Sources of Vit. D

00:54:01

Toxicity

- 4000 IU
- Infants > 50 mg / day → toxicity

Active space

- Deposition of $Ca^{2+}$ in soft tissues and blood vessels
  (calcinosis) (metastatic calcification)
  ↓
  Contraction of blood vessels
  ↓
  HTN

Assay
- 25 (OH) cholecalciferol (Ideal level : 20 – 100 ng / ml)
- Serum Osteocalcin

Sources
- Sunlight
- Fish

* Richest source : – HALIBUT LIVER OIL

## Vitamin E

00:57:27

Chemistry
- Stereo isomers of tocopherols
- ∝ – TocOpherol → most potent active form.
- Chromane ring + isoprenoid unit

Functions
- Naturally occuring most potent antioxidant vitamin.
  (Chain breaking anti oxidant)
- Lipid phase antioxidant
  ↳ In biomembranes, prevents oxidation of PUFA
- Prevents oxidation of LDL.

## Deficiency of vitamin E

01:02:16

(1) Axonal degeneration → • Posterior column affected
• ↓ Position & vibration sense.
(2) Spino cerebellar symptoms → ataxia
(3) Peripheral neuropathy
(4) Skeletal myopathy
(5) Pigmented retinopathy + ophthalmoplegia.
(6) RBC : – ↑ free radical injury
↓ antioxidants
} Hemolytic anaemia

Active space

Toxicity

- Least toxic fat soluble vitamin.
- ↓ Platelet aggregation.
- Interface with vit K

## Sources & RDA of vitamin E

01:06:29

Sources

- Wheat germ oil
- Cotton seed oil
- Sunflower oil

RDA

- Male : 10 mg / day
- Female : 8 mg / day
- Pregnancy : 10 mg / day
- Lactation : 12 mg / day

## Therapeutic uses of high does of Vit. E

01:06:29

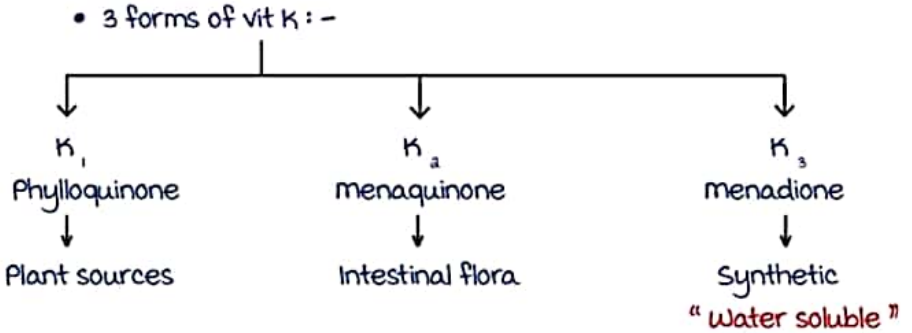
- Retrolental fibroplasia
- Bronchopulmonary dysplasia
- Intraventricular hemorrhage
- Rx for intermittent claudication.

## Vitamin K

01:09:29

Chemistry

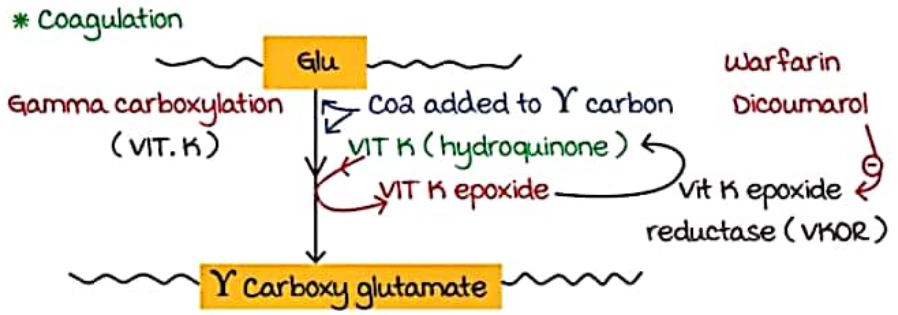
- Naphthoquinone derivative + isoprenoid chain
- 3 forms of vit K : –



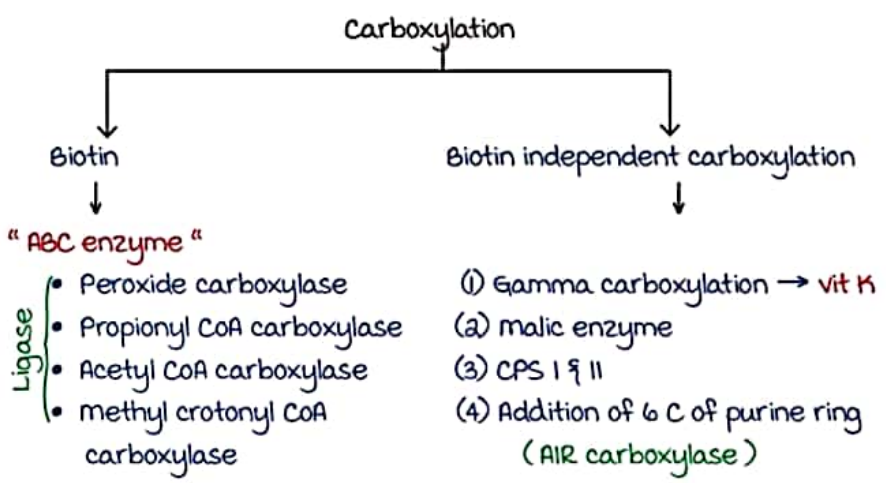

Active space

## Functions of vitamin K

01:12:17




- Gla → Gamma carboxylated glutamic acid
- Gamma carboxylated proteins : -
  - (1) Factor II , VII , IX , X → For $Ca^{2+}$ binding .
  - (2) Protein C , protein S
  - (3) Osteocalcin ( Bone )
  - (4) Nephrocalcin ( Kidney )
  - (5) Product of gas - 6 - gene ( gene arrest specific gene )
  - (6) Matrix Gla protein




## Deficiency & Toxicity of vitamin K

01:24:12

Deficiency

- ↑ Bleeding time
- ↑ Prothrombin time
- Common in premature of neonates : -
  - Immaturity of liver
  - Sterile gut
  - Breast milk poor in vit K
  - Poor placental transport
  - Low body stores

Active space

Toxicity

- Hemolysis
- Jaundice
- Hyperbilirubinemia.

Active space

# WATER SOLUBLE VITAMINS

## Vitamin B1 ( thiamine ) 00:02:23

- Structure – Pyrimidine ring
- Source – Unpolished rice / wheat, Parboiled rice, yeast

↓

Thiamine is present in Aleurone layer
( between white and brown layer of cereals )

↓

required for carbohydrate metabolism

Coenzyme – role

- Thiamine active form – Thiamine pyrophosphate ( TPP )

or

Thiamine Diphosphate

1) Pyruvate dehydrogenase
2) ∝ – Ketoglutarate dehydrogenase
3) Branched chain Ketoacid dehydrogenase
4) Transketolase ( non oxidative phase of HMP )

} Require – TPP

## Deficiency of Vitamin B1 00:08:18

1) Wet beriberi – associated with cardiovascular manifestation

2) Dry beriberi –
affects peripheral nervous system and central nervous system

↓

Symmetric motor and Sensory neuropathy

↓

- Pain, paresthesia, loss of reflexes especially in lower limbs
- Muscle cramps
- Severe cases – muscle atrophy

3) Wernicke's encephalopathy – in central nervous system

↓

Horizontal nystagmus
ophthalmoplegia(ptosis)
Truncal ataxia
Confusion

Active space

4) Wernicke's – Korsakoff syndrome

↓

Wernicke encephalopathy + memory loss (dementia)
Confabulatory psychosis

Nerve conduction and Thiamine

- Thiamine phosphorylates chloride channels in nervous system

↓

helps in nerve conduction

## Biochemical assessment of thiamine deficiency 00:13:17

1) Erythrocyte – Transketolase
2) Urinary – thiamine

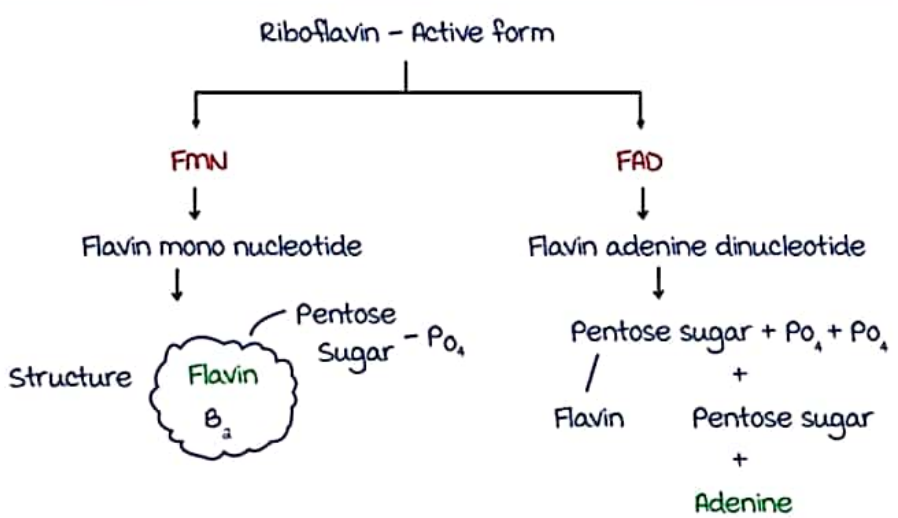
Required daily allowance (RDA)

- 1 – 1.5 mg/day

## Riboflavin - Vitamin B2 00:16:43

- Light sensitive vitamin → Supplemented during phototherapy
- Pigmented vitamin – A/K/A Warburg yellow enzyme – gives urine yellow colour.
- $B_2$ (Riboflavin) & $B_3$ (Niacin) – redox vitamins
- Heat stable vitamin – Cooking food will not destroy

## Co-enzyme role – Riboflavin 00:19:14




Active space

FMN – coenzyme role

1) Complex I in Electron Transport Chain (ETC)
   (NADH Q oxidoreductase)
   (NADH dehydrogenase)

2) L. Amino acid oxidase

FAD – coenzyme role

1) Succinate dehydrogenase (Complex II in ETC)
2) D. Aminoacid oxidase
3) Acetyl CoA dehydrogenase
4) Xanthine oxidase

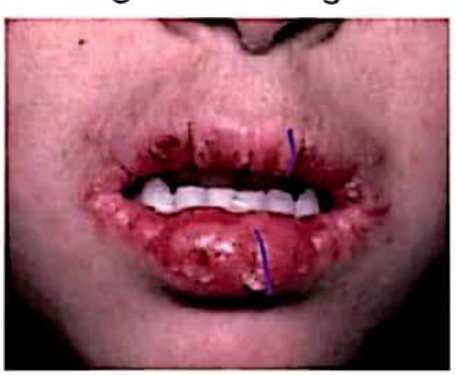
**Warning** : Not all points are covered in the notes, especially conceptual explanations. Please use the notes in conjunction with Marrow Edition 4 videos.

## Riboflavin - deficiency

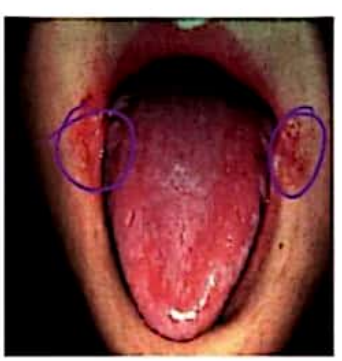
00:24:32

- Initially asymptomatic

1) Cheilosis
   - Pallor in angles of mouth
   - ↓
   - Thinning and maceration of epithelium
   - ↓
   - Fissuring extends radially to skin

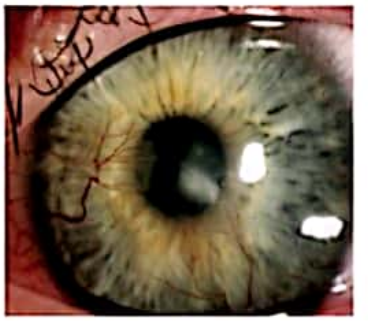


2) Glossitis
   - Magenta coloured tongue
   - Tongue becomes smooth, loss of Papilla



Active space

3) Eyes
- Keratitis
- Conjunctivitis
- Photophobia
- Lacrimation
- Corneal vascularisation



4) Other features
- Seborrheic dermatitis
- Normocytic normochromic anemia

## Biochemical assessment of Riboflavin 00:28:02

- Erythrocyte – Glutathione reductase
  ↓
  by providing FAD invitro

- Urinary excretion of riboflavin

RDA
- 1.5 mg/day
- No reported toxicity

## Niacin / Nicotinic Acid - Vitamin B3 00:29:25

- An endogenously synthesised vitamin from an amino acid (Tryptophan)

Coenzyme role
- Niacin – active form

| $NAD^+$ | $NADP^+$ |
|---|---|
| Nicotinamide adenine dinucleotide | Nicotinamide adenine dinucleotide phosphate |

$NAD^+$ Coenzyme role
- Every dehydrogenase require $NAD^+$

↓

Expect enzymes that require FAD or $NADP^+$

Active space

## Niacin coenzyme role - NADP+ requiring & NADPH requiring

00:33:08

NADP+ requiring

1) Ist two enzymes of oxidative phase of Hexose monophosphate Pathway - Glucose - 6 - Phosphate dehydrogenase
6 - Phospho gluconate dehydrogenase

2) Cytoplasmic isocitrate dehydrogenase

3) Malic enzymes

NADPH requiring

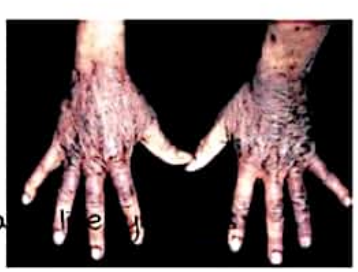
- Almost all reductase require NADPH
  Enoyl reductase, Ketoacyl reductase } for fatty acid synthesis
  HMG CoA reductase - for cholesterol synthesis
  } Reductive biosynthesis of Fatty acid & cholesterol
- Glutathione reductase - free radical scavenging
- Ribonucleotide reductase - conversion of Ribonucleotide to deoxyribonucleotide
- Folate reductase - 1 carbon metabolism

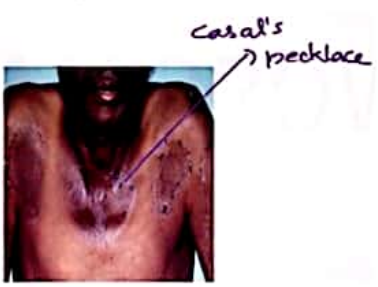
## Niacin - deficiencies

00:38:41

- Initially - Present with vague symptoms .
  ↓
  Progresses to - Pellagra
  ↓
  Cutaneous manifestations
  ↓
  1) Photosensitive dermatitis (Casal's necklace)
  ↓
  2) Diarrhea
  ↓
  3) Dementia
  ↓
  4) Depressive psychosis
  ↓
  5) Advanced cases - Death

Pella






Active space

1) $B_6$ deficiency – inhibit Kynureninase
↓
↓ Niacin

2) Carcinoid syndrome

3) Hartnup's

4) Sorghum vulgare (Jowar) – has high leucine
↓
inhibit Q PRTase

5) Maize / Corn – Niacin is in bound form
↓
Niacytin

- 60 mg of tryptophan synthesize → 1 mg niacin

## Niacin toxicity

00:43:52

- Prostaglandin mediated – Cutaneous flushing
- Most fatal manifestation – Fulminant hepatitis
- Gastric irritation
- Glucose intolerance
- Hyperuricemia

Treatment

- Laropiprant – Prostaglandin $D_2$ inhibitor
- Premedication with Aspirin

Niacin can be used as a lipid modifier drug

- ↓ Triglycerides, ↑ HDL, ↓ LDL

Active space

## Pantothenic acid ( Vitamin B-5 ) 00:46:31

- Contains beta alanine
- " Pantos " - means everywhere
- Endogenously synthesised in intestinal flora

Coenzyme role

Vitamin B5
↓
Present in Coenzyme A → contains beta alanine
↓
Acyl carrier protein
↓
Required for
↓
Succinyl CoA, Acetyl CoA → TCA cycle
HMG CoA → Cholesterol, Ketone body, Leucine catabolism

Deficiency

- Nutritional melalgia / Gopalan's burning foot Syndrome

## Biotin / Vitamin H / Vitamin B7 00:50:39

- Avidin in raw egg - has strong affinity to biotin .

Coenzyme role

Biotin - active form
↓
Carboxy biocytin
↓
required for
ABC enzymes
↓
For carboxylation
↓
1) Pyruvate carboxylase
2) Acetyl CoA carboxylase
3) Propionyl CoA carboxylase
4) methyl crotonyl CoA carboxylase
→ Ligases ↓ require ATP+CO2+Biotin (ABC enzymes)

Active space

Biotin independent carboxylation

1) Malic enzyme
2) Gamma carboxylation ( Vitamin K )
3) 6th carbon in purine ring " AIR carboxylase "
4) Carbomyl phosphase synthetase I & II

Deficiency of biotin

- mental changes ( Depression, hallucination )
- Scaling, seborrheic and erythematous rash around nose eyes and mouth .
- Biotidinase → release active form of biotin

↓

deficiency

↓

Leiner's disease or erythroderma desquamativum

Other uses of biotin

- Streptavidin + 4 biotin

↓

isolated from

↓

Streptomyces avidinii

↓

used for - ELISA test

Biotin labelling of DNA

# HEMATOPOIETIC B COMPLEX VITAMINS

① Folic Acid – $B_9$
↓
derived from " Folium " → rich in Green leafy vegetables
② Cobalamin – $B_{12}$
↓
Animal origin

## Folic acid

00:03:11

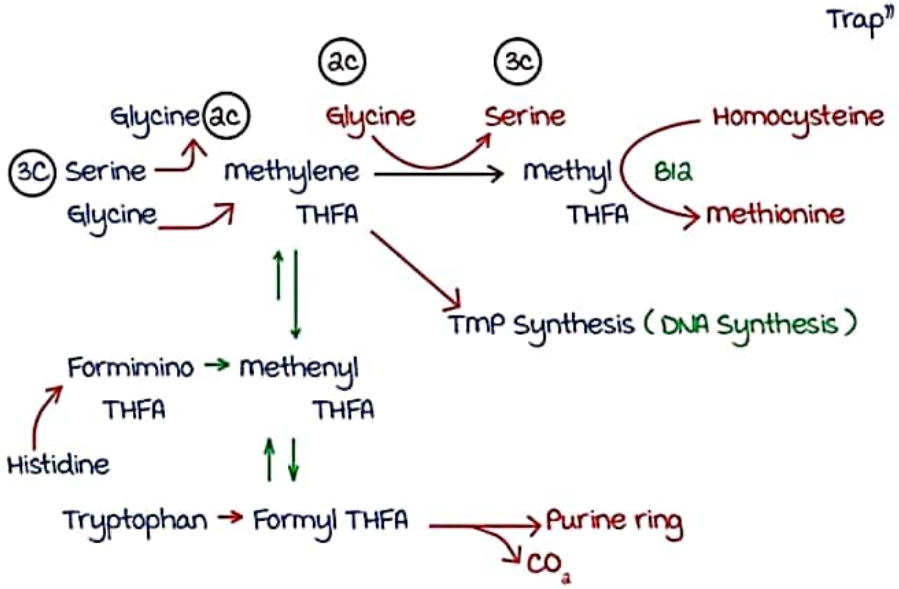
- Active Foam :- THFA
  ↓
  Carrier of 1 – Carbon group

## 1 Carbon group & their metabolism

00:04:49

- methyl $CH_3$–
- methylene – $CH_2$ –
- methenyl – CH =
- Formyl – CHO
- Formimino – CH = NH

" Folate Trap"




Active space

## Folic acid deficiency & assay 00:16:47

- Megaloblastic anaemia
- Accumulation of Homocysteine
  ↓
  Homocysteinemia
  (Homocystinuria)
- Neural tube defects → Spina bifida, Anencephaly

Assay

- Serum Folate
- Red cell folate
- Serum Homocysteine ↑
- Peripheral Smear →
  - hypersegmented neutrophils
  - anisopoikilocytosis
  - Tear drop cells
  - Macrocytes.
- Histidine Load test.

## Folinic acid 00:21:11

- Methotrexate inhibit DHF Reductase +
  ∴, THFA ↓ ↓ ↓ § hence affects DNA Synthesis.
- ∴, We give folinic acid ~ 5 Formyl THFA (stable)
- Leucovorin → Racemic isomer of Folinic Acid

## Cobalamin - $B_{12}$ 00:23:41

- 4 pyrrole rings bound to cobalt (4.35 %)

Coenzyme role "Cobamide enzymes"

↙ ↘

Methyl B12 Ado B12 (Adenosyl B12)

Methyl B 12
↓
- Methionine Synthase / Homocysteine methyl transferase.

Ado B12
↓
- [ methyl Malonate
  Ado B12 ↓ Methyl Malonyl CoA Mutase
  Succinyl Co A
- Leucine amino mutase

Active space

## Metabolism of $B_{12}$ 00:27:44

I) mouth : (Salivary gland) → Cobalophilins / Haptocorrin / R Binders

From diet : Cobalamin + Protein → Pepsin digest the protein of cobalamin is free.

II) Stomach : (Body + Fundus) (Parietal cells) → Intrinsic factor of castle

Cobalamin + Haptocorrin

III) Duodenum : Pancreatic enzyme digest Haptocorrin → Cobalamin + Intrinsic factor of Castle

IV) Ileum : has receptor for Intrinsic factor → Cubulin
∴, gets absorbed.

## $B_{12}$ transport 00:33:29

- Transport protein :-
  - Transcobalamin I ⇝ for cobalamin analogs
  - Transcobalamin II ⇝ most imp

## $B_{12}$ deficiency 00:35:10

- " Folate Trap " → ↓ THFA → ↓↓ DNA synthesis → Megaloblastic anaemia
- methyl malonyl Aciduria
- Homocysteinemia
- ↓ methylation of myelin basic protein → Demyelination → Pyrimidal Tract, Posterior Column

" Subacute Combined Degeneration "

Active space

## Assessment & causes of $B_{12}$ deficiency

00:39:11

- Serum Homocysteine ↑
- Serum Cobalamin ↓
- Serum Folate ↓
- Serum methyl Malonic Acid ↑
- Schilling test
- Peripheral smear → Macrocytes
- Bone marrow study → Megaloblast

Causes

- Nutritional
- Gastric :-
  - Autoimmune gastritis → ↓ Intrinsic factor
    "Pernicious anaemia"
    ↓
    Malabsorption of $B_{12}$
  - Gastrectomy
- Intestinal :
  (1) Ileal resection
  (2) Crohn's disease
  (3) Ileocolic fistula
  (4) Stagnant loop syndrome
  (5) Diphyllobothrium latum (Fish tapeworm)

## Sites of absorption of different nutrients

00:45:07

- Duodenum → Iron
- Jejunum → Folic Acid
- Ileum → Cobalamin

Active space

# VITAMIN B6 AND VITAMIN C

## Vitamin B6 00:01:23

Active form and Co - enzyme role :

→ Pyridoxine ( ring structure )

→ Active form → Pyridoxal phosphate (PLP)

→ Needed for Amino acid metabolism

1) Transamination reaction .

- AST
- ALT
- Glyoxylate → Glycine (Glyoxylate Alanine amino transferase ↓ require PLP .)

Alanine → Pyruvate

In case of PLP deficiency

↓

Glyoxylate → Oxalate

↓

result in " Oxaluria"

2) Amino acid decarboxylation

- Histadine $\xrightarrow{DLP}$ Histamine
- 5-Hydroxytryptophan $\xrightarrow{DLP}$ 5-Hydroxytryptamine ( Serotonin )
- Glutamate $\xrightarrow{PLP}$ GABA ($CO_2$)

## Transsulfuration, Tryptophan metabolism, Heme synthesis and Glycogenolysis 00:05:57

3) Transsulfuration

Homocysteine + Serine

PLP | Cystathionine beta synthase ↓

Cystathionine

PLP | Cystathionase ↓

Homoserine + Cystine

- Transfer of SH group from homocysteine to serine .

Active space

4) Tryptophan metabolism

3-Hydroxykynurenine
PLP ↓ Kynureninase
3-hydroxyanthranilate
Ketogenic fate NADPH metabolism

- In case of PLP deficiency
↓
3-Hydroxykynurenine → Xanthurenic acid
-can lead to pellagra.

5) Heme synthesis.

Succinyl Co A + Glycine
PLP ↓ ALA synthase
delta ALA
↓
Heme

- In PLP deficiency : Microcytic hypochromic anemia

6) Glycogenolysis
↓
- Rate limiting enzyme : Glycogen phosphorylase
- Site : Liver and muscle (80 % stored)

## Vitamin B6 deficiency manifestations 00:12:00

→ Neurological :
- Peripheral neuropathy
- Personality changes (depression & confusion)
- Convulsion

→ Microcytic hypochromic anemia.
→ Pellagra

Urinary analytes excreted in PLP deficiency :
1) Homocysteine.
2) Xanthurenic acid
3) Oxalate (causing oxaluria)

Active space

PLP and hormone dependent cancer :

-Vitamin $B_6$ - inhibit binding of

↓

Hormone receptor complex

to hormone receptor elements .

-In deficiency of vitamin $B_6$

↓

enhanced binding

↓

↑ action of hormone .

Toxicity :

Sensory neuropathy

Biochemistry assay :

- Erythrocyte transaminase
- Tryptophan load test .
- Measurement of PLP in blood .

→ RDA : 1 - 2 mg / day

## Vitamin - C

00:17:42

- Water soluble .
- James Lind

↓

used lemon for treatment

of scurvy .

- Most animals synthesize vitamin C from glucose.
- Humans cannot synthesize vitamin C

↓ due to

absence of " L Gulonolactone oxidase "

Functions : " Hydroxylase "

1) Coenzyme for copper containing hydroxylases .
   - Dopamine $\beta$ hydroxylase
   - Peptidyl Glycine hydroxylase
2) Coenzyme for $\alpha$ Ketoglutarate linked iron containing enzyme .
   - Proline and lysyl hydroxylase .

→ Decreased hydroxylation of proline & lysine

↓

defective collagen → bleeding manifestation

↓

Anemia

Active space

→ Ferrireductase(need Vitamin C)

↓

$Fe^{3+}$ ⟶ $Fe^{2+}$ (ferrous form)

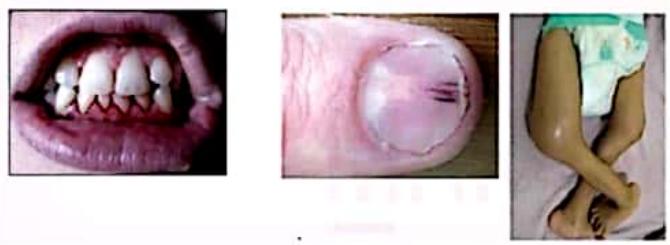
↓

absorbed from intestine

∴ Vitamin C deficiency causes anemia.

## Clinical manifestations of Vit. C

00:23:14



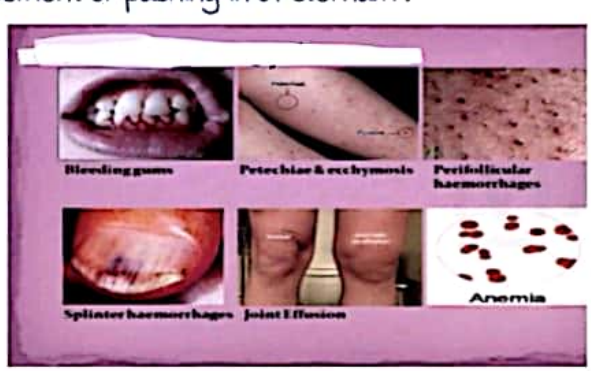
1) Bleeding gums, petechiae, ecchymosis
2) Splinter hemorrhage, perifollicular haemorrhage
3) Hemarthrosis
4) Pseudoparalysis – " Pithed frog leg " appearance
5) Scorbutic rosary

Scorbutic rosary

→ Sharp angulation with or without beading due to backward displacement or pushing in of sternum.

Rachitic rosary

→ Bead like enlargement of costochondral junction




Active space

Infantile scurvy :

→ A/K/A Barlow's disease .

→ Infants between 6 – 12 months,
when weaning starts Vitamin C deficiency occurs .

→ So they should be supplemented with vitamin C sources .

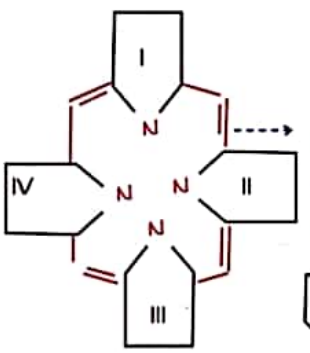
Active space

# HEME SYNTHESIS

Heme — metal + Porphyrin

- metal → $Fe^{2+}$
- Porphyrin → 4 Pyrrole rings joined by methenyl bridges

Pyrrole




= CH –

⇑

methenyl Bridges [∴ one side double bond other side single bond]

→ '5' membered ring

'N' → Nitrogen

## Types of porphyrins

00:03:32

3 types

Based on the side chains

⇓

3 types of porphyrins

U → Uroporphyrin

C → coproporphyrin

P → Protoporphyrin

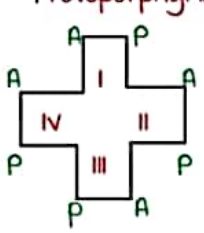
side chains

m → methyl

P → propionyl

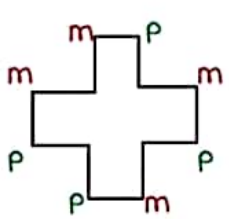
V → Vinyl

A → Acetyl




Uroporphyrin

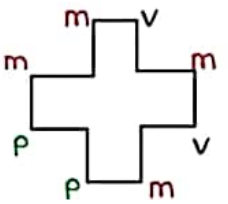
Acetyl – 2 C.




Coproporphyrin [Acetyl – methyl]

methyl – 1 C

Active space




Protoporphyrin
[ Propionyl – Vinyl ]

Different isomeric forms for porphyrin

∴ in that, 2 isomers are important

| Type – III isomers | Type – I isomer |
|---|---|
| • Present in our body<br>• Belong to IX series | • Not normally present in our body |

∴ If Enzymatically Type III isomers not able to be synthesized →
Then, Only Type – I isomer will come .

∴ Heme → $Fe^{2+}$ + Protoporphyrin
→ Ferroprotoporphyrin

## Introduction to heme synthesis 00:11:21

Heme containing proteins

- Hemoglobin
- myoglobin
- Cytochrome P450
- Cytochrome C
- Catalase
- Tryptophan pyrrolase
- Nitric oxide synthase

Site :

All organs [Except – Mature erythrocyte]
⇓
Predominantly
1. Erythroid precursors of bone marrow
2. Liver

Organelle :

Both cytoplasm and mitochondria

Active space

Steps :

1. Synthesis of [ PBG ]
   [ Monopyrrole ]
2. Synthesis of porphyrin
   Uroporphyrinogen → Coproporphyrinogen → Protoporphyrinogen
3. Chelation of iron

Succinyl co-A + Glycine
↓ ALA synthase [ ALAS ]
δ - amino levulinate [ δ - ALA ]

ALA synthase:
- → Rate Limiting step
- → Requires 'PLP' vitamin
- → Two Isoforms

| ALAS-I | ALAS-II |
|---|---|
| - Present in all organ [liver] | - Erythroid precursors |
| - Inducible enzyme ↓sed Heme induces expression of ALAS -I | - Heme + globin ⇓ "Hb" |

- depending upon globin "Hb" will be produced.

2 x δ Aminolevulinate [∴ 2 molecules δ ALA → PBG]
↓ ALA dehydratase [H2O]
Porphobilinogen [PBG] [monopyrrole]

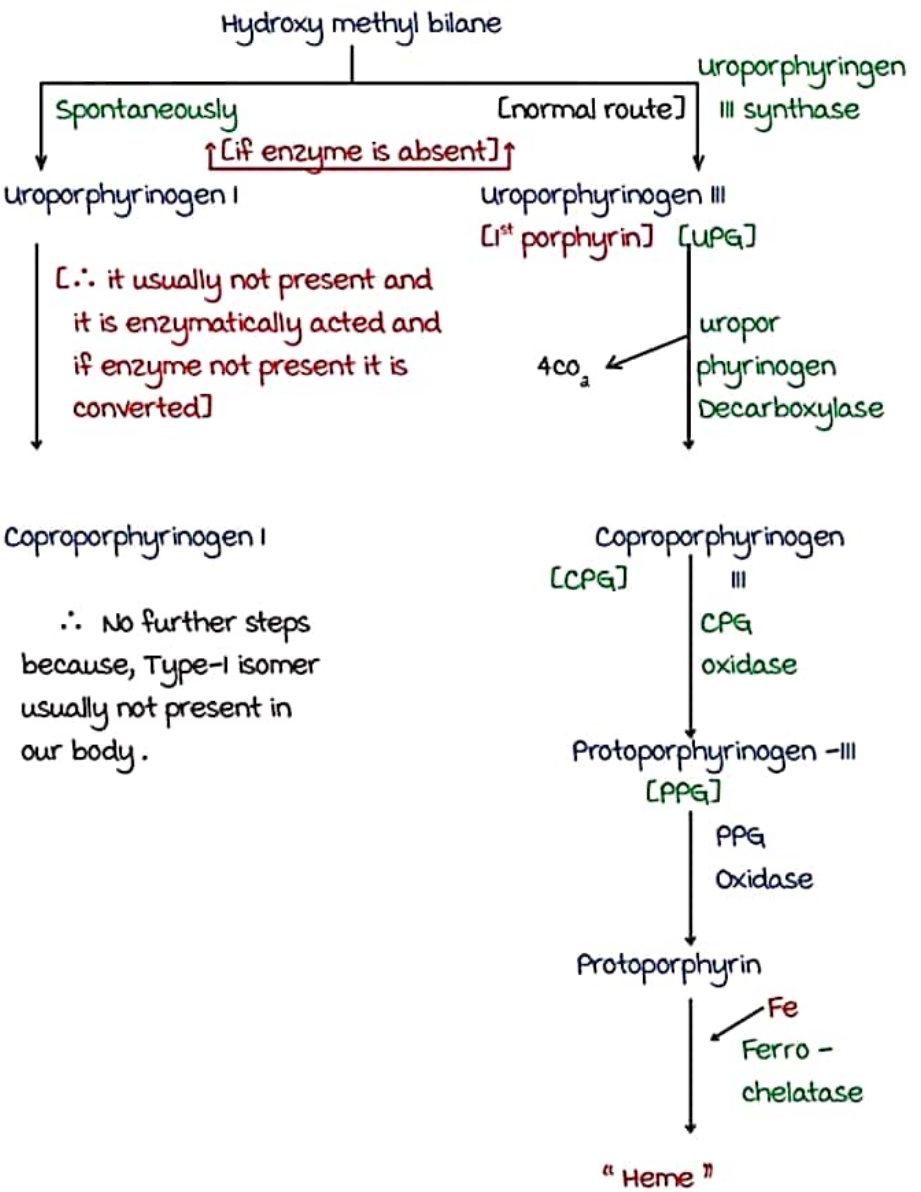
4x Porphobilinogen [PBG] [∴ 4 molecules PBG → HMB]
↓ (4 $NH_3$)
Hydroxy methyl Bilane [HMB]

- PBG Deaminase
- HMB synthase
- Uroporphyrinogen I synthase

Active space

## Fate of HMB and uroporphyrinogen - 1

00:25:11




## Factors affecting heme synthesis

00:36:19

- Lead affect 2 enzymes
  - ALA dehydratase →[Principle lead binding enzyme]
  - Ferrochelatase

- Heme ⟶ Regulator of ALA synthase
  ∴ ↓ ↓ Heme → ALA synthase ↑ ↑ expression
  [gene for ALA synthase is increasingly transcribed]

Active space

- Drugs → Barbiturates ; Griseofulvin → [metabolized in our body using cytochrome]

↓

if that cytochrome are used by these drugs

↓

↓ sed cytochromes [containing Heme]

↓

↓ Heme

↓

↑ ALA synthase I expression

↓

Aggravate "Porphyria". [∴ so, be cautious in using these drugs]

## Lead and heme synthesis

00:40:18

- Lead inhibit ALA Dehydratase

δ ALA [Amino levulinate]

ALA dehydratase (-) [Lead]

↓

Porphobilinogen [PBG]

∴ δ ALA accumulate

↓

Excreted in urine

∴ if PBG is not there ↓ sed Heme

(-) ↓

↓

"Heme"

↑ sed Expression of ALAS -1

## Heme synthesis disorders

00:43:16

Porphyria

- Group of disorders associated with deficiency of enzyme that synthesize "Heme"
- Intermediates of heme synthesis accumulate

## Concept of porphyrias

All porphyrias are autosomal dominant except .

1. X - Linked protoporphyria [XLP]

Active space

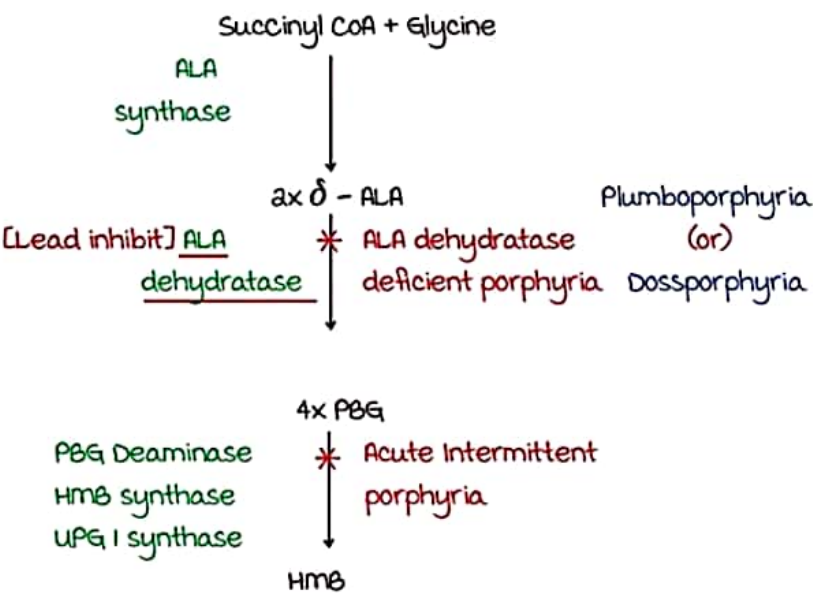
2. Congenital Erythropoietic porphyria [CEP]
3. ALA dehydratase deficient porphyria [ADP]
4. Erythropoietic protoporphyria

Autosomal dominant manifest in [Late Ages]

Autosomal Recessive manifest in [Young Ages]

## Introduction to porphyrias

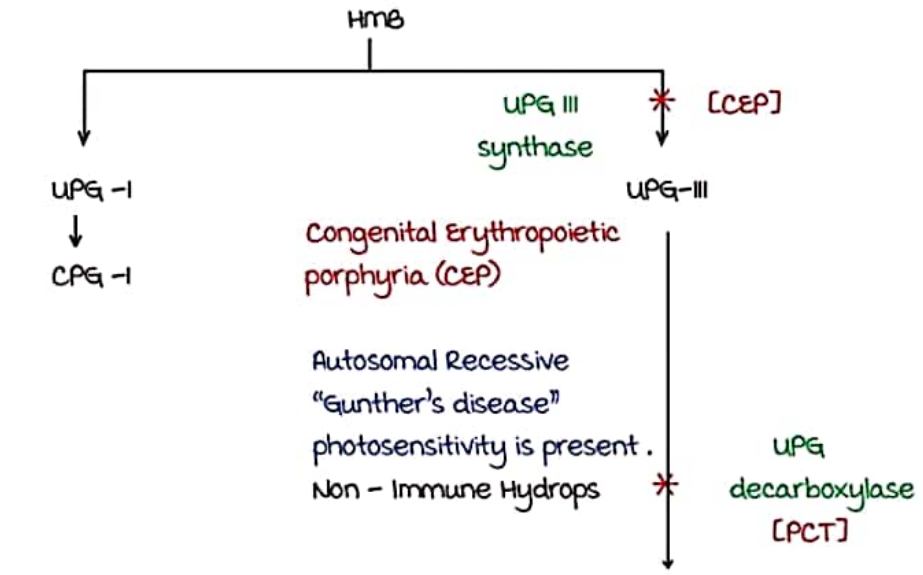
00:47:18




Acute Intermittent porphyria

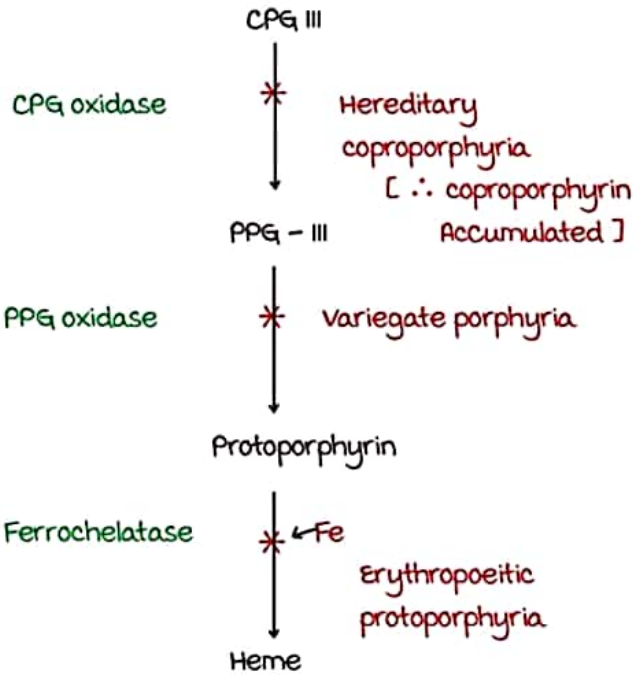
m/C Acute porphyria
manifest as "Pink urine"
m/C symptom Abdominal pain
m/C sign "Tachycardia"
Present as "Neurovisceral symptom"
No photosensitivity

Active space




[Readily Treatable] ← Porphyria cutanea Tarda [PCT] CPG III

m/C porphyria 80% Sporadic
Blistering photosensitivity
m/C sporadic porphyria inhibited by Iron
Associated with "Hemochromatosis"




Erythropoeitic protoporphyria

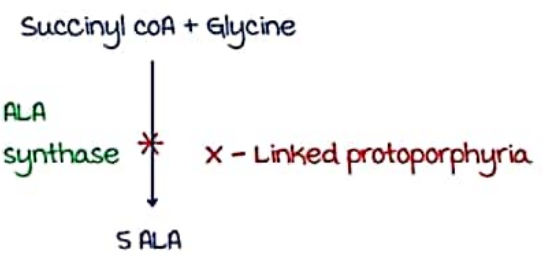
m/C porphyria in children
Autosomal Recessive
photosensitivity present

Active space

[Non - Blistering]

- Skin swelling
- Erythema
- Eczema.

## X-linked protoporphyria and X-linked sideroblastic anaemia 01:04:50



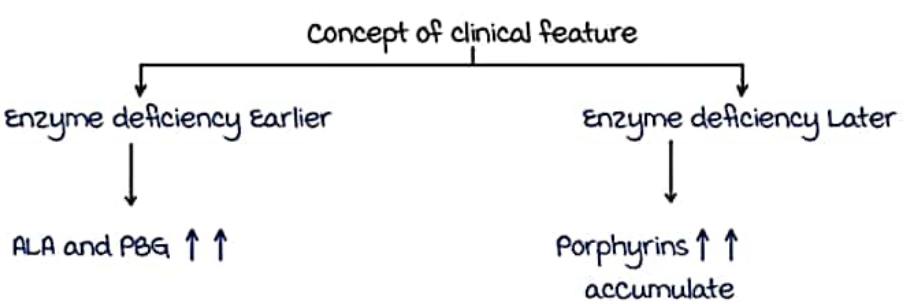

X - Linked Protoporphyria

- "Gain of mutation" Function → ↑sed ALA synthase Expression
- Protoporphyrin Accumulated Because ↑sed expression of ALA synthase all porphyria are synthesised upto protoporphyrin. after protoporphyrin; there is a ↓sed availability of Iron lead to "X - Linked protoporphyria".
- Iron always combine protoporphyrin to form Heme. Iron it is stringently regulated in body. It is not present in Excess level because it is toxic and it will produce Free radical damage.

X - Linked sideroblastic Anaemia

- Due to Loss of Function of ALA synthase

## Clinical features of porphyrias 01:09:04




Active space

Neuro  Visceral

seen in AIP

[Acute intermittent porphyria]

[ So, Photosensitivity seen ]

## Lab diagnosis of porphyrias

01:12:31

1. Illuminate tissue / blood ↑ sed porphyrin

↓

By using Long UV light

↓

Red Fluorescence

2. Ehrlich Test

UBG  PBG

↓

Heme catabolism

∴ PBG accumulate in "AIP"
Ehrlich Test detect "AIP"
by PBG accumulation

3. Watzon Schwartz Test :

To differentiate between UBG, and PBG

4. Soret Band

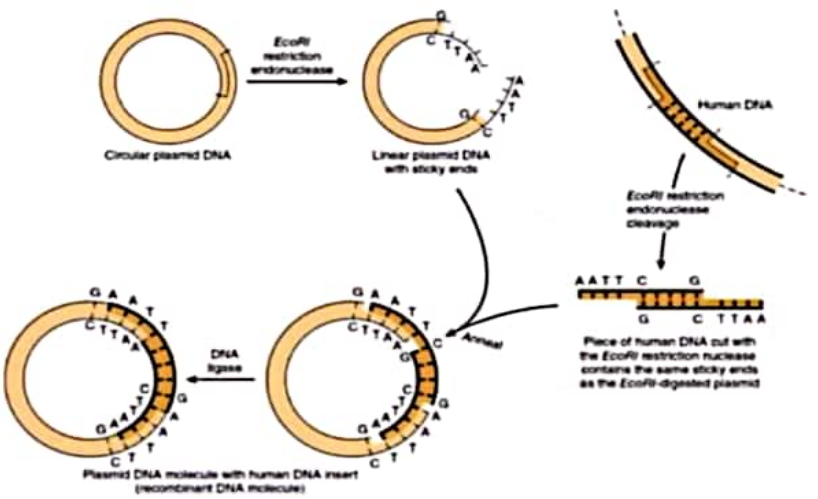
Porphyrins will produce absorption band at "400 nm" called "Soret Band".

Active space

# UPDATES IN BIOCHEMISTRY

## Chimeric DNA

00:00:59




→ Vector DNA Cut and attached to target DNA

↓

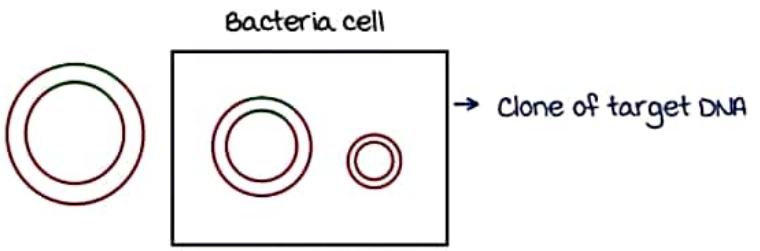
Recombinant / Chimeric DNA

↓

Has its own plasmid DNA ⊕ foreign DNA

Artificial chromosomes :

| BAC | PAC | yac |
|---|---|---|
| ↓ | ↓ | ↓ |
| Bacteria | Phage | - Yeast<br>- most involved<br>- large DNA insertion size |




Active space

## Blue white screening

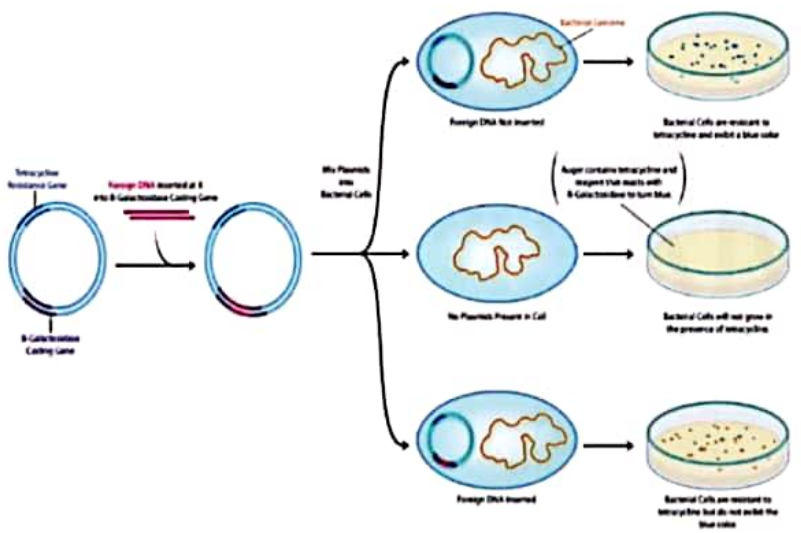
00:05:27

"Plasmid DNA is Complementary to the Carboxyl terminal of β galactosidase enzyme".

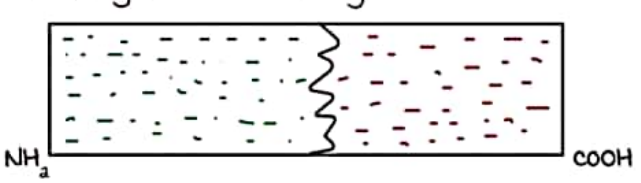
↓

In successful ligation it cannot occur.

→ Based on "principle of complimentation"

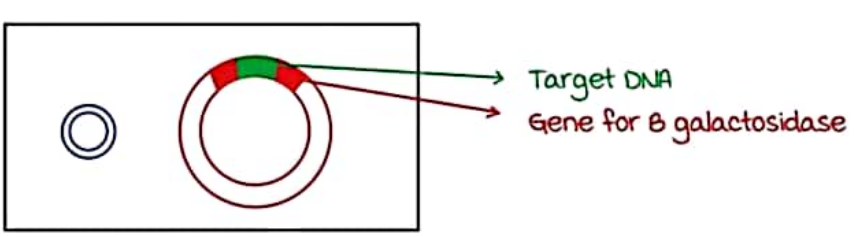


Bacterial β- galactosidase Enzyme




coded by "Bacterial DNA

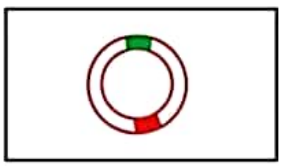
coded by "Plasmid DNA"

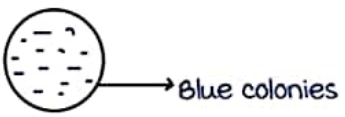



→ In case of defective :- β galactosidase Enzyme is not complete

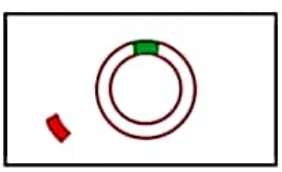

Active space

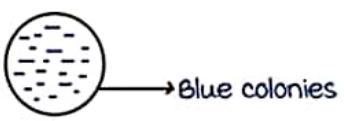
→ (-) β galactosidase
→ Complementation absent
↓
White colonies.
→ Ideal/succesful ligation of target DNA

② 




→ (+) β galactosidase
→ (+) gene coding for β - galactosidase - active
→ (+) Complementation
↓
Blue colonies
↓
Discarded

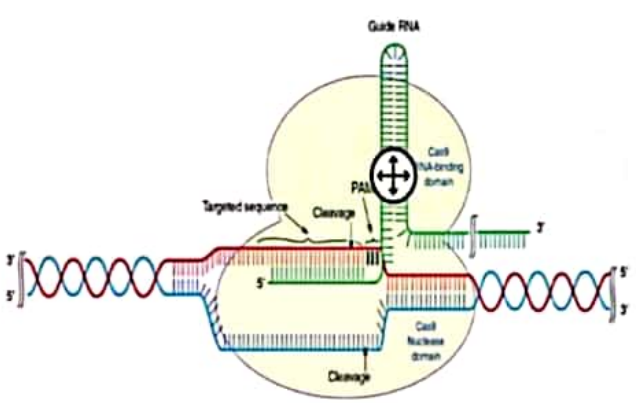
③ 




→ (+) β galactosidase
→ (+) complementation
↓
Blue colonies
↓
discarded

## Novel genome editing mechanism 00:17:15

" CRISPR cas 9 "

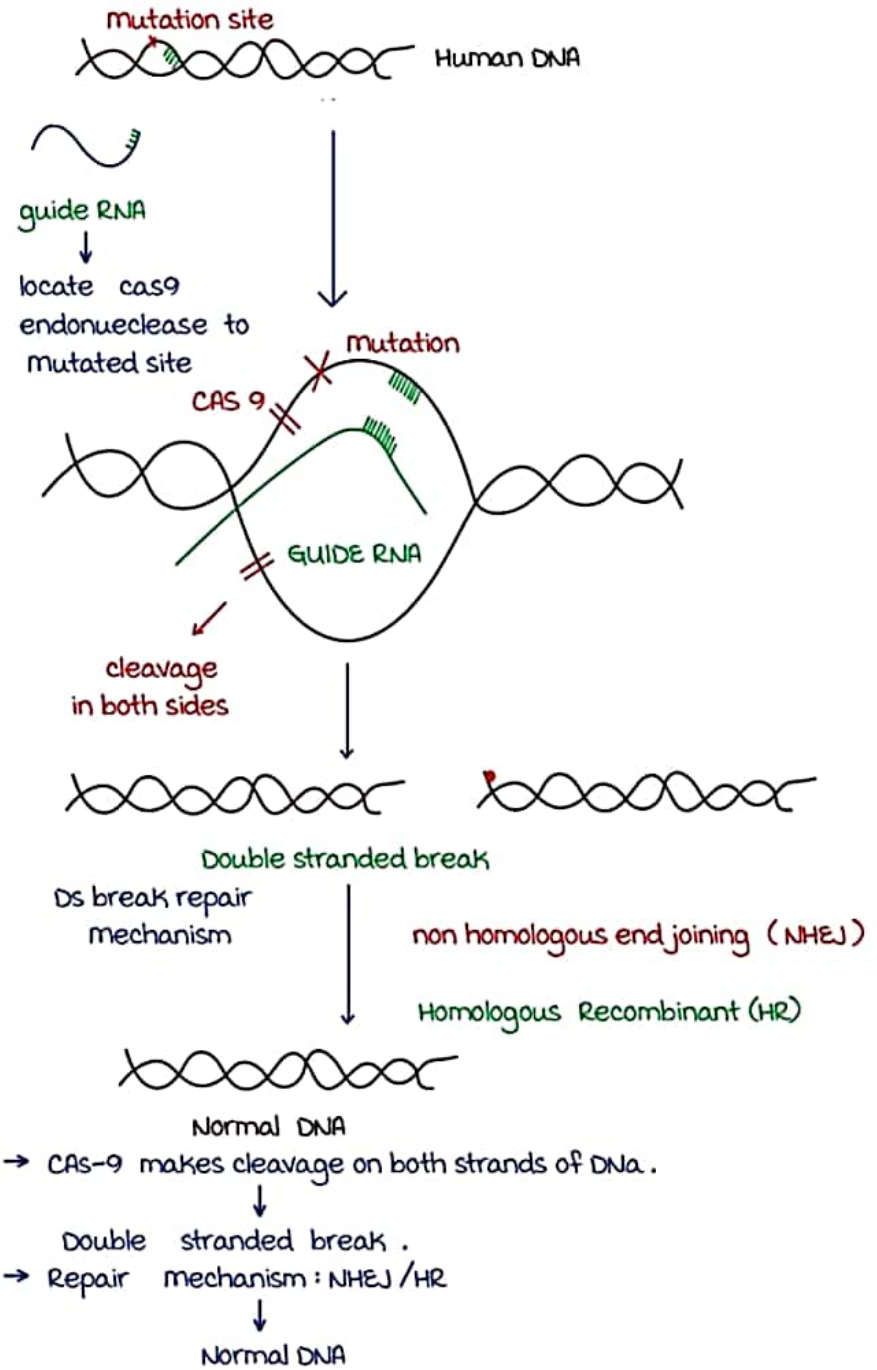



Active space

"CRISPR cas9" gene.

↓

"Defence mechanism in bacteria against phages"
codes enzyme "Endonuclease cas 9"




→ CAs-9 makes cleavage on both strands of DNa.

↓

Double stranded break.

→ Repair mechanism: NHEJ/HR

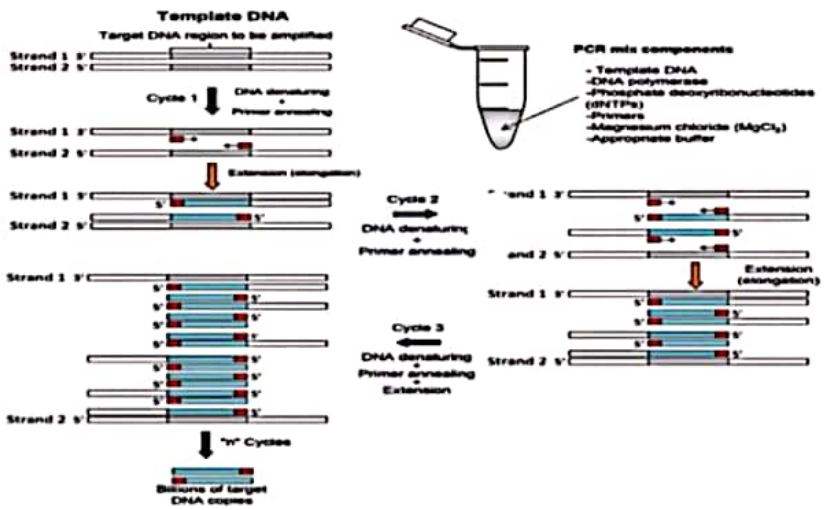
↓

Normal DNA

Active space

## Polymerase chain reaction and hybridoma technique 00:23:59

polymerase chain reaction :

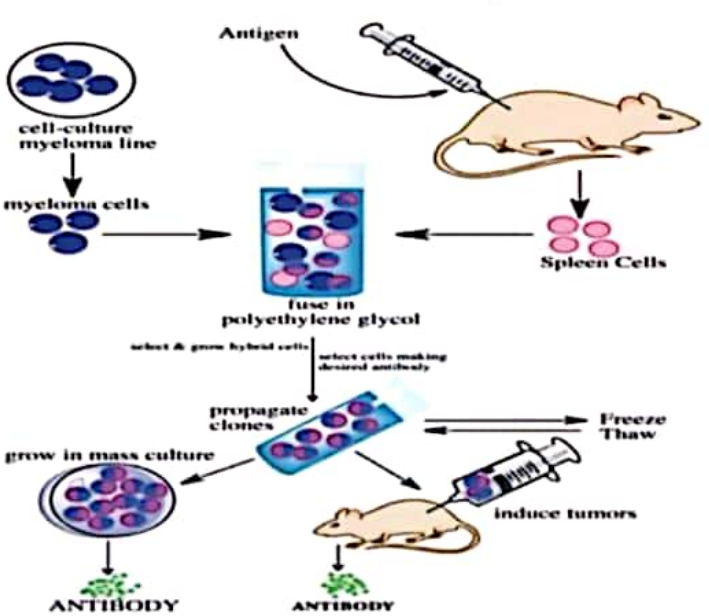



- Inverted by : Dr. Kary B mullis
  Noble Prize 1993
  Hybridoma technique :
- Produce monoclonal Antibiotics

Normal B cells + myeloma Cells

↓ Polyethylene glycol

Fused myeloma Cells.




HAT MEDIUM : [ Hhypoxanthine, aminopterin, thymidine]

- To identify correctly fused cells .

Active space

① unfused cells

B cell — myeloma cell.

- Perish in HAT medium.

② Fused cells.

↓

HGPRTase ⊕ immense replicating potential

↓

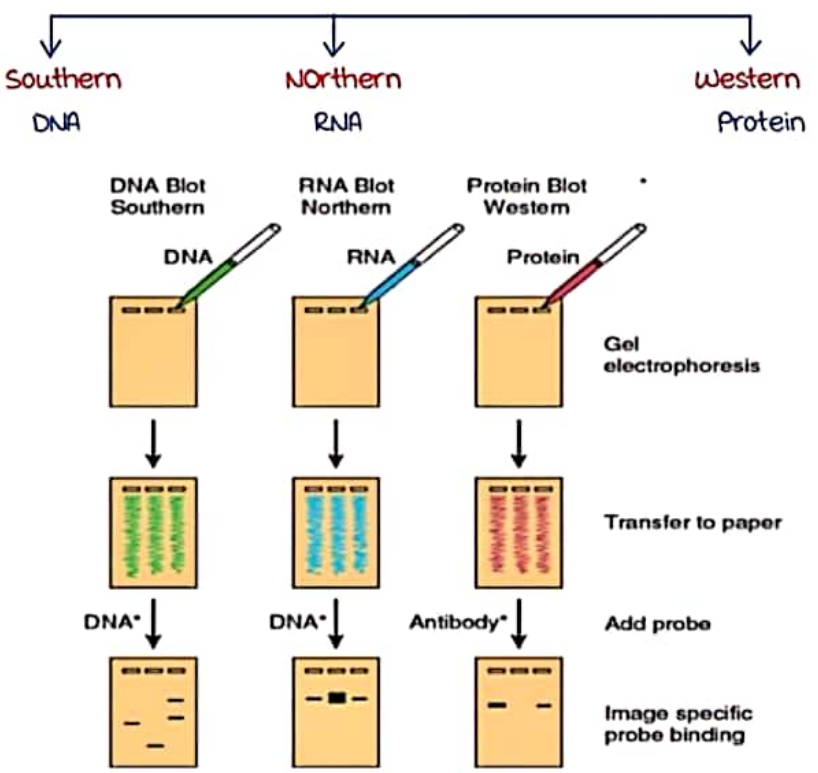
Survive in HAT medium.

→ HGPRTase- Hypoxanthine-guanine Guanine phosphoribosyltransferase

## Blot technique and Ame's test

00:30:54

Blot technique :




overlay :

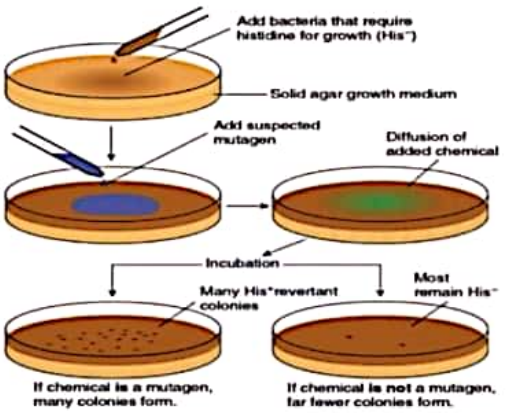
" south western Blot "

" study protein DNA interaction "

-western blot – " Immuno blot "

Active space

Ame's test :




Bacteria : Salmonella Typhimurium

↓

requires histidine to grow

↓

⊕ suspected mutagen ⊕ chemical

↓

Incubated

↓

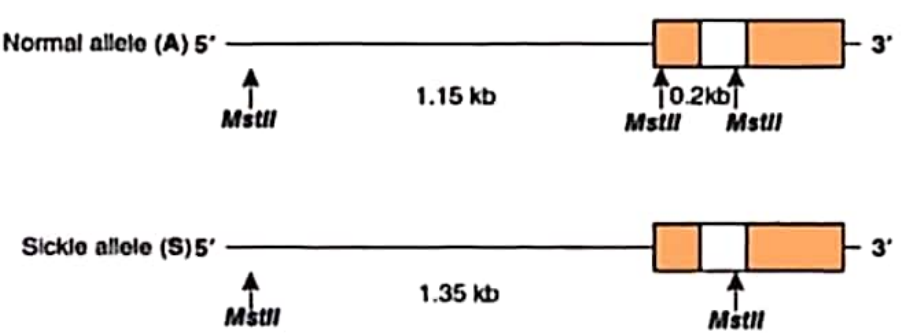
If mutagen → many colonies

not a mutagen → fewer colonies

## Restriction fragment length polymorphism 00:35:32

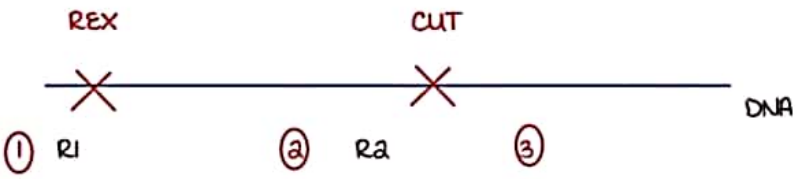
'Analysing fragments which is produced by a restriction endonuclease - unique for a particular allele"

A. MstII restriction sites in and around the $\beta$-globin gene



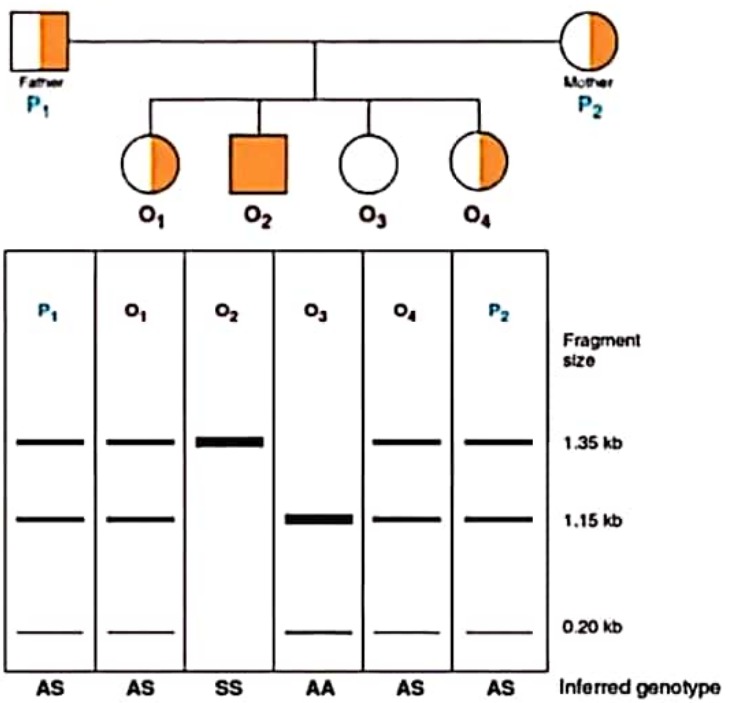

Active space

Pedigree analysis of sickle cell disease.




" Restriction map "

Pedigree analysis of sickle trait mother and sickle cell trait father




→ $O_1$ – $O_4$ : Heterozygous .
- number of bands are same as parent.

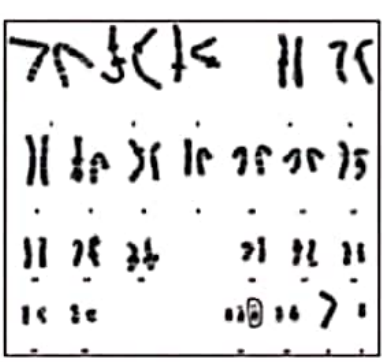
→ $O_2$ : only 1.35 Kb band present .
-Homozygous for sickle cell disease .

→ O3 – Normal child .
- 1. 15 icb, 0.20 Kb seen .

## Karotyping

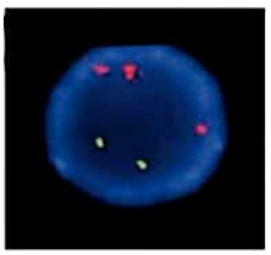
00:42:16

- Karyotyping of trisomy 21



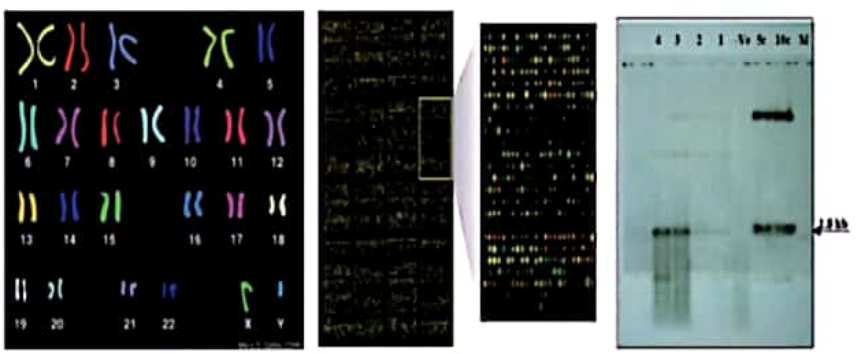
Active space

- Red colour unique for chromosome 21



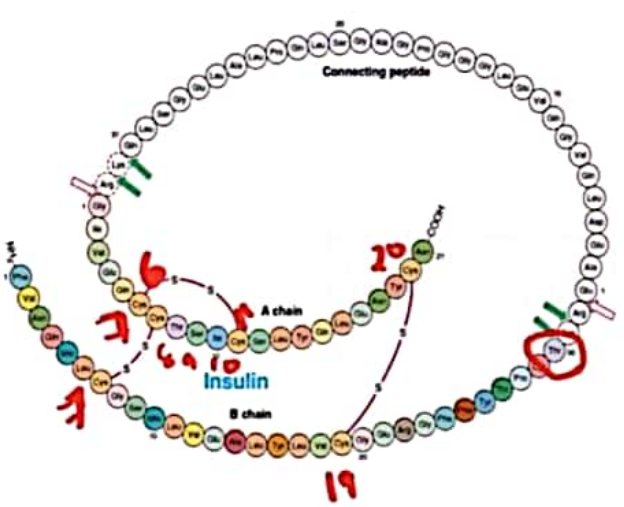
Intraphase FISH

- Aneuploidy can be identified



A B C

| A | B | C |
|---|---|---|
| → Metaphase FISH<br>→ Multicolour FISH Karyotyping<br>→ A chain – 21 aa | → Microarray technique<br>→ Comparing test genome with normal genome | → Electrophoresis of DNA stain ( Ethidium bromide ) used<br>→ done after southern Blotting |




Structure of insulin

Active space

→ A chain – 21 amino acid

B chain – 30 amino acid

→ Collecting peptide – absent in ideal Insulin.

2 Di – Sulphide bonds (between 2 cysteine)

Intra chain

between 6th cysteine and 11th cysteine residue

Intra chain .

(1) between 7th amino acid of A chain and 7th amino acid of B chain.

(2) between 20th aa of A chain with 19th amino acid of A chain

Bovine Insulin :

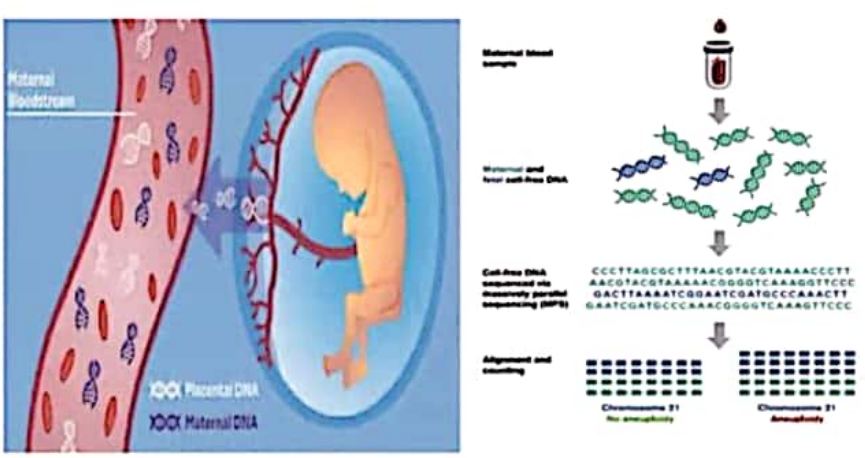
- species variation confined to terminal amino acid of B chain (30th)
- A chain – 8/9/10th amino acid

Porcine Insulin :

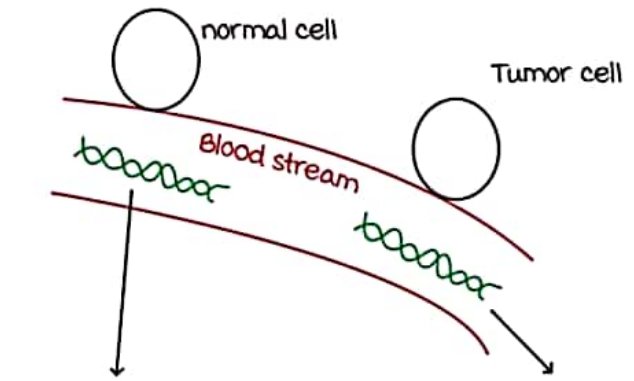
- only in 30th amino acid of B chain.

## Cell free DNA

00:50:17




Active space




→ Based on these patient tailored treatment can be given to cancer patients

→ some foetal DNA enters maternal blood

↓

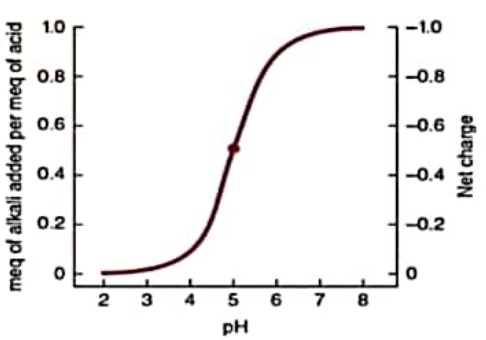
aspirate

↓

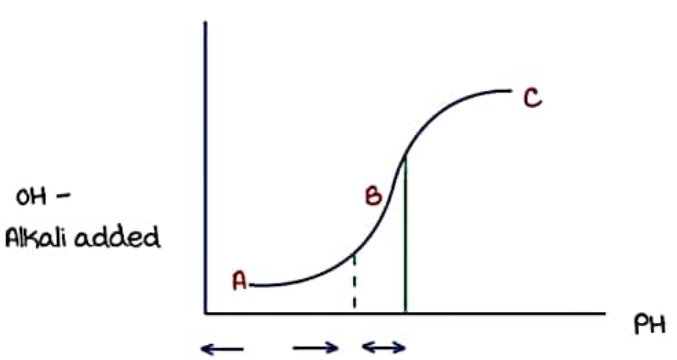
Study DNA of fetus

## Titration curve

00:55:41




**E 2–5 Titration curve for an acid of the type HA.**
e center of the curve indicates the $pK_a$, 5.0.




Active space

- point A – completely unionised.
  point B – midpoint-maximum buffering.
  – Partially ionised

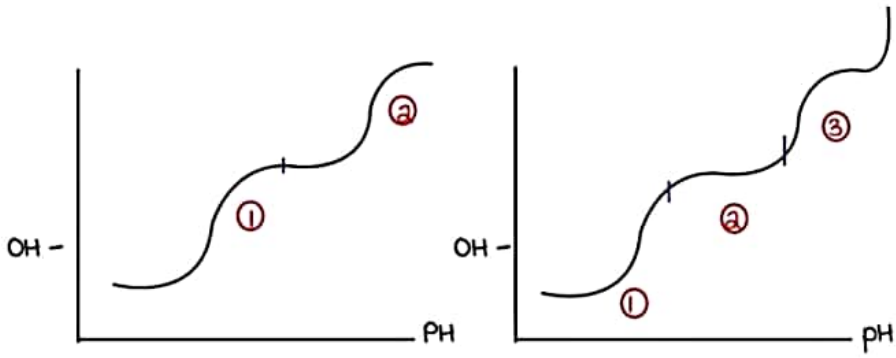
$$[CH_3COO^-] = [CH_3COOH]$$

$$PH = PKa + \frac{\log \text{base } [CH_3COO^-]}{\log \text{ acid } [CH_3COOH]}$$

$$PH = pKa + \log 1$$

$$\boxed{PH = pKa}$$

point C – completely ionised




→ Titration curve of compound with 2 ionisable group

→ titration curve of compound with 3 ionisable group

→ e.g – Aminoacid
– α aminogroup
– α carboxylic group

Active space