Biochemistry

BIOCHEMISTRY OF FED STATE

Fed state/absorptive phase :

- Also called as post prandial state.
- Starts within 2-4 hours of food intake.
- Digestion & absorption of food takes place.
- Plasma level of glucose, amino acids, fatty acids & triacyl glycerol level increases.
- Chylomicrons which carry triacyl glycerol also increases.

Insulin secretion

00:02:45

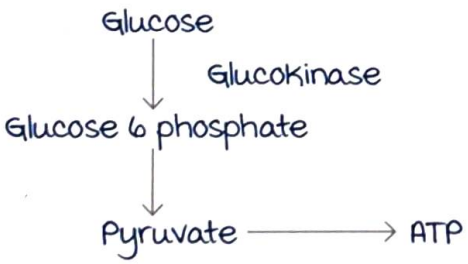
Hormone of fed state : Insulin.

Insulin starts to rise when blood glucose level > 3.9 mmol/ L or 70 mg/dl.

Glucose is transported to beta cells of pancreas through GLUT 2 transporter.

GLUT 2 : Low affinity transporter.

Once inside the beta cells :



ATP/ADP ratio increases which results in closure of ATP sensitive K^+ channels.

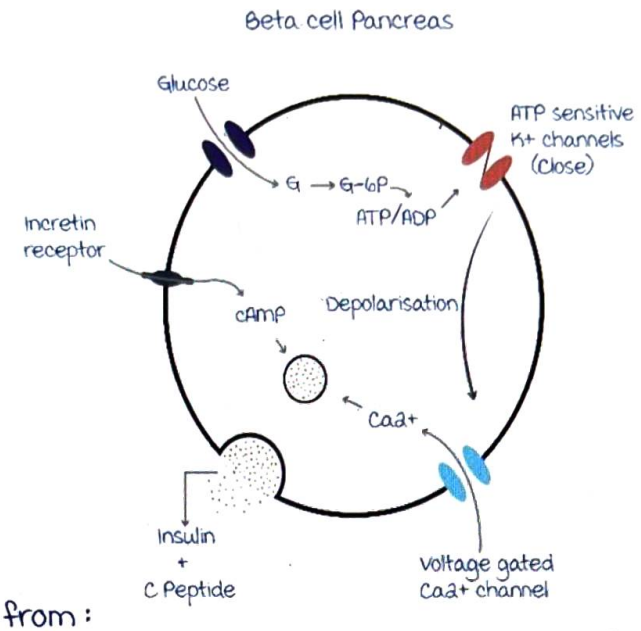
Resulting in depolarisation of membrane.

Opening of voltage gated calcium channels opens, resulting in Ca^{2+} influx.

Calcium stimulate secretory vesicles carrying insulin, resulting in its secretion.

C peptide is co-secreted along with insulin.

Active space



Incretin :

Secreted from :

- Neuro endocrine cells of GIT (L cells).
- Intra islet alpha cells.

2 types :

- Glucose dependent insulinotrophic peptide (GIP).
- Glucagon like peptide (GLP-1).

Action :

- Increases the cAMP levels inside beta cells resulting in secretion of insulin.
- Hence incretin analgoues are used in the treatment of diabetes mellitus.

Mechanism of action of insulin 00:07:53

Insulin binds to insulin receptors (IR), a heterotetramer receptor with 2 types of subunits (2 alpha & 2 beta subunits).

Insulin binds to the extracytoplasmic alpha subunit.

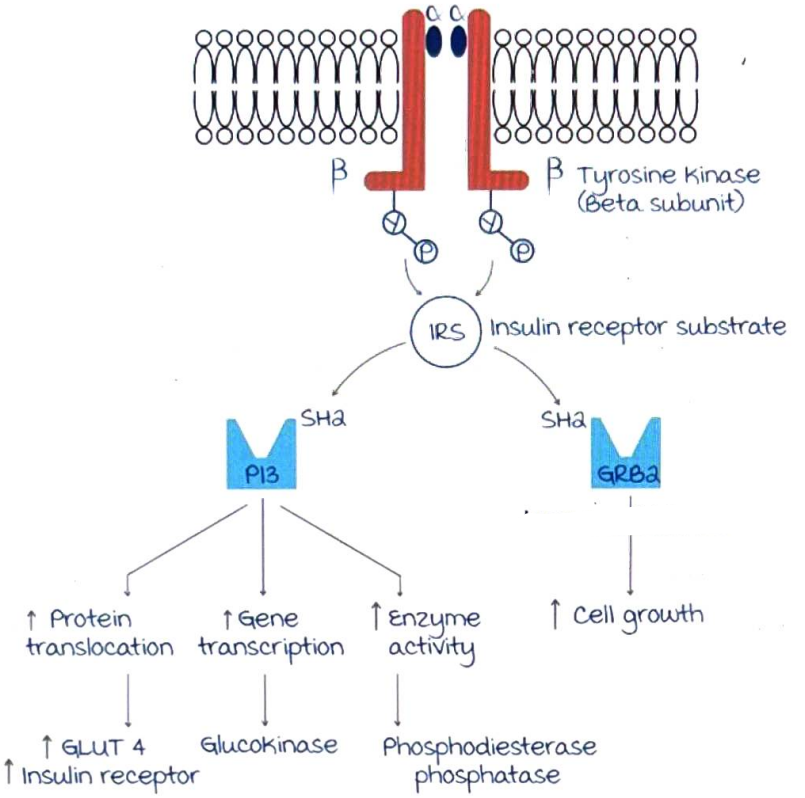
Stimulation of intrinsic receptor tyrosine Kinase in beta subunit.

Resulting in autophosphorylation of tyrosine residues in beta subunit.

Phosphorylation ignites a complex signal cascade.

Stimulates insulin receptor substrate (IRS).

- Binds to SH2 (Src homology 2) domain of PI3 Kinase (phosphatidyl inositol triphosphate) (IRS 1-4).
- Binds to SH2 domain of GRB2 receptor.

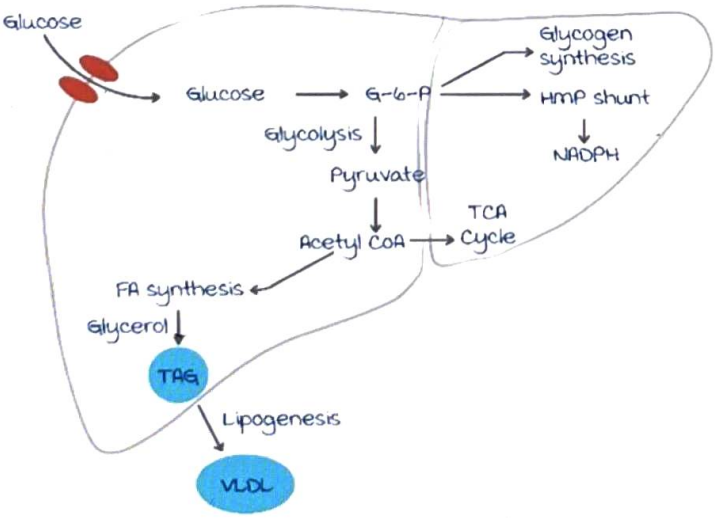


Liver in fed state

00:14:58

Glucose enters liver through GLUT 2 transporter.

Glut 2 : Low affinity transporter.



Amino acids enter into liver and undergoes :

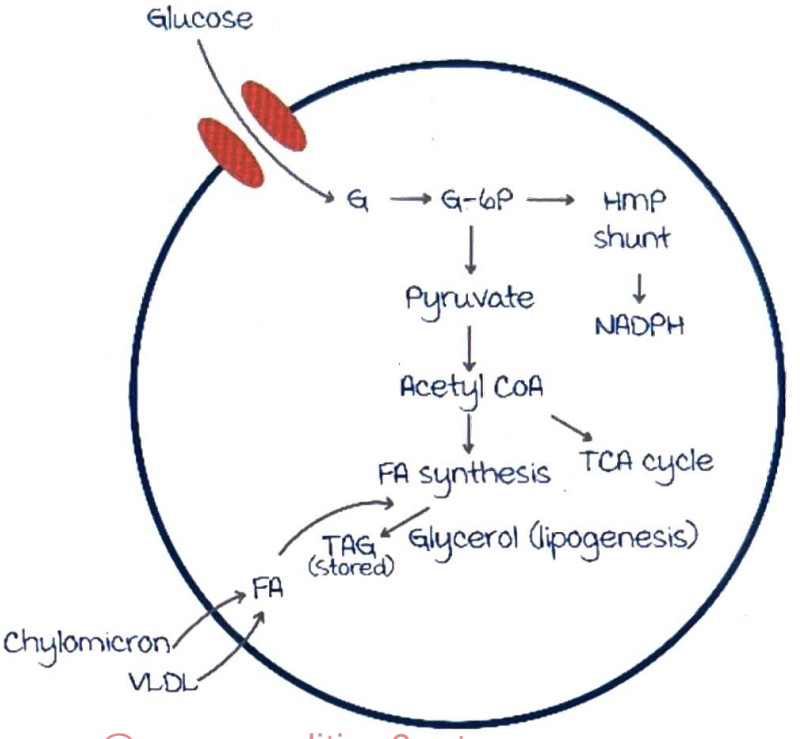
- Protein synthesis (major portion).
- Oxidative deamination.
- Carbon skeleton entering into anabolic fate.

Active space

Adipose tissue in fed state

00:19:58

Glucose enters adipose through GLUT 4 transporter.



In the fed state when there is excess glucose, ~~its transported~~ into the skeletal muscle.

Glucose metabolism in skeletal muscle :

Glucose → Glucose-6-phosphate → Glycogen

Glucose-6-phosphate → Glycolysis → Pyruvate → Acetyl CoA → TCA cycle

Amino acid metabolism in skeletal muscle :

Enters muscle cells and result in preotein synthesis.

Brain :

Two glucose transporters :

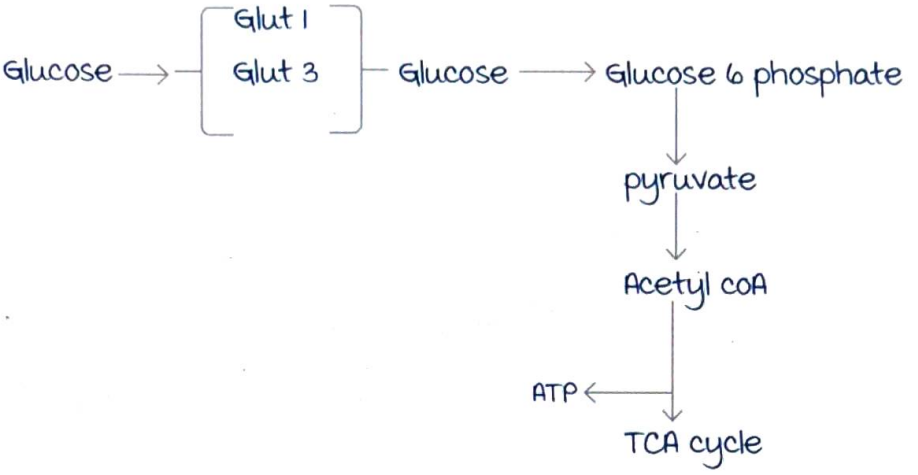
- Glut 1 (blood brain barrier).
- Glut 3 (neurons).

Both are high affinity glucose transporters.

Transport glucose into the brain in all stages.

Active space

Brain depends on oxidative pathways.



Metabolic fuels : Fed state

00:26:42

Organ	metabolic fuel
Brain	Glucose
Liver	Glucose >>> Free fatty acids
Skeletal muscle	Glucose >> Free fatty acids
RBC	Glucose
Adipose tissue	Glucose >> Free fatty acids
Heart	Glucose << Free fatty acids (low glycolytic activity)

- Most organs depends on glucose.
- RBC & brain solely depend on glucose.

Active space

BIOCHEMISTRY OF FASTING STATE

Stages of post absorptive state 00:00:55

Feed fast cycle :

Well-fed state	2-4 hours after food
Early fasting	4-16 hours without food
Fasting	16-48 hours without food
Prolonged fasting (starvation)	2-5 days without food intake
Prolonged starvation	5 days without food intake

Early fasting : metabolic changes :

- 4 -16 hours without food intake.
- Source of blood glucose : Glycogenolysis.
- End product of glycogenolysis is glucose (hepatic glycogenolysis).
- Muscle lacks glucose 6 phosphatase : It cannot be a source of blood glucose in this state.
- Indirectly muscle provides blood glucose.
- In 16-18 hours, glycogen stores are depleted.

Fasting : Metabolic changes 00:06:02

16 hours to 48 hours without food intake.

Source of blood glucose :

Non carbohydrate substrates are converted to glucose (gluconeogenesis).

Source of ATP for gluconeogenesis : TAG gets converted into fatty acids + Glycerol.

Fatty acid undergoes beta oxidation providing ATP.

Glycerol : Non carbohydrate substrate which can be converted into glucose.

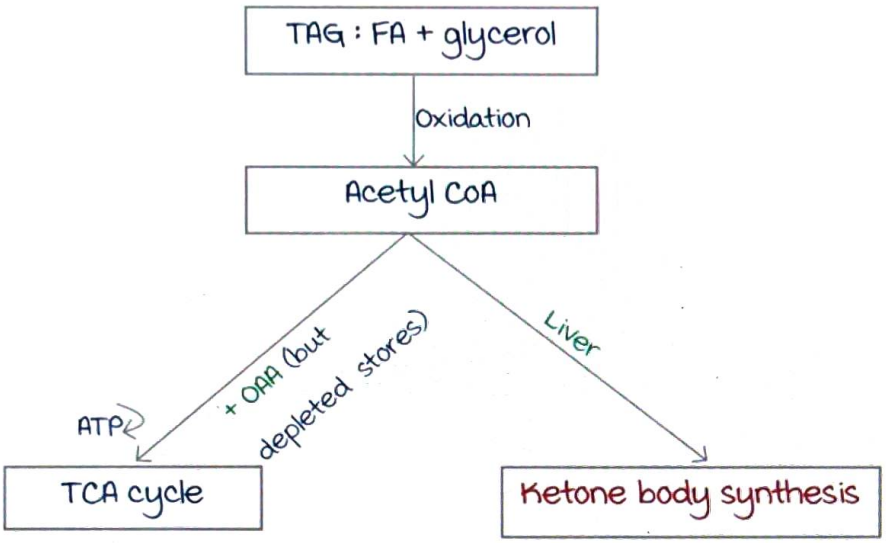
Prolonged fasting/starvation : metabolic changes :

2-5 days without food intake.

Decreased gluconeogenesis.

Active space

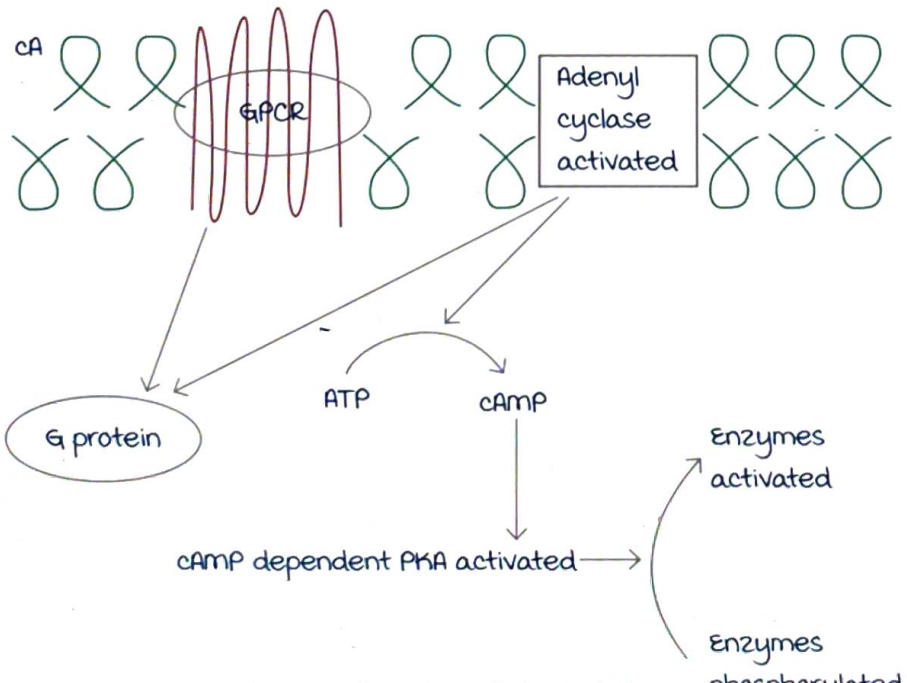
Only source : TAG gets converted into FA + glycerol.



Prolonged starvation : Metabolic changes 00:10:52

more than 5 days without food intake, all TGA stores are depleted, fatty acids are low.
Ketone body synthesis : Decreasing.
Muscle proteolysis → Cachexia.

Mechanism of action of glucagon and epinephrine :
Glucagon is secreted when blood glucose level is <50 mg/dl.

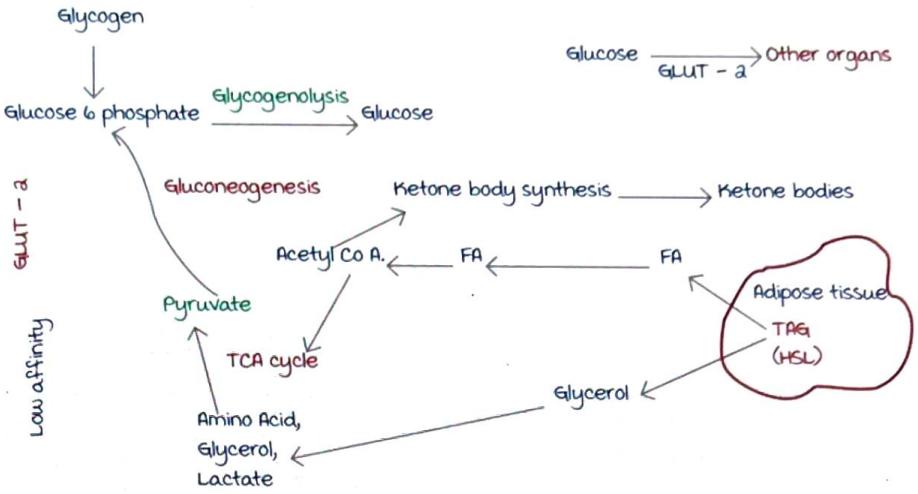


Enzymes are active in phosphorylated state.

Active space

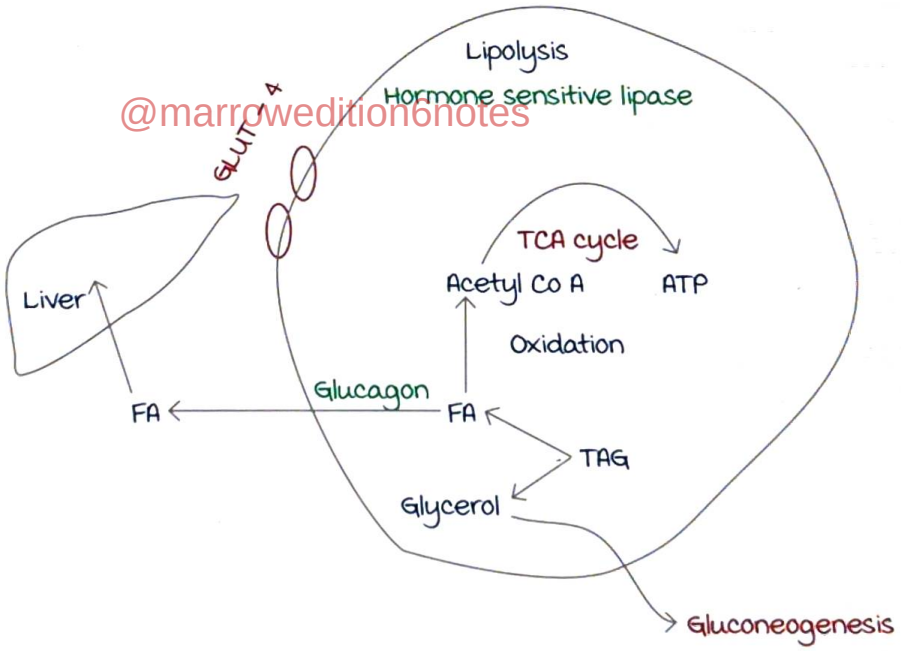
Organ Mapping

00:16:02



Metabolic pathways acting in adipose tissue during various stages of fasting

00:21:28



GLUT-4 : Insulin dependent glucose transporter.
more GLUT-4 in the periphery.
Glucagon cannot translocate through GLUT-4.
There will be less amount of GLUT-4 on the surface of the membrane (not allowing glucose to enter adipose tissue).
Hormone sensitive lipase active under the influence of glucagon.

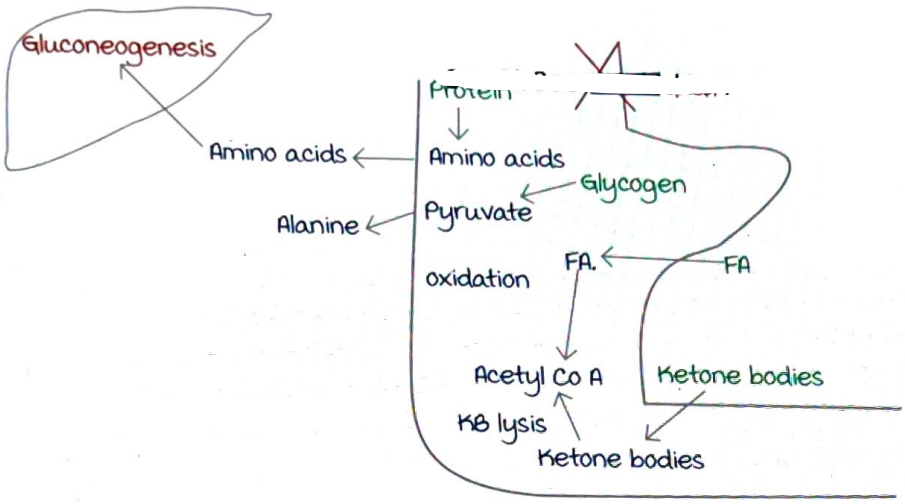
Active space

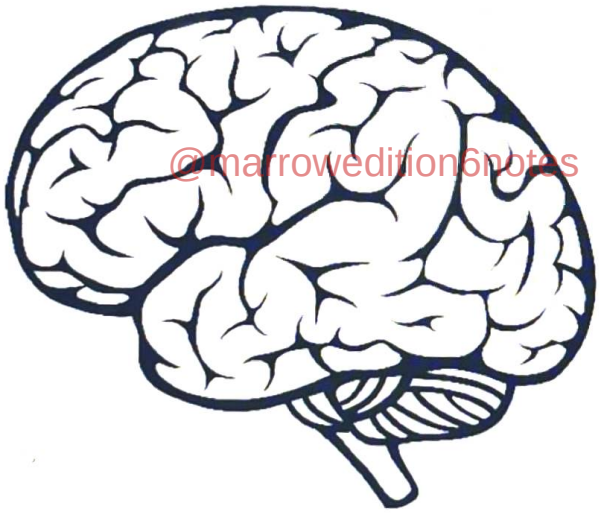
Resting skeletal muscle :

GLUT is GLUT-4.

But cannot act in fasting state.

Amino acid transported as alanine.





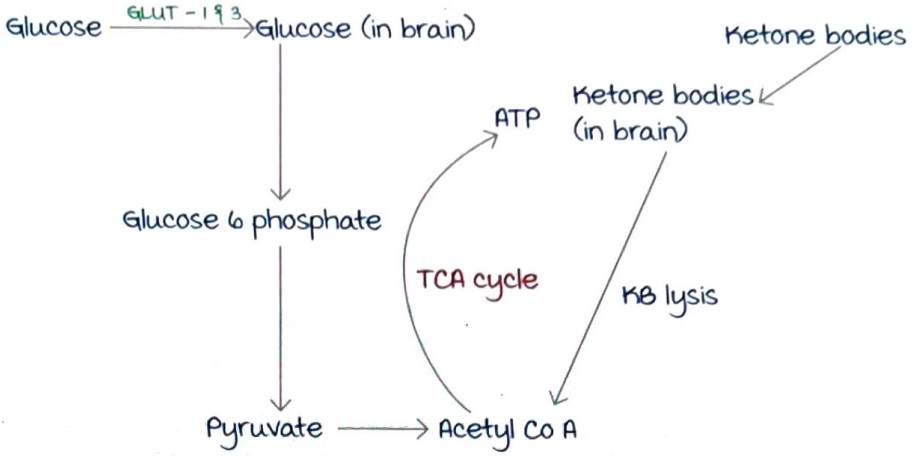
Brain :

2 GLUTs : GLUT 1, GLUT 3 (neurons).

KB can only provide 20% energy.

Prolonged starvation : Brain depends on KB.

Active space



Metabolic fuels of fed fast stages

00:30:16

Organ	Fed	Early fasting / fasting	Starvation
Liver	Glucose > FFA	FFA > Glucose	AA/FFA (KB not used)
Heart	FFA > Glucose	FFA	FFA/KB
Brain	Glucose	Glucose	Glucose/KB (20% energy)
Skeletal muscle	Glucose > FFA	FFA > Glucose	FFA/KB
RBC	Glucose	Glucose	Glucose
Adipocytes	Glucose > FFA	FFA > Glucose	FFA

Major metabolic fuel used : Free fatty acid > Glucose, KB lysis.

Exceptions : RBC and liver.

Brain cannot use free fatty acid.

A person had his dinner at 8 pm. Before having his breakfast at 9 am his blood glucose was checked, it was 100 mg/dl. Which pathway is the source of his blood glucose?

- Gluconeogenesis.
- Hepatic glycogenolysis.
- Muscle glycogenolysis.

Active space

CONCEPT OF ENZYME REGULATION

Hormonal regulation.

Allosteric regulation.

Hormonal regulation 00:01:18

Well fed/absorptive/post prandial state → Insulin.

High insulin glucagon ratio.

Fasting state → Glucagon.

Low insulin glucagon ratio.

Action of glucagon :

Glucagon binds to G protein coupled receptor.

↓

Activates G protein.

↓

Activates adenylyl cyclase.

↓

ATP → cAMP

↓

Activates cAMP dependent protein kinase A.

ATP

↓

ADP

Enzyme → Phosphorylated enzyme.

Action of insulin :

- Activation of phospho diesterase :
  - Converts cAMP to 5' AMP.
  - Insulin reduces cAMP level.
- Converts enzyme into dephosphorylated state.

Active space

Insulin	Glucagon
Well fed state. Enzymes active under the influence of insulin is active in the dephosphorylated state.	Fasting state. Enzymes active under the influence of glucagon is active in the phosphorylated state.

Examples	Active state
Glycolysis	Dephosphorylated state
Gluconeogenesis	Phosphorylated state
Glycogen synthesis	Dephosphorylated state
Glycogen degradation	Phosphorylated state
Pyruvate dehydrogenase. (Connects glycolysis to TCA cycle)	Dephosphorylated state
Fatty acid synthesis	Dephosphorylated state
Cholesterol synthesis	Dephosphorylated state

Allosteric regulation

00:14:04

Substrate favour forward reaction. AKA Feed forward regulation.

Product inhibit forward reaction. AKA Feed back inhibition.

Examples	Allosteric activator	Allosteric inhibitor
Phosphofructokinase I (Converts Fructose 6 phosphate to fructose 1,6 bisphosphate)	Fructose 6 phosphate 5' AMP	ATP Low pH Citrate
ALA synthase (heme synthesis)		Heme
Acetyl coA carboxylase (fatty acid synthesis)	Citrate (source of acetyl coA needed in fatty synthesis)	Acyl coA (fatty acid is synthesized as co enzyme A derivative)

Active space

INTRODUCTION TO ENZYMES

Introduction 00:01:50

Definition : Enzymes are specialised proteins that act as catalyst in biological reactions.

In 1850, Louis Pasteur discovered that sugar can be converted to alcohol. This process is known as fermentation. He termed the likely vitalism that catalyses this rection as ferments.

In 1897, Edward Buchner discovered that cell free yeast extract could catalyse fermentation.

Frederick W Kuhne coined the word enzyme which is derived from Greek word enzymos meaning leavened.

Types of enzymes 00:05:19

Simple enzyme :

- Only amino acid residues take part in the reaction.

Complex enzyme :

- Amino acid residue + Chemical component is required for the reaction to occur.
- Amino acid component is known as apoenzyme.
- Non enzyme component can be :
  Inorganic molecule like metals : Fe^{2+}/Fe^{3+}, Cu^{2+}, Zn^{2+} → Co-factor.
  Organic component like organic molecule or metallo-organic molecule → Co-enzyme.

Properties of enzyme

00:08:43

1. Enzymes are proteins.
   Exception : Ribozyme : RNA with catalyst property.
   Examples of ribozymes :
   - 28 rRNA in ribosome has enzymatic activity peptidyl transferase and is required for peptide bond synthesis.
   - snRNA (Spliceosome). } RNA splicing
   - Group II introns (hnRNA).
   - RNAse P : Post transcriptional modification of tRNA.
2. 16% by weight nitrogen.
3. Precipitated by protein precipitating agents.
4. Heat labile.

Coenzymes

00:12:50

- Heat stable.

- Low molecular weight non-protein organic compounds.
- Considered as second substrates or cosubstrates.

Examples : Most of the coenzymes are water soluble vitamins.

	Active form	Reaction involved
Vitamin B1	Thiamine Pyrophosphate (TPP)	• Oxidative decarboxylase • Transketolase
Vitamin B2 (riboflavin)	FAD/FMN	• Dehydrogenases • Redox reactions
Vitamin B3 (niacin)	NAD+/ NADP+	• Dehydrogenases
Vitamin B5 (pantothenic acid)	CoA	• Transfer of acyl groups
Vitamin B6 (pyridoxine)	Pyridoxal phosphate (PLP)	• Transfer reactions like 1. Transamination 2. Transulfuration
Vitamin B9 (folic acid)	Tetrahydrofolic acid (THF)	• One carbon reactions

Active space

Vitamin B12 (Cobalamin/ cobamide enzymes)	Adenosyl B12 Methyl B12	• Transfer of alkyl groups/ H atom
Lipoate		• Oxidative decarboxylation as part of multienzyme complexes like pyruvate dehydrogenase, alpha-keto glutarate dehydrogenase, branched chain keto acid dehydrogenase
Vitamin C	Ascorbate	• Hydroxylation reactions

Cofactor 00:21:04

Metals as co-factor :

Metalloenzyme :

Metal is tightly integrated by covalent or non-covalent bonds to the apoenzyme.

E.g. : Zn in carbonic anhydrase.

Cu in tyrosinase.

Metal activated enzyme :

Metal is not an integral part of the apoenzyme, but its presence is required in the surroundings for the enzyme to act.

E.g., Lipase requires presence of Ca^{2+} to act.

Prosthetic group 00:23:58

Chemical component (coenzymes/cofactor) which is tightly integrated to the apoenzyme by covalent bonds or non-covalent bonds.

Holoenzyme : Apoenzyme + Chemical component (coenzyme/ cofactor).

Active space

Zinc	• Carbonic anhydrase : Transfer of gases. • Carboxypeptidase A and B : Digestion of proteins. • Alcohol dehydrogenase : Metabolism of alcohol. • Alkaline phosphatase. • ALA dehydratase. • Adenosine deaminase. • Cytosolic Superoxide Dismutase (SOD) : Free radical scavenging. • Lactate dehydrogenase.
Magnesium (usually coupled with ATP)	• Phosphotransferases (Kinases). • Phosphatase (phosphohydrolase). • Mutase. • Enolase.
Iron (Fe^{2+}/Fe^{3+})	Heme iron : • Complex IV cytochrome c oxidase, Complex III cytochrome c oxidase } Electron transport chain • Tryptophan pyrolase/ dioxygenase : For catabolism of tryptophan and required for synthesis of niacin • Peroxidase, Catalase } Free radical scavenging Non Heme iron : • Complex I, Complex II } Fe-S complex
Manganese : Required along with Mg^{2+}	• Kinases. • Phosphatases. • Arginase : Urea synthesis. • Ribonucleotide reductase : Ribonucleotides are converted to deoxyribonucleotide : Required for DNA synthesis. • Mitochondrial superoxide dismutase : Free radical scavenging.
Molybdenum. MnO^{2-}_3	• Xanthine oxidase : Synthesis of uric acid. • Sulphite oxidase • Dinitrogenase.
Potassium (K^+)	• Pyruvate Kinase. • Na^+, K^+ ATPase.

Copper (Cu^{a+})	• Tyrosinase : Cu^{a+} deficiency causes hypopigmentation as tyrosine required for melanin production • Complex IV of ETC Cyt C oxidase : Energy depletion in Cu^{a+} deficiency. • Lysyl oxidase : Required for covalent crosslinking of collagen : Menke Kinky disease/ Steely hair syndrome. • Cytoplasmic Superoxide dismutase.
Nickel	• Urease
Calcium	• Lecithinase • Lipase

Clinical problems

00:40:17

Vitamin deficiency (coenzyme) :

- Energy depletion : Coenzymes are part of oxidoreduction reactions which result in formation of reducing equivalents that goe into electron transport chain for generation of ATP. Coenzyme deficiency hence causes energy depletion.
- Collagen depletion : Vitamin C acts as coenzyme of hydroxylation of lysine and proline which is required for collage synthesis.
  Vitamin C deficiency can cause scurvy.
- Enzyme defect : PLP defect can halt homocysteine metabolism.

Mineral deficiency (cofactor) :

- Hypopigmentation (Cu^{a+} deficiency).
- Oxidative stress : minerals are required for free radical scavenging; deficiency of minerals can cause oxidative stress that leads to atherosclerosis, cancers etc.,
- Collagen defect : Cu^{a+} required for lysyl oxidase needed for covalent crosslinking.
- Vision : Zinc is cofactor for retinol dehydrogenase which is involved in Wald's visual cycle.

Active space

CLASSIFICATION OF ENZYMES

Trivial System : Inadvertent naming of the same enzyme by multiple names.

International Union of Biochemistry and Molecular Biology / IUBMB

00:02:20

7 classes of enzymes (August 2018)

Enzyme classes :

Mnemonic : Operation theatre has low-intensity light.

I – Oxidoreductase.

II – Transferase.

III – Hydrolase.

IV – Lyase.

V – Isomerase.

VI – Ligase.

VII – Translocase.

Class I – Oxidoreductases

00:05:10

Catalyze oxidation and reduction reactions.

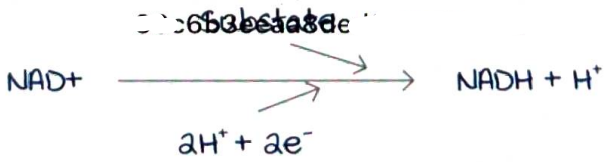
Subclasses :

1. Dehydrogenase :

Catalyze transfer of hydrogen element H^+, H^-, H_a and electrons to an acceptor.

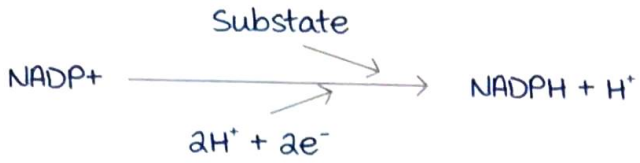
Acceptors are :

Nicotinamide coenzymes :



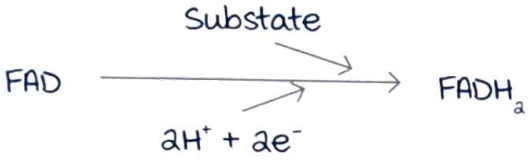
Active space

NADP :



- Glucose 6 phosphate dehydrogenase.
- 6 Phosphogluconate dehydrogenase.
- Cytoplasmic isocitrate dehydrogenase.
- Malic enzymes.

Flavoprotein (Riboflovin):



- Succinate dehydrogenase (Complex II).
- Acyl CoA dehydrogenase.
- Mitochondrial Glycerol 3 phosphate dehydrogenase.

2. Oxygenase :

Add oxygen directly to the substrate :

2a. Monooxygenase / Mixed function oxidase : Add 1 atom of oxygen.

Hydroxylase :

$$AH + O_a + 2H_a \longrightarrow AOH + H_aO + Z$$

E.g. :

- Tryptophan Hydroxylase.
- Tyrosine Hydroxylase.
- Phenylalanine Hydroxylase.

} Aromatic amino acid Hydroxylase.

- 7 - Hydroxylase.
- Cytochromes

Cytochrome P450 and Cytochrome b5 are involved in modifying and degrading drugs.

Active space

Are present in the Liver & Intestine.

Are present in the Endoplasmic reticulum & Mitochondria.

Barbiturates induce cytochromes, which aggravates Porphyria.

2b. Dioxygenase : Add both atoms of oxygen.

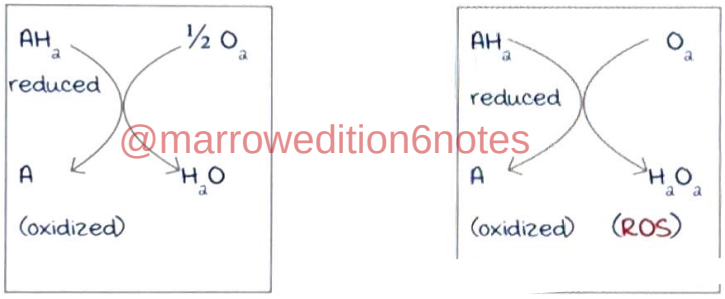
$$A + O_a \longrightarrow AO_a$$

E.g. :

- Homogentissate oxidase/ dioxygenase.
- Tryptophan pyrolase / dioxygenase.

3. Oxidase :

Transfer hydrogen element and donate to acceptor O_a



ROS – reactive oxygen species.

E.g. :

- Cytochrome C oxidase.
- Monoamino oxidase.
- Flavoproteins (FAD/FMN) are acceptors :
  - L amino acid oxidase.
  - Xanthine oxidase.

4. Hydroperoxidases :

- Peroxidase.
- Catalase.

} Hemoproteins

Substrate : Hydrogen peroxide / any oxygenic peroxide.

Results in free radical scavenging.

Active space

4a. Peroxidase :

Electron acceptors :

- Ascorbate
- Quinones.
- Glutathione.
- Cytochrome C.

} Free radical scavenging

$H_2O_2 + AH_2 \longrightarrow 2H_2O + A.$

E.g. : Glutathione peroxidase : a Selenium containing enzyme.
It is present in erythrocytes and other tissue.

4b. Catalase :

Electron acceptor : 1 molecule of H_2O_2.

$2H_2O_2 \longrightarrow 2H_2O + O_2.$

Catalase is present in Peroxisomes of :
Mucous membrane, Kidney, Liver, Blood, Bone marrow.

Peroxisomes containing :
Catalases scavenge reactive oxygen species.
Oxidases generate reactive oxygen species.

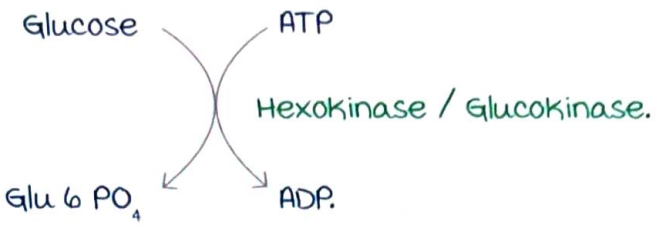
5. Reductases :
They require NADPH.
Are involved in the reductive biosynthesis of steroids, Fatty acids, and Cholesterol.

Class II – Transferase 00:29:37

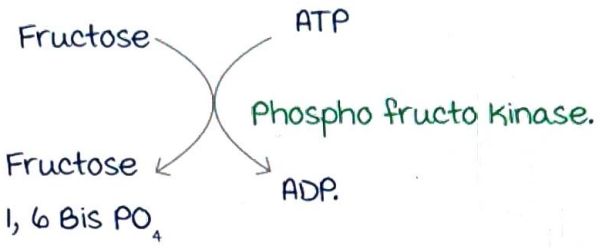
Transfer the functional group/ moieties like methyl, and amino groups. Example :

- Any enzyme with TRANS in its name : Transaminase, transketolase, transaldolase, transmethylase.
- Any enzyme that trasfer phosphoryl groups – Kinases.
  Donor is ATP : Hexokinase, phosphofructokinase
  Donor is inorganic phosphate : phosphorylase

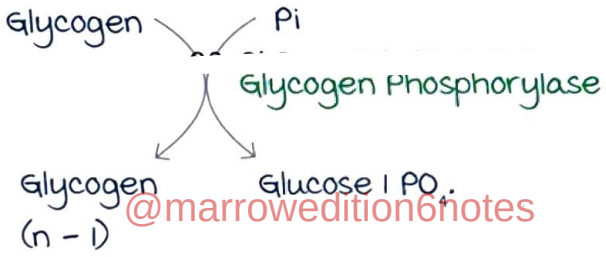
1. Hexokinase :



2. Phospho fructo kinase :



3. Phosphorylase :



Class III : Hydrolases 00:34:26

Hydrolytic cleavage of covalent bonds like C-C, C-N, and C-O. A covalent bond is a bond of the primary structure of macromolecules.

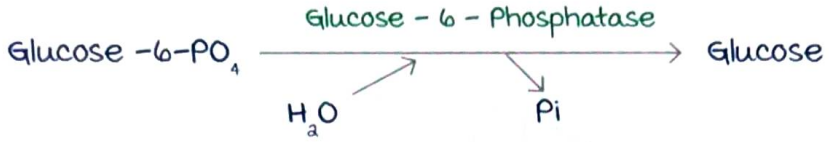
Macromolecule	Covalent bonds	Hydrolase
Carbohydrate	Glycosidic bond	Amylase, maltase, Sucrase.
Protein	Peptide bond	Peptidases Proteases (Trypsin, Chymotrypsin, elastase).
Nucleic acid	3'-> 5' Phosphodiester bond	Nucleases (Endonucleases and Exonucleases).
Lipids	Ester bond	Esterase/Lipases.

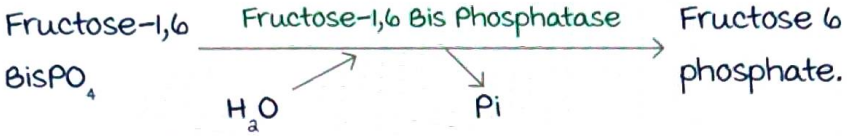
Active space

Other hydrolases:

Arginase.

Phosphatases.





Class IV : Lyases. 00:41:10

Cleavage of covalent bonds like C-C, C-N, and C-O without adding water, by atom elimination and forming a double bond or adding groups to double bond.

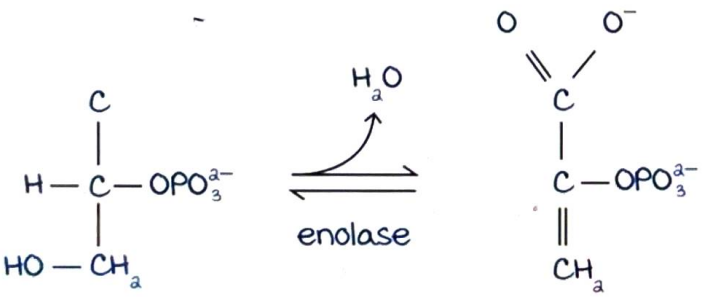
- Any enzyme with Lyase in the name:
  1. HMG Co-A lyase.
  2. Arginosuccinate lyase.
  3. ATP citrate lyase.

- Aldolase :

Fructose - 1, 6 - bis PO_4 → Glyceraldehyde- 3 - PO_4 + DHAP
6C 3C 3C

Break / make double bond by atom elimination.

- Enolase :

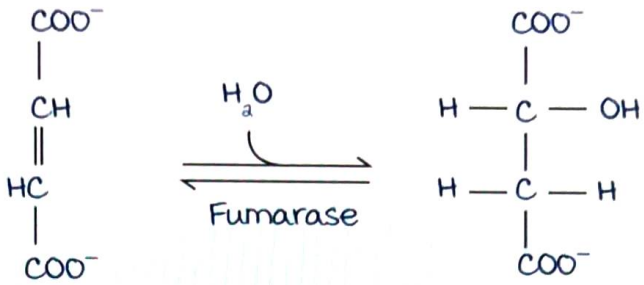


2-Phosphoglycerate

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

Active space

- Fumarase :



Fumarate L'malate

- Aconitase : Converts citrate to isocitrate
- Decarboxylase : They are of two types

1. Simple decarboxylation reactions :

Histidine → Histamine.

Glutamate → GABA.

Tryptophan → Tryptamine.

These reactions :

- Liberate CO_2.
- Require co-enzyme → PLP (class - Lyase).

2. Oxidative decarboxylation reaction :

Pyruvate —(Pyruvate dehydrogenase)→ Acetyl CoA

α Ketoglutarate —(α Ketoglutarate dehydrogenase)→ Succinyl CoA.

Branched-chain Ketoacid / BCK —(BCK dehydrogenase)→ Corresponding Acyl CoA.

In these reactions :

- Liberate CO_2.
- Reduce one carbon atom of the substrate: n (carbon atoms) → n-1 (carbon atoms).
- In these reactions: NAD^+ → NADH: hence these dehydrogenase enzymes belong to class 1 oxidoreductases.

Active space

- These dehydrogenase enzymes are multienzyme complexes & require 5 coenzymes :
  - TPP / Thiamine pyrophosphate (Vit B).
  - NAD+ / Vit B_3.
  - FAD / Vit $B1_a$.
  - Lipoate.
  - CoA / Vit B_5.

Class V – Isomerase

00: 51: 45

Catalyze structural and Geometric isomers.

Any enzyme with isomerase in its name.

Glucose – 6– PO_4 $\xleftarrow{\text{Phosphohexose isomerase}}$ Fructose – 6 – PO_4

Glyceraldehyde – 3 – PO_4 $\xleftarrow{\text{Phosphotriose isomerase}}$ DHAP.

Racemase :

D Glucose $\longleftrightarrow$ L Glucose.

D Alanine $\longleftrightarrow$ L Alanine.

Mutase :

Intramolecular transfer of groups :

Glucose – 6 – PO_4 $\xleftarrow{\text{Phosphoglucomutase}}$ Glucose – 1 – PO_4.

3 Phosphoglycerate $\xleftarrow{\text{Phosphoglycerate mutase}}$ 2 Phosphoglycerate

Class VI – Ligase

00:56:25

Joins 2 molecules coupled with the hydrolysis of ATP.

E.g.:

1. Carboxylase.

Active space

2. synthetase. E.g.: Glutamine synthetase, Carbamoyl PO_4, Synthetase.

Carboxylase :

E.g.: Carboxylases.

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

Acetyl Co-A (2C) —Acetyl Co-A carboxylase→ Malonyl Co-A (3C)

Propionyl Co-A (2C) —Propionyl Co-A Carboxylase→ methyl Malonyl Co-A (3C)

In all these reactions :

Enzymes: ligases.

Mnemonic for ligases - ABC enzymes

Hydrolysis of ATP is required, it is converted to ADP.

Coenzyme: Biotin.

CO_2 is added.

Class VII – Translocase. 01:00:50

Belonged to class III Hydrolase previously.

Transport of molecules and ions across the membrane.

E.g. :

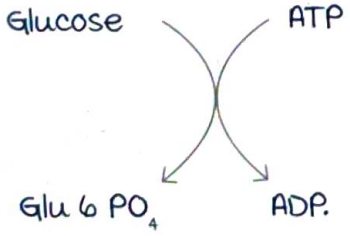
H^+ pump.

K^+ channel.

Ca^{2+} channel.

Exercise :

Q) what is the name of enzyme in the following reaction ?



Answer: Hexokinase / Glucokinase.

Kinase (Class II - Transferase)

Transfer of phosphate.

The substrate is Glucose / Hexose.

Active space

Q) Identify the enzyme involved.

1. Glucose -6-PO4 → Glucose.
   (H2O added; Pi removed)

Answer: Glucose – 6 – Phosphatase.

H_2O is added and inorganic Phosphate is removed.

An example of Hydrolase is Phosphatase.

Adding substrate to the name: Glucose – 6 – Phosphatase.

2. Glucose – 6 – PO_4 ⟷ Fructose – 6 – PO4.

Answer: Phosphohexose isomerase.

Glucose and Fructose are isomers; hence enzyme is Isomerase.

Both are hexose phosphates.

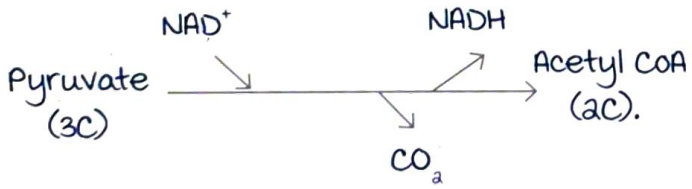
3. Glucose – 6 – PO_4 ⟷ Glucose – 1 – PO_4.

Answer: Phosphoglucomutase.

A type of isomerase: Mutase.

The substrate is Glucose phosphate.

4.

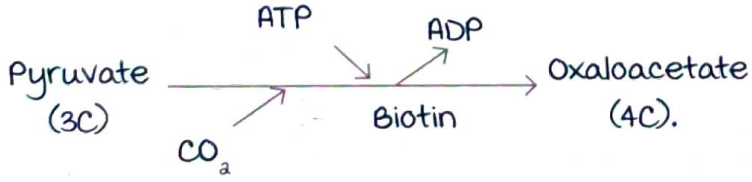


Answer: Pyruvate dehydrogenase.

In these reactions: $NAD^+ \rightarrow NADH$: hence the enzyme is dehydrogenase.

The substrate is Pyruvate.

5.



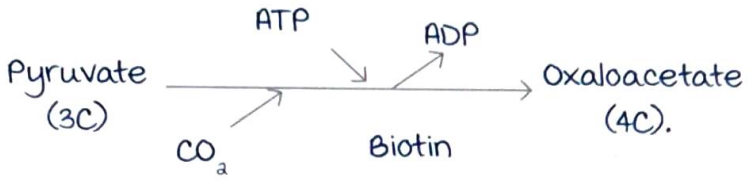
Answer: Pyruvate carboxylase.

Active space

Probable Clinical questions :

Q) A person consuming Raw eggs develops Hypoglycemia, Tiredness. Reason?

Answer: Raw eggs contain Avidin, which Inhibits Biotin.
Hence all the reactions catalyzed by Biotin are inhibited.
Example :



The enzyme Pyruvate carboxylase is inhibited.
This reaction is a step of Gluconeogenesis.
Hence when the blood glucose is reduced, Glycogen cannot be used as an energy source.
This results in Hypoglycemia and tiredness.

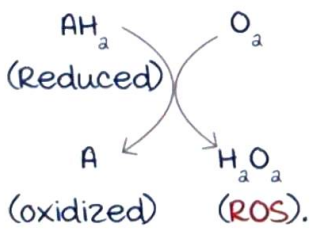
Q) Drugs.

Answer: Most drugs are acted upon by Cytochrome P450 and Cytochrome b5.
These are Hydroxylation reactions catalyzed by the Monooxygenase enzyme.

Q) Free radical scavenger.

Answer: Enzymes are Peroxidase and Catalase.
In these reactions, H_2O_2 is converted to H_2O.
Catalase is present in Peroxisomes.

Q) Free radical generator.
Answer: Enzyme Oxidase.
In Oxidation reactions :



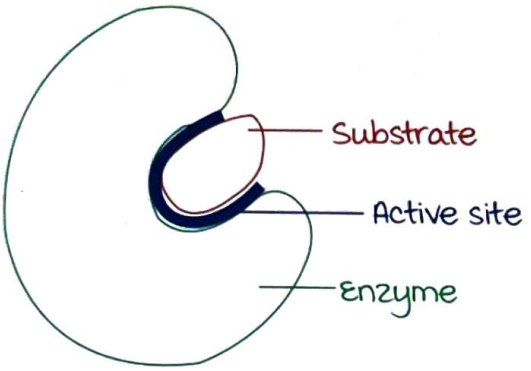
Active space

MECHANISM OF ACTION OF ENZYMES

Active site

00:01:08

The reaction is not occurring in the entire enzyme. it takes part only in certain site called active site.



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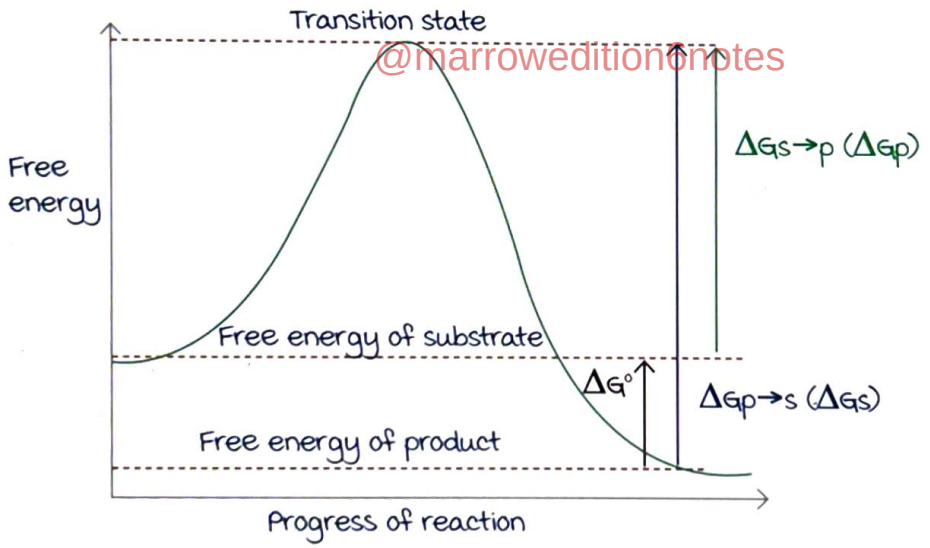


Activation energy :

The difference between the free energy of substrate and the transition state.

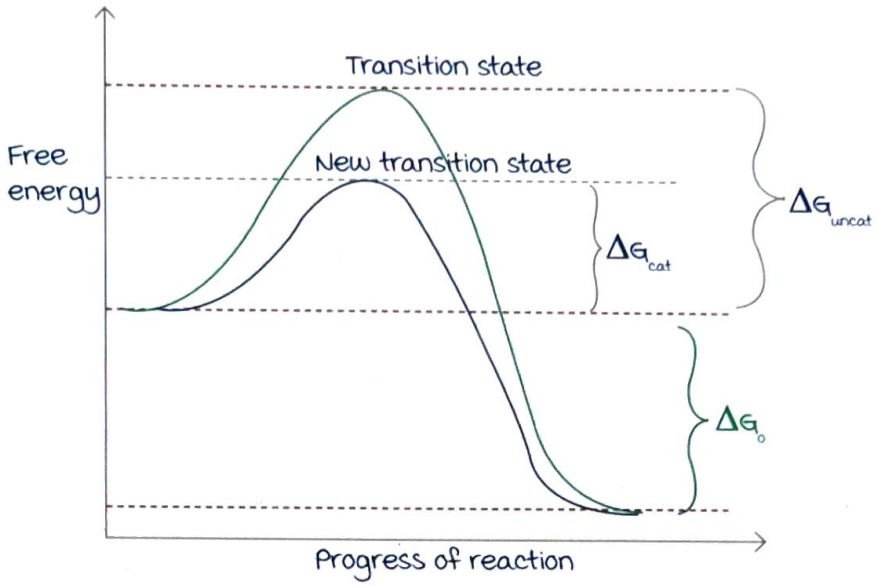
The standard free energy change : ΔG_0

$\Delta G_0 = \Delta G_p - \Delta G_s$

Gibb's free energy : G

Active space

Action of enzymes :



ΔG_{uncat} – ΔG of uncatalysed reaction

ΔG_{cat} – ΔG of catalysed reaction

Enzymes lower the activation energy.

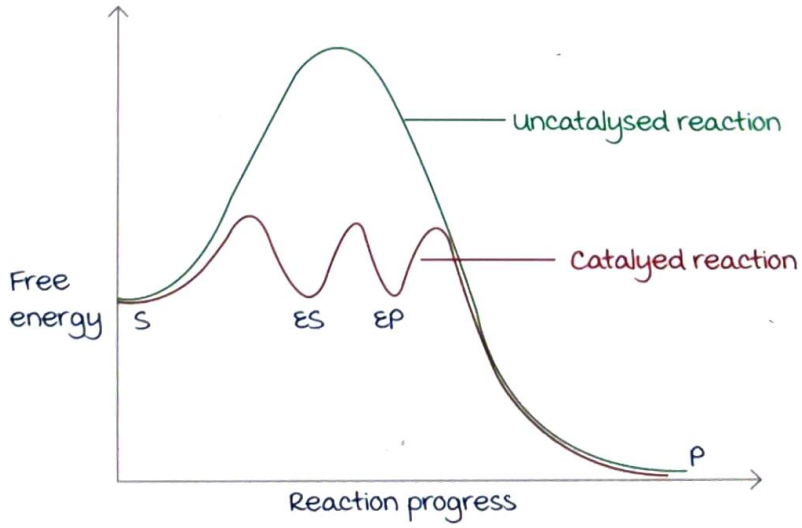
Enzymes do not alter ΔG_0 standard free energy change.

E+S → ES → EP → E+P

E : Enzyme, S : Substrate, P : Product.

Another way of reaction coordinate of catalysed reaction

00:19:14



Active space

Energy barriers	Enzymes
1. Entropy (disorderly) of system.	Reduce the entropy.
2. Solvation shell of hydrogen bonded water around the substrate.	Desolvation of the substrate.
3. Improper alignment of reactive groups, Enzyme to the substrate.	Proper alignment of reactive groups in the active site to the substrate.

Proper alignment of reactive groups in the active site to the substrate It's explained by various theories.

Various theories 00:28:30

1. Emil Fischer's template theory/ Lock and Key model :
   Fixed shape for the active site, so that substrate with complementary shape can bind to the active site of the enzyme.
2. William Jenk and Linus Pauling theory:
   Active site of the enzyme is complementary to the transition state so that more products are formed.
3. Induced fit theory: by Daniel Koshland (1958).
   Enzymes undergo conformational change when substrate binds to the active site, which is induced by multiple weak interaction between substrate and active site.

Mechanisms by which the reaction takes place 00:36:15

- Catalysis by proximity.
- Acid base catalysis.
- Covalent catalysis.
- metal ion catalysis.
- Catalysis by strain.

1. Catalysis by proximity :
   - For the reaction to occur, the reactant should come in bond forming distance with the active site.

2. Acid base catalysis :
   - Catalytic residues in the active site, either as a proton donor (Acids) or proton acceptor (Bases).
   - Example : Aspartate protease – they are the enzymes whose catalytic residue in the active site is aspartate (Pepsin's active site has aspartate)

3. Catalysis by strain :
   - Applicable for lytic reactions (catalyst by Lyases and Hydrolases).
   - Enzyme bound to the substrate in such a way that it strain and weaken the bonds to be broken.

4. Covalent catalysis :
   - In Group transfer reactions. Examples : Transferases (transamination).
   - Involves formation of covalent bond between enzyme and the substrate transiently.
   - Example : Serine proteases (Chymotrypsin, trypsin, elastase).

5. Metal ion catalysis :
   - Usually in case of metalloenzymes.
   - Example : Zinc in carbonic anhydrase.
   - Metal ion is helping for the proper alignment of substrate to the active site.

Q. Which of the following regarding lowering of activation energy are true except?

A. Entropy is lowered.

B. Desolvation of the substrate.

C. Confirmational change at the active site during binding of the substrate.

D. Active site of the enzyme and substrate are always complementary to each other.

Active space

ENZYME KINETICS

Enzyme kinetics

00:01:20

Deals with 2 things.

- Rate of reaction.
- Factors that affect the rate of enzyme catalyzed reaction.

Rate of reaction :

$$A + B \underset{r\,2}{\overset{r\,1}{\longleftrightarrow}} P + Q.$$

$r_1 \propto [A][B]$. $\quad r_1 = K_1[A][B]$.

$r_a \propto [P][Q]$. $\quad r_a = K_a[P][Q]$.

K_1 = Rate constant of forward reaction.

K_a = Rate constant of backward reaction.

At equilibrium, it is a dynamic state where forward and backward reaction takes place, at the same rate.

$r_1 = r_a$

$K_1[A][B] = K_a[P][Q]$.

$\frac{K_1}{K_a} = K_{eq}$ (Equilibrium constant).

$K_{eq} = \frac{K1}{K2} = \frac{[P][Q]}{[A][B]}$.

K_{EQ} = Concentration of products / Concentration of substrates.

- Enzymes don't alter equilibrium constant (Keq).
- Enzymes lower activation energy.
- Enzymes don't alter free energy state [ΔG°].

$\Delta G^\circ = -RT \log K_{eq}$ (R : Gas constant. T : Absolute temperature).

Active space

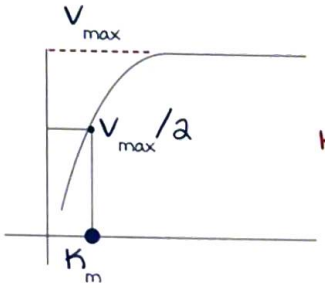
Factors that affect rate of enzyme catalyzed reactions

00:07:34

- Substrate concentration.
- Temperature.
- H^+ ions Concentration.
- Enzyme concentration.

Substrate Concentration :

Increase in substrate concentration → Increase in rate of reaction → Till it reaches V_{max} Then no further increase in rate of reactions.



Hyperbolic curve

Till V_{max} : $V\alpha [S]$ → 1st order kinetics (As Substrate concentration increases Rate of reaction also increases).

Above V_{max} : $V\alpha$ x [S] → Zero order Kinetics (Active sites are utilized : No increase in rate of reaction).

K_m : Michaelis constant.

Leonor Michaelis & Maud Leonora Menten's derivation

00:15:07

V_i (V_o)

$$Vi = \frac{V_{max} X[S]}{K_{m+}[S]}$$

K_m = Substrate concentration at ½ V_{max}

If [S] at ½ Vmax = 20mmol = K_m, Find V_{max}, V_{max} = 40 mmol.

If S] = K_m, Velocity of reaction = X then V_{max} = 2x.

Significance of Km :

- Independent of enzyme concentration.
- Denotes affinity of an enzyme towards substrate.

Active space

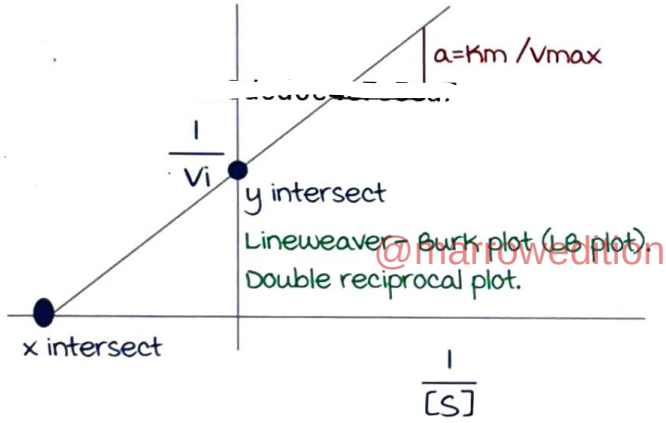
- Unique for an enzyme substrate pair.
  E.g. : Km value of glucose hexokinase differs from K_m value of fructose hexokinase.
  "Signature" of an enzyme substrate pair.
- Higher the K_m, Lower is the affinity of the enzyme towards the substrate.
  Km $1/\alpha$ Affinity.
  Affinity $1/\alpha$ [S] $\rightarrow$ $K_m \alpha$ [S].

$$Vi = \frac{V_{max} X[S]}{Km+[S]} \rightarrow \frac{1}{Vi} = \frac{Km + [S]}{Vmax\,[S]}.$$

$1/V_i$ – Variable 'y'
$K_m / Vmax$ – 'a'
$1/[S]$ – Constant
$1/V_{max}$ – constant

$$\frac{1}{Vi} = \frac{Km}{Vmax} X \frac{1}{[S]} + \frac{1}{Vmax}$$ (V_i = Initial velocity).

Y=ax + b $\rightarrow$ Equation of line.



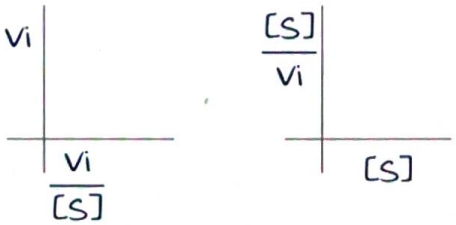
At x intersect, y = 0
Y = ax + b
X= -b/a
$= \frac{-1 / V_{max}}{K_m / V_{max}}$
$X = -1 / K_m$

At y intersect, x=0
Y = ax + b
Y = 0 + b
$Y = 1 / V_{max}$

Alternatives to LB plot

00:29:00

Single reciprocal plot :
a) Eadie –Hofstee plot.
b) Hanes–Woolf plot.



Catalytic constant (AKA Turnover number) :
K_{cat} : measure of efficiency of a homogenous mixture of enzyme.

Active space

$K_{cat} = V_{max} / \varepsilon_{t.}$ (ε_t : Total enzyme concentration).

$E + S \longleftrightarrow ES \longrightarrow E + P$.

$\varepsilon_t = E + ES$.

$$K_{cat} = \frac{V_{max}}{\varepsilon_t}.$$

To compare efficiency of enzyme : Calculated as Kcat / K_m (AKA "Specificity constant").

Dissociation constant : $K_d = \frac{K_{-1}}{K_1}$

Association constant : $K_a = \frac{K_1}{K_{-1}}$

Q. Pancreatic lipase is an an enzyme that digest dietary fats. If its an enzyme that follow michaelis menten Kinetics. Which one of the following best describes a characteristic feature of pancreatic lipase?

A. Velocity of the is half maximal when 100 % of substrate binds to the enzyme.

B. Velocity of the is half maximal when 50 % of substrate binds to the enzyme.

C. Velocity of enzyme is maximal when 50% of enzyme bound with substrate.

D. Velocity of enzyme is independent of substrate concentration.

Q. Expression of tissue specific isoenzyme is a method of regulation ofenzymes.The glucose metabolism differ in RBC and liver as one metabolise glucose and other store glucose.In RBC first step use Hexokinase I whereas liver glucokinase. What is the reason behind this ?

A. The Km of HK I is higher than GK.

B. The Km of HK I is lower than GK.

C. The Km of HK I is same as GK.

Temperature

00:49:55

Most optimum temperature in Human : 35° to 40° C.

Maximum stability : 45° to 55° C.

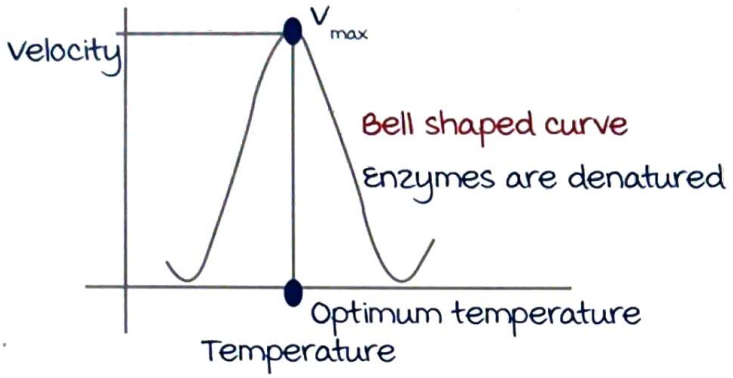
Temperature Co-efficient (Q_{10}) :

Every 10° rise in temperature : Rate of reaction is doubled.

$Q_{10} = 2$

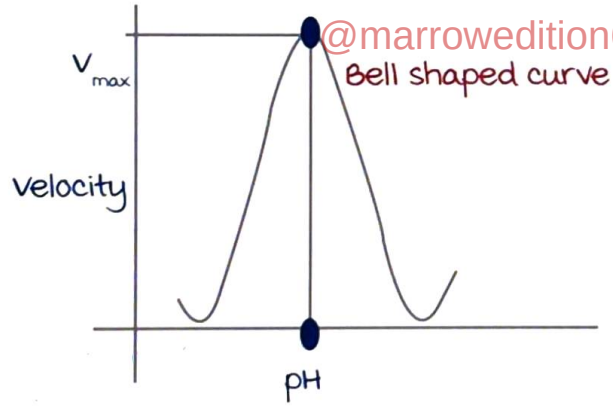
Rate of reaction is doubled because :

- Increased Kinetic energy.
- Increased Collision frequency.



H^+ ion concentration

00:55:07



Optimum pH : Normal : 5 to 9.

Changed state of Amino acid residues varies with pH.

pK^a : Ionization constant. pH : "Charge of ionizable group varies".

Aspartate protease → pepsin

Active in acidic pH.

Aspartate $pK_a \cong 2$.

↓

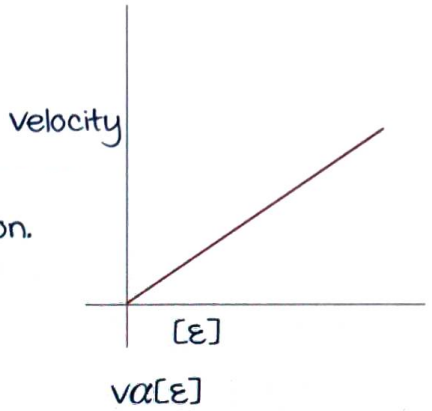
Intestine

Chymotrypsin : Catalytic residue : Histidine ($pK_a = 6$, active in alkaline pH).

Enzyme concentration

01:02:00

Concentration is directly proportional to velocity of reaction.



Q. Enzyme with highest catalytic efficiency is:
(INICET March 2020)

A. Km of an enzyme = 10 micromole and Kcat = 20 per sec.

B. Km of an enzyme = 2000 nanomole and Kcat = 50 per sec.

C. Km of an enzyme = 2 micromole and Kcat = 200 per sec.

D. Km of an enzyme = 4 micromole and Kcat = 200 per sec.

Q. In an enzyme catalysed reaction substrate concentration was 1000 times Km.1% of substrate metabolised to 12 micromole of the substrate in 9 min. If in the same reaction mixture ,enzyme concentration is reduced to 1/3 rd and substrate concentration is doubled. How much time is needed for the same amount of substrate to get converted to product.

A. 9 min.

B. 13.5 min.

C. 18 min.

D. 27 min.

Active space

ENZYME INHIBITION

Enzyme inhibition 00:02:44

All cellular processes need enzymes. Control of these processes can be done via an enzyme inhibitor.

Types :

Reversible inhibition :

- Competitive inhibition.
- Uncompetitive inhibition.
- Mixed inhibition.

Irreversible inhibition :

- Suicide inhibition.
- Transition state analogues.

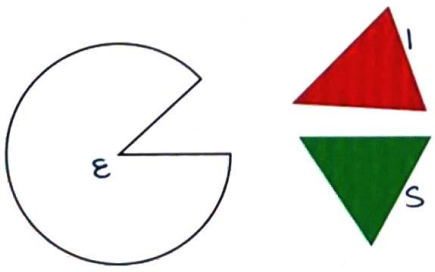
Non-competitive inhibition can be both reversible and irreversible.

Competitive inhibition 00:05:24

It is a type of inhibition in which the inhibitor is a structural analogue of the substrate and hence it competes with the substrate for binding to the active site.

$E + S \leftrightarrow ES \rightarrow E+P$

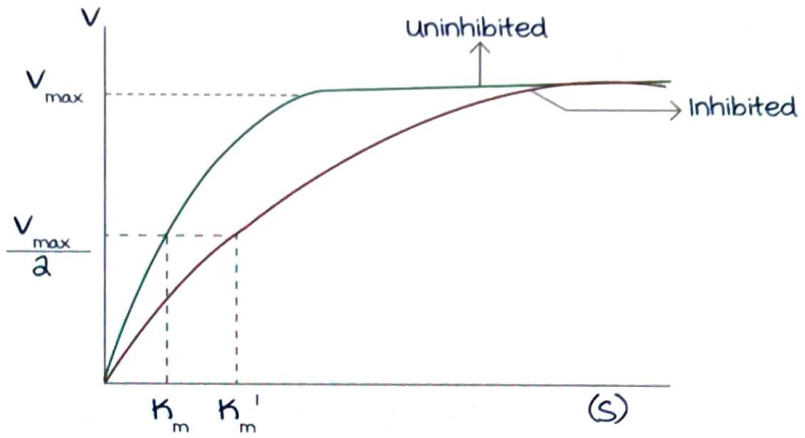
E + I (inhibitor) → Enzyme – Inhibitor Complex.



Properties of competitive inhibition :

- Inhibitor is a structural analogue of the substrate.
- Addition of excess substrate can replace the inhibitor from the active site.

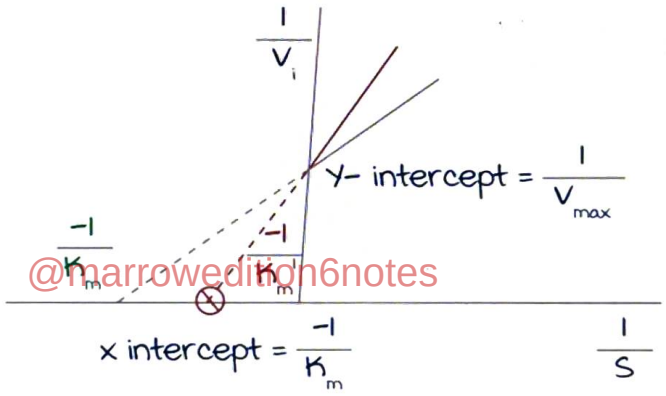
Active space



V_{max} remains constant.

Apparent $K_m = K_m' = \alpha K_m$.

i.e., K_m increases.



X-intercept moves towards zero.

Y-intercept remain same.

Plot is shaped like 'X'.

Case scenario :

A 5 year old child playing in the garden accidentally drank an unknown solution kept in a bottle. Later he was not feeling well. He told his mother and she took him to the nearby hospital and she also took the leftover bottle. On the way he vomited twice and was experiencing abdominal cramps.

In the casualty stomach lavage done.

PR : 50/min and BP : 80/50.

Muscle fasciculations present in the legs.

Active space

Ans. Poison was found to be Malathion, an organophosphate.

Malathion
↓
Malaxone
↓
Inhibits Ach esterase (competitive inhibition)
↓
Acetyl choline ⟶✕ Acetate + choline
↓
Accumulates in nervous system
↓
Stimulates autonomic nervous system
↓
Symptoms like vomiting, sweating, increased muscle twitching

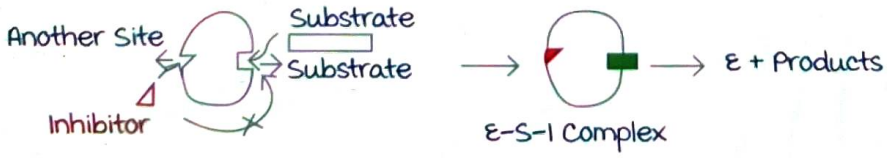
Initially, the inhibition of Ach esterase by malaxone is reversible, but as the dose of organophosphate increases, the inhibition becomes irreversible.

Case scenario :
In Hooch tragedy, many people were hospitalised. Ethanol was given as an antidote. Why?
Ans. Methanol was ingested in hooch tragedy.

Methanol $\xrightarrow{\text{Alcohol dehydrogenase}}$ Formaldehyde ⟶ Blindness.

Ethanol competitively inhibits the enzyme alcohol dehydrogenase and forms acetaldehyde which is relatively less toxic.
Excess ethanol replaces methanol from active binding site and prevents formation of toxic formaldehyde.

Non-competitive inhibition 00:19:31



The inhibitor is not a structural analogue of the substrate.

Active space

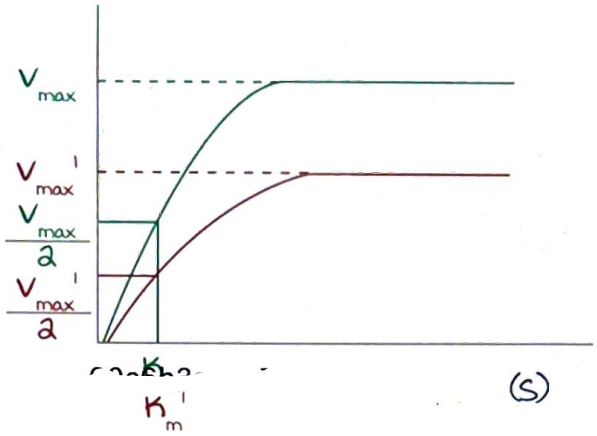
The inhibitor binds to a distinct site.

$E + S \leftrightarrow ES \rightarrow E+P$

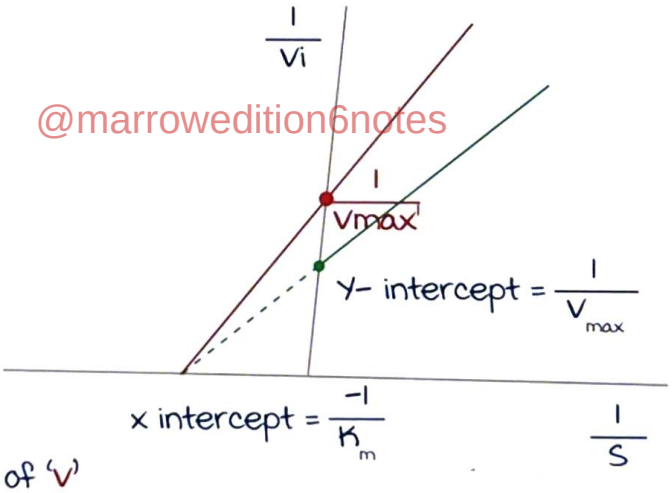
$\uparrow\downarrow$ Inhibitor (I)

Enzyme-Inhibitor complex (EI) $\overset{S}{\leftrightarrow}$ Enzyme-Inhibitor-Substrate complex (EIS) $\rightarrow$ E+ P at a very negligible rate.

Vmax reduces, K_m remains constant.



Lineweaver Burk plot of non-competitive inhibitor :



Shape of 'V'

Kinetic properties of non-competitive inhibition :

Vmax reduced $\rightarrow 1/V_{max}$ (y intercept) increases.

Km constant $\rightarrow (-1)/K_m$ (x intercept) unchanged.

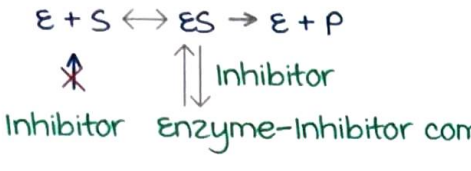
Examples :

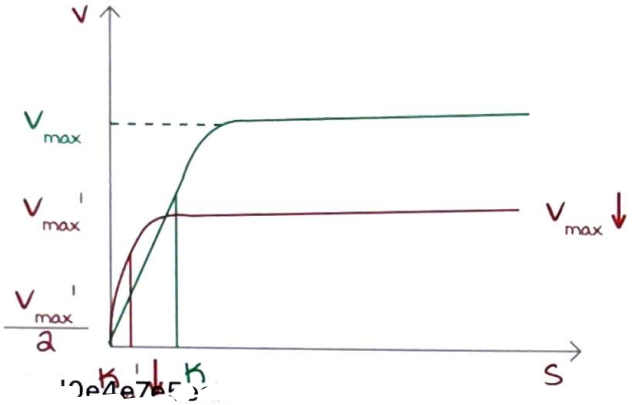
Inhibitor	Enzyme inhibited
CO, CN^-	Cytochrome c oxidase
Fluoride	Enolase
Iodoacetate	Glyceraldehyde 3-PO_4 dehydrogenase

Uncompetitive inhibition

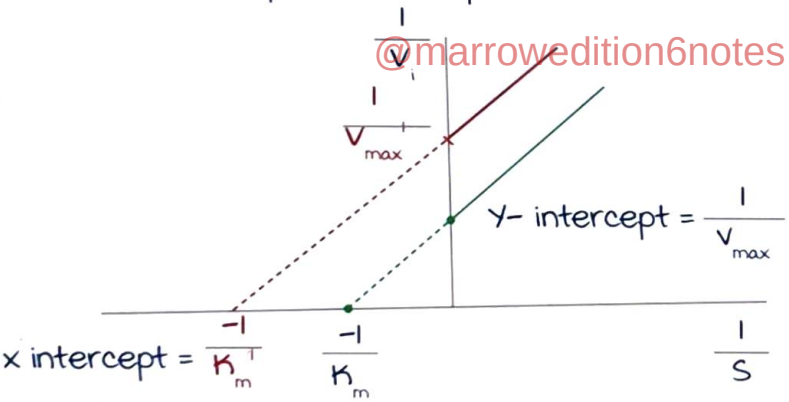
00:28:03

The inhibitor has no affinity towards free enzyme but it binds to the enzyme-substrate complex.





Lineweaver Burk plot of uncompetitive inhibitor :



Shape : Parallel.

X-intercept moves away from zero.

Y-intercept increases.

E.g., Placental ALP inhibited by phenylalanine.

Case scenario :

Following a short circuit, a house was on fire at night. In the morning all the family members were found dead but none of the bodies were charred. What is the reason behind the death of the all the family members following a fire?

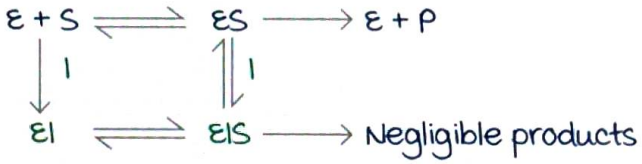
Ans. Fire → Increased carbon monoxide emission.

Active space

1. Carbon monoxide inhibits cytochrome c oxidase (complex IV).
2. CO also has higher affinity for Oxygen.

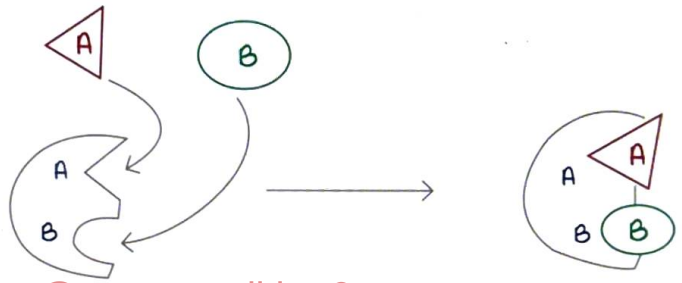
Mixed inhibition

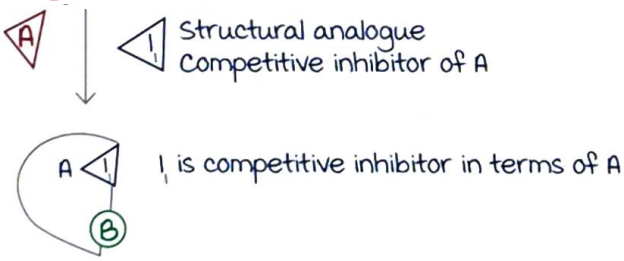
00:35:56

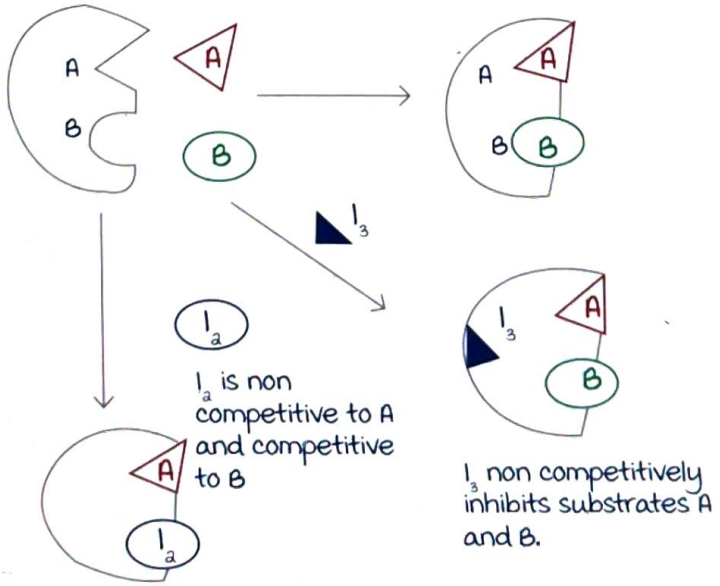


Kinetic property :

- Both V_{max} and K_m changes.
- V_{max} decreases.
- K_m can increase or decrease.







Non competitive inhibition in terms of substrate A. This can be reversed by adding more B but cannot be reversed by adding A.

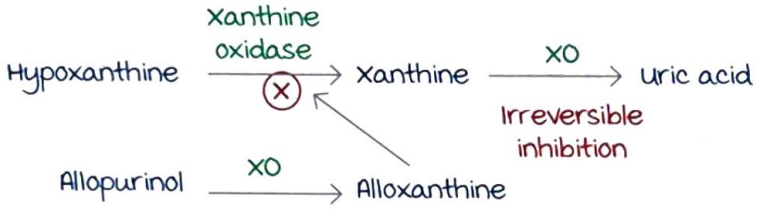
Active space

Suicide inhibition 00:47:54

The inhibitor is unreactive, it hijacks the mechanism of the enzyme to convert itself into a more reactive compound. This compound binds to the active site of enzyme irreversibly.
Also known as mechanism based inhibition.

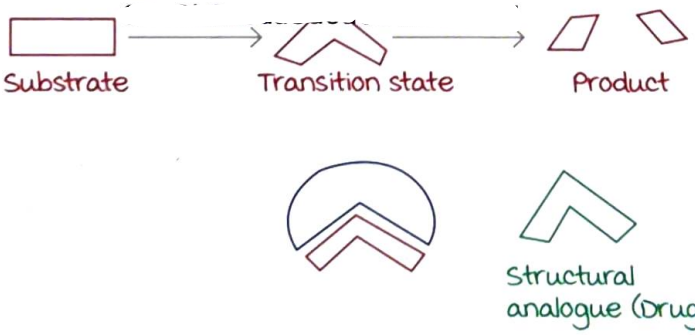
Examples :

- Allopurinol used for treatment of gout inhibits xanthine oxidase.



- Aspirin inhibits cyclooxygenase.
- Difluoro methyl ornithine inhibits ornithine decarboxylase. It is used in the treatment of trypanosomiasis.

Transition state analogue 00:51:58



Substrates gets converted into a transition state before forming the end product.
The active site of the enzyme is complementary to this transition state of the substrate.
Drugs have been developed which are structural analogues of transition state of substrates.

E.g. Penicillin is a structural analogue for the enzyme transpeptidase which is required for cell wall synthesis of bacteria.

Active space

MCQs

Q. An antibiotic newly developed is a close structural analogue of a substrate that participate in DNA synthesis of bacteria. Hence the antibiotic reduces the overall enzyme activity but it can be restored if excess of the substrate is added. Which one of the following best describes the antibiotic?

A. It's a suicide inhibitor.

B. An irreversible inhibitor.

C. A competitive inhibitor.

D. An uncompetitive inhibitor.

Q. Methanol is converted to formaldehyde which is highly toxic. Patients who have ingested methanol is treated with ethanol to inhibit methanol oxidation. Which explains the rationale of this treatment?

A. Ethanol is a structural analogue of methanol hence it can be a non-competitive inhibitor.

B. Ethanol is a structural analogue of methanol hence it can compete for the active site of ADH.

C. Ethanol can alter the V_{max} of oxidation of oxidation of methanol by ADH.

D. Ethanol compete with the formaldehyde binding site of the enzyme.

	Km	Vmax
Competitive	$K_m' = \alpha K_m$ (increase)	Constant
Non-competitive	Remains same	$V_{max}' = V_{max}/\alpha$ (decreases)
Uncompetitive	$K_m' = K_m/\alpha$ (decrease)	$V_{max}' = V_{max}/\alpha$ (decreases)
Mixed	Increase or decrease	$V_{max}' = V_{max}/\alpha$ (decreases)

Active space

ENZYME REGULATION

In 19th century, Claude Bernarde put forward the concept of enzyme regulation.

Regulation

Enzyme Quantity (long term)
- Control of enzyme synthesis (Induction and repression). E.g., Cholesterol synthesis, heme synthesis, cytochromes, urea cycle etc.
- Control of enzyme degradation

Enzyme Quality/intrinsic catalytic activity (short term)
- Allosteric regulation
- Covalent modification

Control of enzyme synthesis

00:03:50

Also known as induction and repression.

E.g. 1) Cholesterol synthesis :

More cholesterol in diet

↓ Represses

Gene for HMG CoA reductase (rate limiting enzyme)

2) Heme synthesis :

Succinyl CoA + Glycine

↓ ALA synthase

δ ALA

↓

Heme.

Increased heme will repress ALA synthase ~~whereas decreased~~ amount of heme will activate the enzyme.

Q. Phenobarbitone induces heme synthesis and aggravates porphyria. Why?

Ans. Phenobarbitone gets metabolised with the help of cytochromes, which are heme containing proteins.
When cytochromes get used up, amount of heme decreases and in turn induces ALA synthase. This causes accumulation of porphyrins (intermediate), which aggravates porphyria.

Active space

Control of enzyme degradation 00:08:30

- Short lived proteins e.g., regulatory proteins like cyclins.
- Aberrant/defective proteins.

These proteins must be degraded which takes place in a proteasomal machinery.

These proteins bind to ubiquitin.

↓

Degraded by Ubiquitin-proteasomal machinery.

If ubiquitin-proteasomal machinery is affected, the regulatory proteins like cyclins are lost, causing dysregulation of cell cycle which can lead to cancers.

Basic pathology of Alzheimer's and Parkinsonism :

Aberrant proteins.

↓

Defective ubiquitin-proteasomal machinery.

↓

Degradation does not occur.

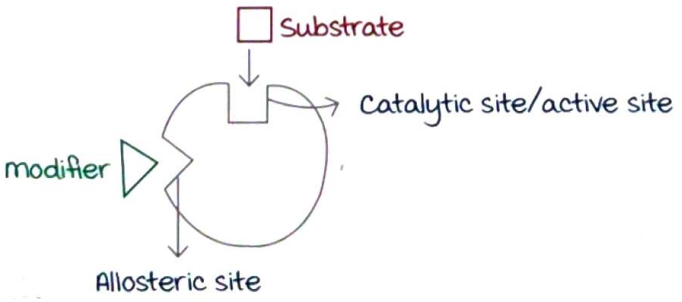
↓

Accumulation of defective proteins → Disease.

Allosteric regulation 00:12:48

Allos : "other".

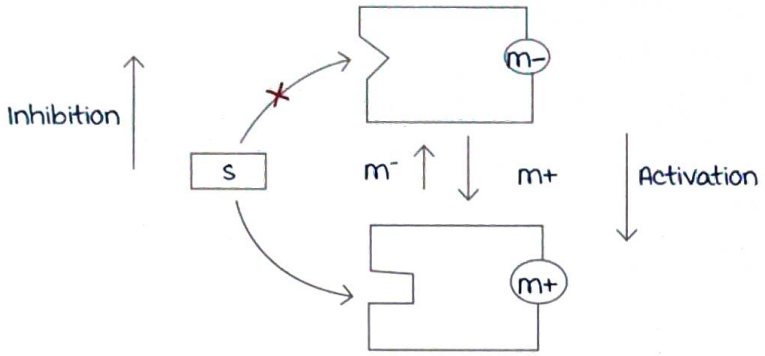
Allosteric enzymes are those enzymes whose activity at substrate binding site/active site is modulated by the presence of a modifier/effector.



Positive modifier : Activator (catalytic activity increases).

Negative modifier: Inhibitor (catalytic activity decreases).

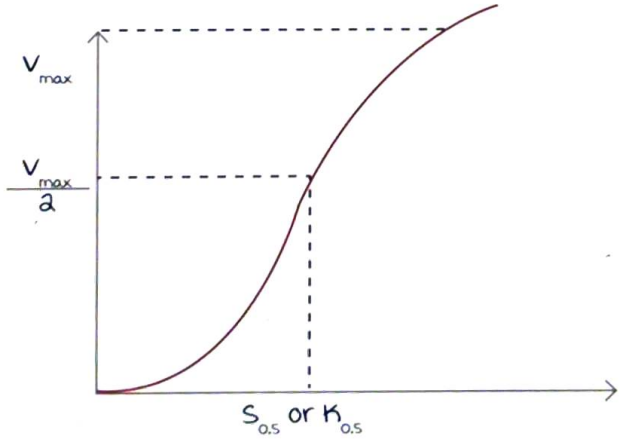
Allosteric activation : Positive modifier (m+) binds to an allosteric site and makes favourable conformational changes in the catalytic site, enabling binding of substrate to the active site.
Allosteric inhibition : Negative modifier (m−) binds to an allosteric site and makes unfavourable conformational changes in the catalytic site, which inhibits binding of substrate to the active site.



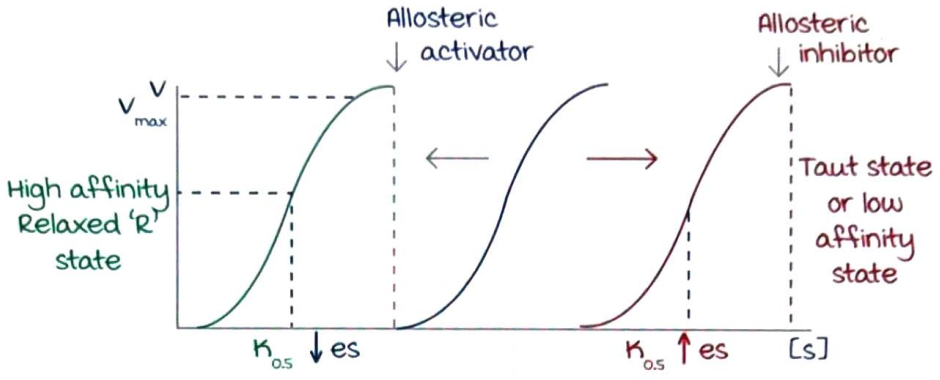
Features of allosteric enzymes (Correlate with hemoglobin) :

- Most allosteric enzymes are multi-subunit enzyme.
- They possess a quaternary structure.
- The modifier need not be a structural analogue of the substrate.

- Substrate saturation curve is a sigmoid curve.



- Follows a sigmoid kinetics as it exhibits cooperative binding.
  Binding of one substrate favour binding of other substrates to the same enzyme.
  Does not follow Michaelis-Menten hyperbolic kinetics. (Which result in a hyperbolic curve).



~~Addition of activator~~ → Graph moves to left, $K_{0.5}$ decreases → High affinity or R state.
Addition of inhibitor → Graph moves to right, $K_{0.5}$ increases → Low affinity or taut state.

Allosteric enzymes are of two types :

K series	V series
$K_{0.5}$ increases	$K_{0.5}$ constant
V_{max} constant	V_{max} decreases
Similar to competitive inhibition.	Similar to non-competitive enzyme inhibition.

Enzyme	Activator	Inhibitor
ALA synthase		Heme
Aspartate transcarbomylase (pyradimine synthesis)	ATP	CTP
HMG CoA reductase		Cholesterol
Acetyl CoA Carboxylase (fatty acid synthesis)	Citrate	Acyl CoA
Carbomyl phosphate synthase-I (urea synthesis)	N-Acetyl Glutamate	

Substrate favours forward reaction.
Product inhibits forward reaction.

Covalent modification

00:32:38

Definition : Enzymes are regulated by addition or removal of functional groups by making or breaking covalent bonds.

Types :

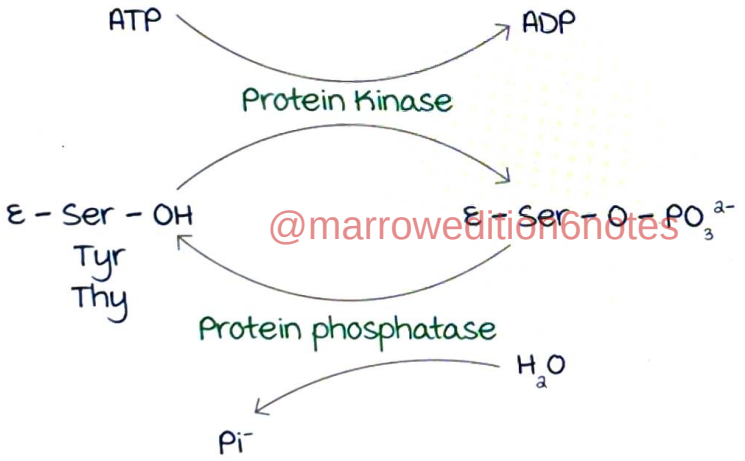
Irreversible : Partial proteolysis (zymogen activation).

- Trypsinogen → Trypsin.
  By chemically cleaving polypeptide chain.
- Chymotrypsinogen → Chymotrypsin.
- In blood coagulation, fibrinogen → Fibrin.

Reversible :

- Phosphorylation de-phosphorylation (most versatile).
- Acetylation.
- methylation.
- ADP ribosylation.
- Ubiquitin addition.

most common site for phosphorylation : OH group-containing amino acids (serine, threonine, and tyrosine).

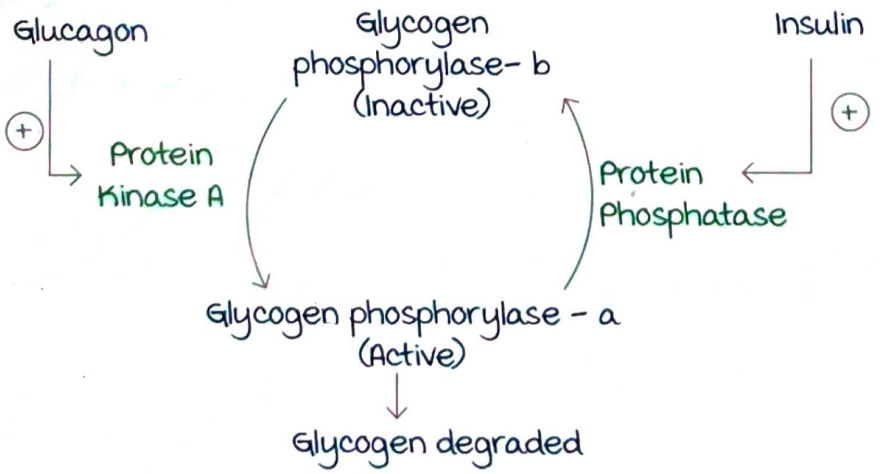


In process of glycogen degradation :

Glycogen phosphorylase degrades glycogen in its active phosphorylated state.

Insulin (fed state)

Glucagon (fasting state)



Active space

Acetylation :
Happens in lysyl residues.
Enzyme is acetyl transferase.
Acetylation happens in histone and other proteins.

Histone acetylated by Histone Acetylate Transferase (HAT) → Positive charges in histone reduced → Interaction of histone with DNA decreases → Euchromatin formation (less condensed form)
This is an epigenetic modification.

ADP ribosylation :
Donor : NAD+

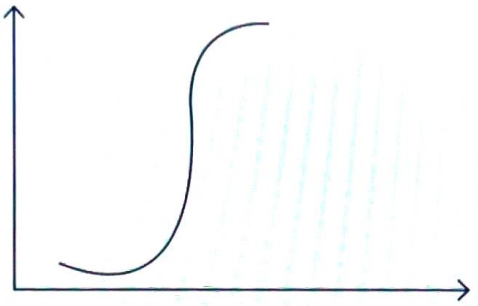
Q. Cholera toxin prevents G protein from binding to adenylyl cyclase. How?
Cholera toxin enables ADP ribosylation of G protein which prevents it from binding to adenyl cyclase.

Q. Insulin and glucagon regulate the blood glucose depending on our dietary status. How?
Covalent modification.
Insulin (fed state) dephosphorylates the rate limiting enzyme.
The enzyme active under insulin is active in dephosphorylated state.
Glucagon phosphorylates rate limiting enzyme.
Enzyme active under glucagon is active in its phosphorylated state.

Methylation :
Donor of methyl group : S adenosyl methionine.
Process of methylation : Epigenetic modification where cytosine residues are methylated → Decrease in gene expression.

How does Yersinia pestis cause plague?
Yersinia pestis induces production of enzyme called tyrosine phosphatase which disables the phagocytic machinery of macrophages.

Q. The substrate saturation curve given below characterises an allosteric enzyme system. Which of the following statement is true?



A. Allosteric modifier binds in a concentration dependant manner :
False, binding is not based on concentration.

B. Modifier can affect catalytic site by binding to allosteric site :
True.

C. Adding more substrate can displace the modifier :
False, because they are not structural analogues.

D. Allosteric modifier changes binding constant not Vmax :
False, as allosteric enzyme can belong to K series or V series where $K_{0.5}$ or V_{max} can vary respectively.

Compartmentation 00:50:22

Certain pathways take place exclusively in cytoplasm or mitochondria, or both. This mechanism provides a safe space for certain reactions.

E.g., Fatty acid synthesis → Cytoplasm.
Fatty acid degradation → mitochondria.

Active space

CLINICAL ENZYMOLOGY

Isoenzymes

00:01:18

Physically distinct form of the same enzyme.
Catalyze the same reaction.

Properties :

- May be the product of different genes.
  e.g. : Salivary amylase and pancreatic amylase.
- May be made of different subunits.
  e.g. : Lactate dehydrogenase → H4, H3M1, H2M2 etc.
  Creatine Kinase → CK- MB, CK-MM, CK- BB.
- Different electrophoretic mobility.
  e.g. : Fastest is LDH-1, Slowest is LDH-5.
- Differ in heat stability.
  e.g. : 2 forms of alkaline phosphatase → Heat stable and heat labile.

- Difference in Km or substrate specificity.
  e.g. : Different affinity for hexokinase enzymes from liver and pancreas.
- Cofactor requirement varies.
  e.g. : Cytoplasmic isocitrate dehydrogenase needs NADP as co-enzyme ; mitochondrial isocitrate dehydrogenase needs NAD+ as co-enzyme.
- Different tissue localization.
  e.g. : LDH-1 in heart, LDH- 2 in RBC.

Enzymes :

Functional enzymes :

Have a function in the blood.
e.g. : Lipoprotein lipase, coagulation factors.

Non- functional enzyme :

No function in blood.
Seen in blood due to normal wear and tear of organs, or injury to them.

used as diagnostic biomarkers.

- Helps to find the location of the disease.
- Reflects the nature and severity of the disease.

e.g. : A reversible inflammation in the cells increases cell membrane permeability, and the cytoplasmic enzymes are seen in blood.

In irreversible cell injury and necrosis, mitochondrial membrane permeability is also increased, such that mitochondrial enzymes are also seen in blood.

Lactate dehydrogenase

00:10:20

Pyruvate $\xrightarrow{\text{Lactate dehydrogenase}}$ Lactate.

Location → Cytoplasm.

Structure → Tetramer with two types of subunits H and M.

Based on the subunits, different isoenzymes are seen.

Isoenzyme	Subunits	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	Spleen, lungs, lymph node, leucocyte, platelet.	20
LDH- 4.	H_1M_3	Slow	Liver, skeletal muscle (LDH- 4 predominant).	10
LDH- 5.	M_4	Slowest	Liver, skeletal muscle	5

LD- 6 → Seen in severely ill patients.

LDX/ LDC → Seen in post-pubertal testes.

Blood analysis gives the total amount of LDH activity. To know the specific isoforms, electrophoresis is done.

Active space

Creatine kinase

00:16:29

Creatine $\xrightarrow{\text{Creatine Kinase}}$ Creatine phosphate.

Location : cytoplasm.

Structure : Dimer with 2 subunits, M and B.

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	Skeletal muscle	80%

CK-Mt : Creatine Kinase in mitochondria.

Alkaline phosphatase (ALP)

00:20:56

Removes inorganic phosphate by adding water (hydrolases) in an alkaline pH.

Isoenzymes :

- α-1- ALP :
  Located in epithelial cells of biliary canaliculi.
  Marker of extra-hepatic cholestasis (marked elevation).
  e.g. : Stone in common biliary duct, carcinoma head of pancreas obstructing the common bile duct.
- α-2- heat labile ALP :
  Located in hepatocytes.
  Marker of parenchymal injury.
  Mild to moderate elevation.
  e.g. : Viral hepatitis.
- α-2- heat stable ALP :
  Placental form.
  Most heat stable ALP.
  Inhibited by phenyl alanine.
- Pre-β ALP :
  Seen in osteoblasts.
  Marker of bone formation.
  Elevated in Paget's disease, vitamin D deficiency, osteomalacia, 1° and 2° hyperparathyroidism.
  ALP is normal in multiple myeloma.

- Gamma ALP :
  Seen in mucosa of intestinal cells.
  Elevated in Ulcerative colitis.
  Cleared by sinusoidal cells of liver.
  In case of hepatitis, it will be elevated.
- Leucocyte ALP :
  Marker of leucocyte disorders like leukemia.
- Regan isoenzyme :
  Also called carcino-placental enzyme.
  Similar to placental ALP.
  Germ cell origin.
  Depressed in conditions of malignant tumors.
- Nagao isoenzyme.
- Kasahara isoenzyme.

} Seen in malignant tumors.

Cardiac Biomarkers 00:30:36

Rise when there is injury to heart.
Enzymes and non-enzymatic compounds.

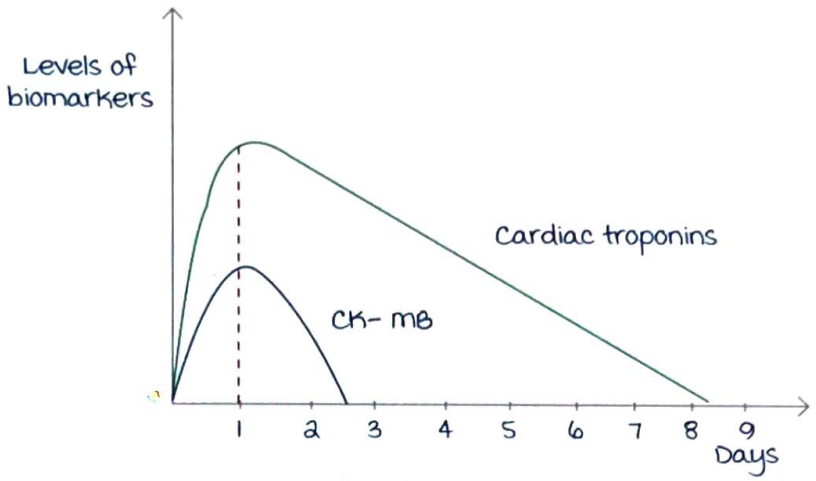
Name	Rise	Peak	Return to base line
CK- MB	4- 8 hours	24 hours	48- 72 hours
Troponin T	4-6 hours	24 hours	7- 10 days
Troponin I	4-6 hours	24 hours	7- 10 days
LDH	24 hours	3- 6 days	2 weeks
AST	12 hours	48 hours	4- 5 days

LDH and AST are not clinically used.

Cardiac troponins are more specific than CK- MB.
First biomarker to rise → myoglobin (2 hours) → Non specific.
High sensitivity monoclonal Ab based assay :
Detects < 1 ng/L of troponins.

Flipped LDH pattern :
In normal individual → LDH-2 > LDH- 1.
In MI → LDH-1 > LDH-2.

Active space



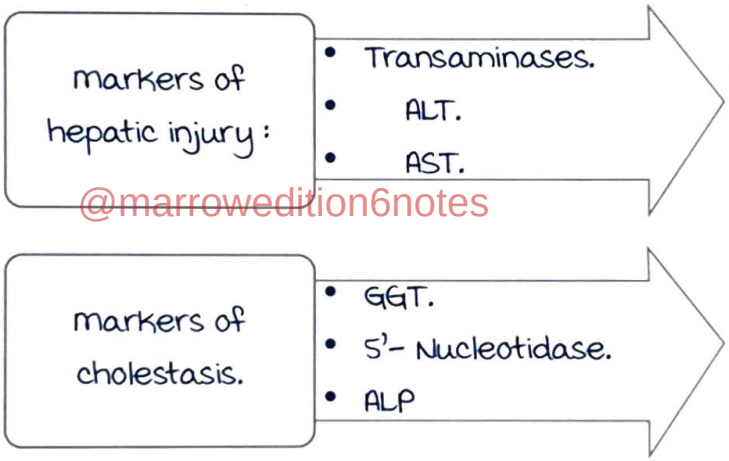
Brain natriuretic peptide (BNP) :

Not a marker of ischemia.

marker of volume overload and circulatory failure.

Enzyme profile in liver disease

00:38:19



markers of hepatic injury :

Tissue	AST	ALT
Heart	7800	450
Liver	7100	2850
Skeletal muscle	5000	300
Kidney	4500	1200
Pancreas	1400	130
Spleen	700	80
Lungs	500	45
Erythrocyte	15	7

From the previous table.

ALT is more specific for liver injury.

(Very high values only for liver injury)

AST has very high values for heart, liver, skeletal muscle, kidney and pancreatic injuries- non-specific.

ALT is exclusively intra-cytoplasmic.

AST is located in cytoplasm and mitochondria.

AST : ALT ratio (AAR) :

AAR < 1 : rise in ALT > Rise in AST.

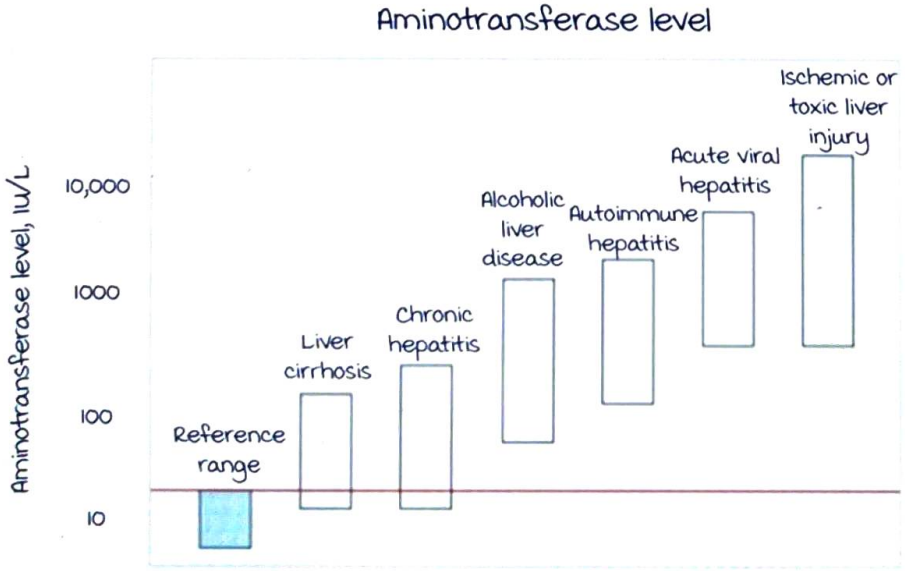
- Chronic viral hepatitis.
- Non-alcoholic fatty liver disease.
- Toxic hepatitis.
- Acetaminophen toxicity.

AAR > 1 :

- Alcoholic hepatitis.

  Damage to mitochondria results in rise of AST levels.
- Hepatic cirrhosis.

  AST levels increase as the sinusoidal cells of liver are injured, and AST is not cleared from the plasma.
- Liver neoplasia.



Acute injury or inflammation of liver → marked elevation of transaminases.

Chronic conditions → mild elevation of transaminases.

All liver enzymes within normal limits in an asymptomatic person, with only mild elevation of ALT → Fatty liver.

Active space

Markers of cholestasis

00:48:15

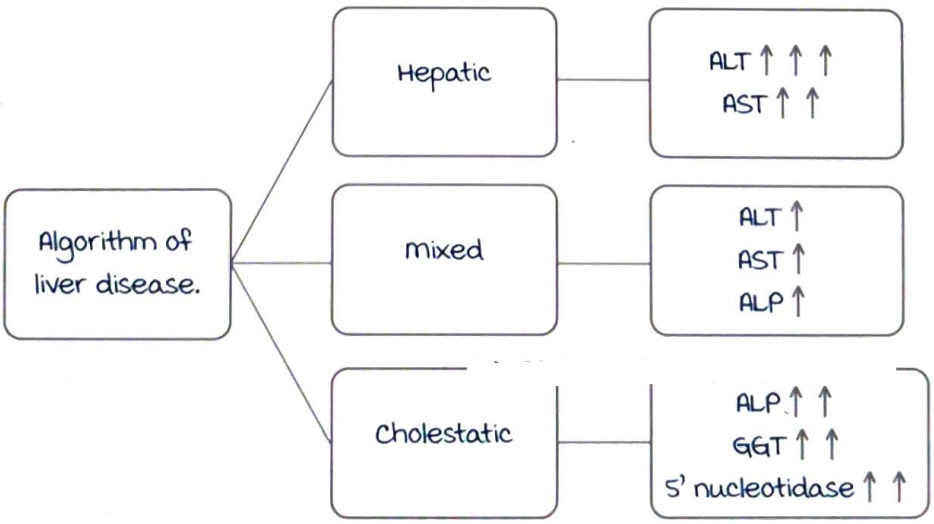
Alkaline phosphatase (ALP) :
Present in the cell membrane of epithelial cells of biliary canaliculi.
α -I- ALP.
Marked elevation signifies extra- hepatic obstruction.
Non- specific marker.

5' nucleotidase :
Specific marker of cholestasis.
Present in cell membrane of biliary canalicular cells.

Gamma glutamyl transpeptidase (GGT) :
Located in cell membrane and smooth endoplasmic reticulum (microsomes) of the hepatobiliary system.
Less specific for cholestasis.
Elevated in intra-hepatic and extra- hepatic cholestasis.
Marker of :

Alcoholic hepatitis.
Heavy drinkers.
— Involvement of microsomes.

Drugs like phenytoin and phenobarbitone.



Enzyme profile in prostate

00:55:15

Acid phosphatase :
Inhibited by tartrate ion → Tartrate labile.
(That is found in spleen, leucocytes → Tartrate resistant).

Not a specific marker (also elevated in osteoclastic injuries).

Prostate specific antigen :

Also called Kallikrein related peptidase-3 (KLK- 3).

Cut off → <4ng/ml.

Enzyme profile in pancreatic disease 00:56:55

Serum Amylase :

Non- specific as it is elevated in other intra-abdominal conditions, salivary gland disorders, genitourinary disorders like ectopic pregnancy.

Lipase :

Elevated almost 5000 times.

Markers of bone :

Bone formation	Bone resorption
Originates from osteoblasts. • Pre- beta ALP. • Osteocalcin. • Pro- peptide of type I collagen.	Originates from osteoclasts. • N- telopeptide of type I collagen. • C- telopeptide of type I collagen. • Urine free deoxypyridinoline.

Novel markers of acute kidney injury :

- Kidney injury molecule (KIM- 1).
- Neutrophil gelatin associated lipocalin (NGAL).
- IL- 18.
- ALT.
- Glutathione- S- transferase.
- GGT.
- Beta 2 microglobulin.
- Alpha 2 macroglobulin.
- Retinol binding protein.
- Cystatin C.
- Microalbumin.
- Osteopontin.
- Liver fatty acid binding protein.
- Sodium hydrogen exchange isoform.
- Exosomal fetuin.

Marker enzyme of cell organelle

01:00:10

Plasma membrane :

- 5' nucleotidase.
- Adenylyl cyclase.
- Na^+K^+ ATPase.

Endoplasmic reticulum → Glucose-6-phosphatase.

Golgi apparatus → Galactosyl transferase.

Mitochondria → ATP synthase.

Lysosomes →

- Cathepsin.
- Acid phosphatase.

Clinical scenarios

01:01:20

Q : A 50 year old man presented with complaints of squeezing type of chest pain while he climbed up the stairs. On examination, he is anxious and restless, tachycardia present. BP 180/110mm Hg. His ECG shows no ST segment elevation. What is the next investigation to be done?

A. Serum LDH.
B. Serum CK- MB.
C. cTnT.
D. ProBNP.

Suspicion of MI. Troponins elevates earlier.

Q : A 70year old male patient was admitted with generalized weakness, easy fatiguability, itching and jaundice for one month. On examination, patient was emaciated and scratch marks were seen all over the body. He was jaundiced and a mass was palpable in the upper abdomen. Which is the best enzyme marker of this patient?

A. Alanine aminotransferase.
B. Alkaline phosphatase.
C. 5' nucleotidase.
D. Aspartate aminotransferase.

Best marker is 5' nucleotidase.

Q : A 16year old female patient was admitted in medical ward with fever, anorexia, vomiting, generalized weakness and jaundice. She gives history of recent travel to Bangalore. Acute viral hepatitis was diagnosed. Which is the non- functional enzyme elevated in this case?

A. Alkaline phosphatase.
B. Alanine aminotransferase.
C. Gamma glutamyl transferase.
D. Creatine Kinase.
E. Lactate dehydrogenase.

Q : A 38year old lady was admitted following a Road Traffic Accident. During her hospital stay an intern in the ward while going through her case file noticed that her serum ALP was elevated and Skull X-ray showed-Osteoporosis circumscripta, thickening of diploic areas and sclerosis of portions of skull bones.

Out of his curiosity he sends a blood sample to Biochemistry lab. Which of the following is the Biomarker he sent?

A. Serum osteocalcin.
B. ProBNP.
C. ANP.
D. KIM-1.
E. Exosomal Fetuin-A.

Q. A 40year old female with history of inadvertent use of NSAIDs develop oliguria. Her serum creatinine and BUN elevated and Acute Kidney Injury [AKI] was diagnosed. Which of the following are novel markers of AKI which can be done in this case?

A. N terminal pro BNP.
B. Clusterin.
C. Neutrophil Gelatinase Associated Lipocalin.
D. Cross linked N-Telopeptide.

Active space

CHEMISTRY OF CARBOHYDRATES

Definition 00:02:34

Hydrates of carbon.

General formula : $C_n(H_2O)_n$

where n is the no. of carbon atoms.

They are aldehyde/keto derivatives of polyhydroxy alcohol.

Simplest carbohydrate : Glyceraldehyde, Dihydroxyacetone.

Aldehyde group at C_1 Keto group at C_2

Parent alcohol : Glycerol.

Classification of carbohydrates 00:06:15

Monosaccharide : Single sugar unit.

Disaccharide : Two sugar units.

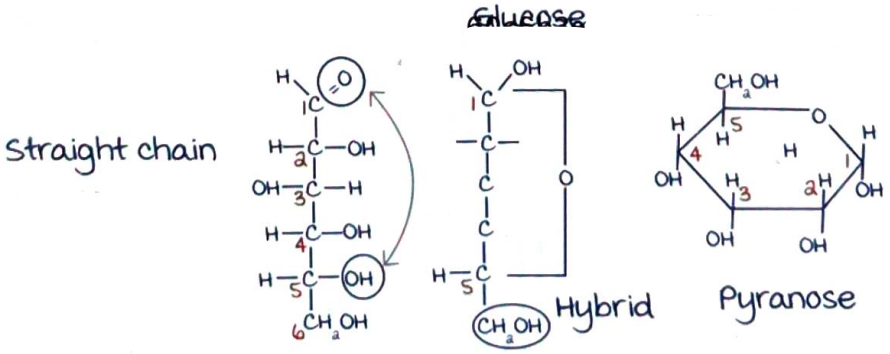
Oligosaccharide : 3–10 sugar units.

Polysaccharide : > 10 sugar units.

Monosaccharide :

No. of carbon atoms	Aldoses	Ketoses
3C : Trioses	Glyceraldehyde	Dihydroxyacetone
4C : Tetroses	Erythrose	Erythrulose
5C : Pentoses	Ribose Xylose	Ribulose Xylulose
6C : Hexoses	Glucose Galactose Mannose	Fructose

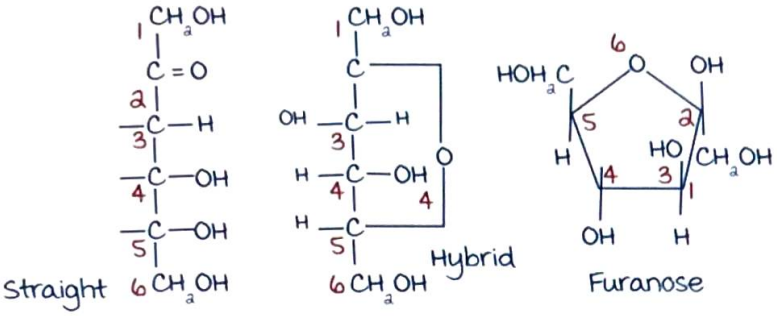
Ring structures of glucose and fructose 00:09:16



Active space

In solution, 99% glucose and fructose exists in ring form.

Fructose :



Disaccharides

00:15:44

Depending on which carbon atoms are engaged in the glycosidic bond formation, they are divided into reducing and nonreducing disaccharides.

Non reducing : Functional groups are engaged in linkage, and no free group present.

Reducing : Free functional group present.

Examples of reducing disaccharides :

Name	Monomer units	Linkage
Maltose	Glucose + Glucose	α1 – 4 glycosidic linkage
Isomaltose	Glucose + Glucose	α1 – 6 glycosidic linkage
Lactose	Galactose + Glucose	β1 – 4 glycosidic linkage
Lactulose	Galactose + Fructose	

Examples of non-reducing disaccharides :

Name	Monomer units	Linkage
Sucrose	Glucose + Fructose	α1 – β2 linkage
Trehalose	Glucose + Glucose	α1 –1 linkage

Polysaccharides

00:21:34

Homopolysaccharide
One type of monomer

Heteropolysaccharide
> 1 type of monomer units.

Glycogen :

- Storage carbohydrate in humans/animals.
- made of α D glucose.
- Branched polymer.

Active space

- Straight chain : α 1,4 linkage.
- At branches : α 1,6 linkage.
- Starting point has a reducing end.
- Multiple non reducing ends present where glucose molecules are released present.

Starch : Storage carbohydrate in plants.

Amylose
(Unbranched : Linear, α1 - 4)

Amylopectin
(Branched, α1 - 4; α1 - 6)

Dietary fibres

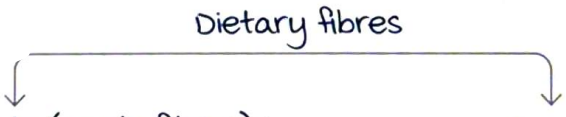
00:27:27

Also called non starch polysaccharide.

Definition : Remnants of edible part of plants and analogous carbohydrates that are resistant to digestion and absorption by human small intestine but completely or partially fermented by human large intestine.

Human intestines lack the enzyme cellulase to hydrolyse the β 1-4 linkage. Most of cellulose is made of β D glucose

Usually fermented to short chain fatty acids like acetic acid, butyric acid & propionic acid (energy source).



Dietary fibres

Insoluble (crude fibres) :
Cellulose
Hemicellulose
Lectin

Soluble :
Pectin
Gums
Mucilage

Lectin cannot be digested, absorbed or fermented.

RDA : 40 g/2000 Kcal.

Energy released per gram : 2 Kcal/g of dietary fibre.

Uses :

- Act as substrates promoting colonisation of probiotic strains (hence are prebiotics).
- Faecal bulking and softening.
- Regularity to bowel movement.
- Reduce cholesterol by binding to bile salts.

Active space

- Favours satiety.
- Fibre particularly gums (fenugreek) and pectin reduce post prandial blood glucose level in the blood.

Inulin :

Fructose, Fructosan.

Inulin clearance test : used to access glomerular filtration test (ideal) but not used now.

Chitin : In exoskeleton of crustaceans.
Made of N-acetyl D glucosamine.

Pectin : Heteropolysaccharide.
Predominantly made of galacturonic acid.

Dextrin : Hydrolytic product of starch.

Dextran : Polysaccharide of α D glucose.

- Used as plasma volume expander.
- Synthetic dextran : Size exclusion chromatography.
- Dental plaque.

Isomerism in carbohydrates

00:38:34

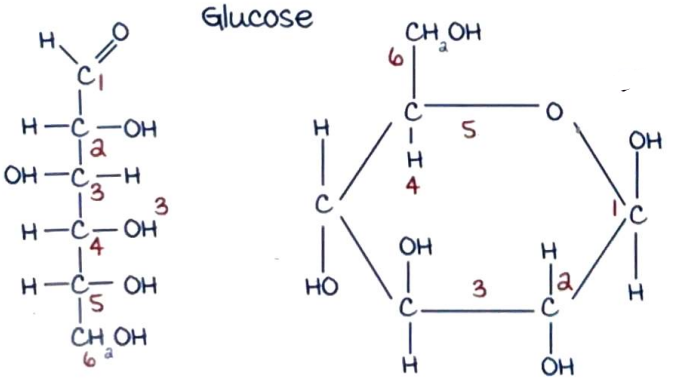
Isomers are compounds with same molecular formula but different structural organisation.

Structural isomerism/
Stereoisomerism :
D & L isomerism (enantiomers)
Anomerism
Epimerism

Optical isomerism :
Dextro or levo rotatory

Asymmetric carbon atom :
4 valencies of carbon atom occupied by 4 different groups.

Glucose



Active space

In straight form of glucose : 4 asymmetric C atoms.

In glucopyranose form : 5 C atoms.

Leber van't Hoff formula : 2^n, where n stands for no. of asymmetric carbon atoms.

D and L isomerism/enantiomers

00:38:34

The organisation of different groups attached to carbon atom changes at the penultimate/reference carbon atom.

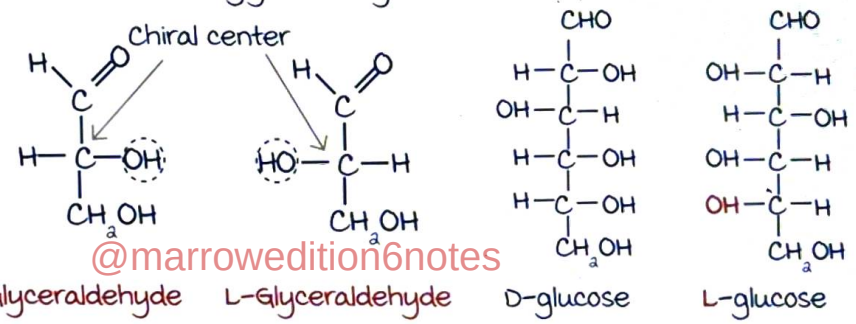
They are mirror images.

most carbohydrates exist in D form.

most amino acids exist in L form.

In glucose, it depends on position of H and OH on 5th C atom.

D and L forms of glyceraldehyde



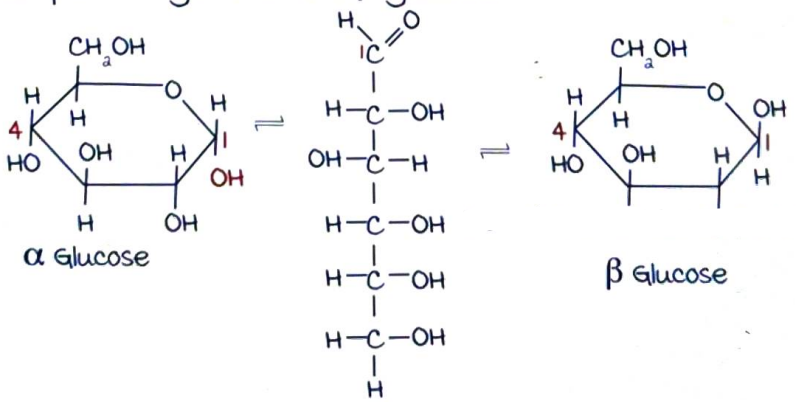
D-Glyceraldehyde L-Glyceraldehyde D-glucose L-glucose

Anomerism

00:49:25

Isomerism at the functional carbon atom.

Examples : α glucose and β glucose.



α Glucose β Glucose

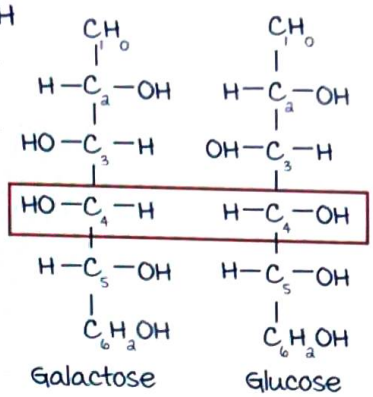
α-D-fructofuranose β-D-fructofuranose

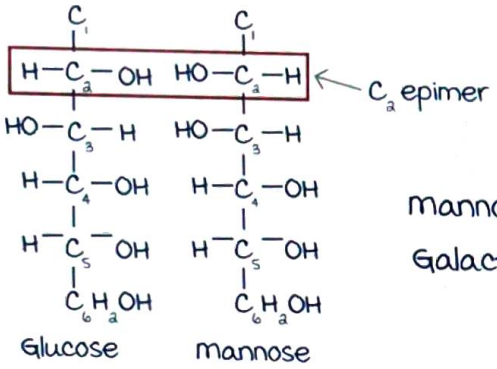
Active space

Epimerism

00:53:47

Difference in orientation of H and OH group at a single carbon atom other than the functional and penultimate carbon atom.





Mannose : 2nd epimer of glucose.

Galactose : 4th epimer of glucose.

Allose : 3rd epimer of glucose.

Diastereoisomers :

Difference in orientation of H and OH groups in > 1 carbon atom other than the functional and penultimate carbon atom.

Mannose and galactose are diastereoisomers.

Optical isomerism

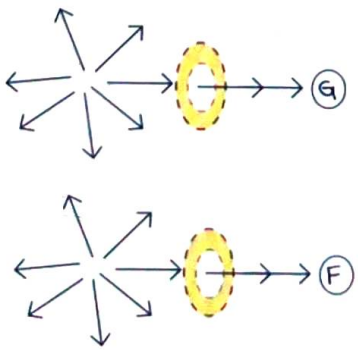
00:57:41

Ability to rotate the plane polarised light.

Occurs due to asymmetric carbon atom.

- Glucose : Right/clockwise direction : Dextrorotation ('d' glucose or '+'). Hence glucose is known as dextrose.
- Fructose : Left/anticlockwise direction : Levorotation ('l' glucose or '-').

Sucrose is initially dextrorotatory and after it is hydrolysed, becomes levorotatory. Hence aka invert sugar.



Active space

One liners

01:01:42

- Monosaccharide with single asymmetric carbon atom : Glyceraldehyde.
- Ketoses have 1 asymmetric carbon less than corresponding aldoses.
- Carbohydrates with no asymmetric carbon atom : Dihydroxy acetone.
- Amino acid with no asymmetric carbon atom : Glycine.
- Most predominant form of glucose : β D glucopyranose.
- Most carbohydrate exist in D form.
- Most amino acids exist in L form.

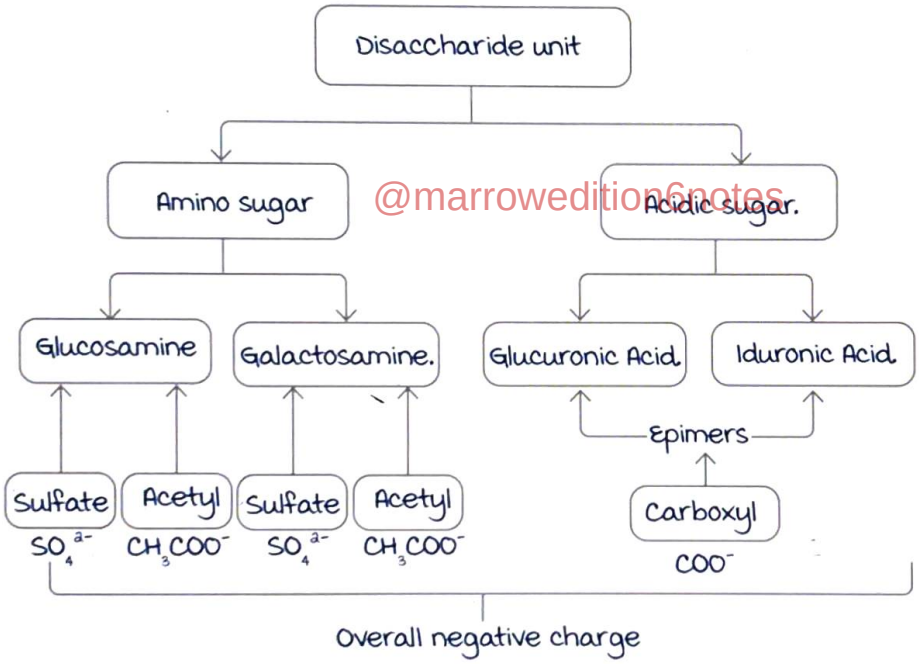
GLYGOSAMINOGYCANS AND MUCOPOLYSACCHARIDOSES

Introduction

00:00:08

Repeating disaccharide unit.

Also called as Heteropolysaccharide/ Mucopolysaccharide
Long unbranched heteropolysaccharide consisting of a repeating disaccharide unit.



Properties of GAGs :

- Negatively charged/Polyanions.
- Form hydrogen bond with water.
- Absorb water and form a hydrated gel acting as a lubricant.
  1. Compressibility of cartilage.
  2. Mobility and resilience of joints.
- Occupy large space → molecular seive
- Selective transport of molecules.

Active space

GAG.	Disaccharide repeating unit.	Location.
Hyaluronic acid.	N-Acetyl Glucosamine & Glucaronic acid.	Skin, Synovial fluid, Bone, Cartilage, Loose connective tissue.
Chondroitin Sulfate.	N-Acetyl Galactosamine & Glucaronic acid.	Cartilage, Bone, CNS.
Keratan Sulfate.	N-Acetyl Glucosamine & Galactose.	Cornea, Cartilage, Loose connective tissue.
Heparin.	Glucosamine & Iduronic acid.	Mast call, Liver, Lung, Skin.
Heparan Sulfate.	Glucosamine & Glucaronic acid.	Skin, Kidney basement membrane.
Dermatan Sulfate.	N-Acetyl Galactosamine & Iduronic acid.	Skin.

Characteristics of major GAGs

00:12:38

Chondroitin Sulfate :

- Most abundant GAG.
- Compressibility of cartilage.

Keratin Sulfate :

- Most heterogenous GAG.
- Repeating unit is Galactose and hence no Uronic acid
- KS I : Cornea (Corneal transparency).
- KS II : Loose connective tissue.

Dermatan Sulfate :

- Widely distributed in the skin.
- Structure of Sclera.
- Atherogenic GAG : Synthesized from arterial smooth muscle cells, hence attaches to low density lipoproteins (LDLs). These LDLs are carriers of bad cholesterol.

Heparan Sulfate :

- Present in synaptic vesicles.
- Present in the renal basement membrane making it responsible for the charge selectiveness of the glomerular basement membrane.
- Gives a net negative charge to the basement membrane, therefore doesn't let negatively charged Albumin to pass through.
- Anchors lipoprotein Lipase to the endothelial surface.
- Acts as plasma membrane receptor.

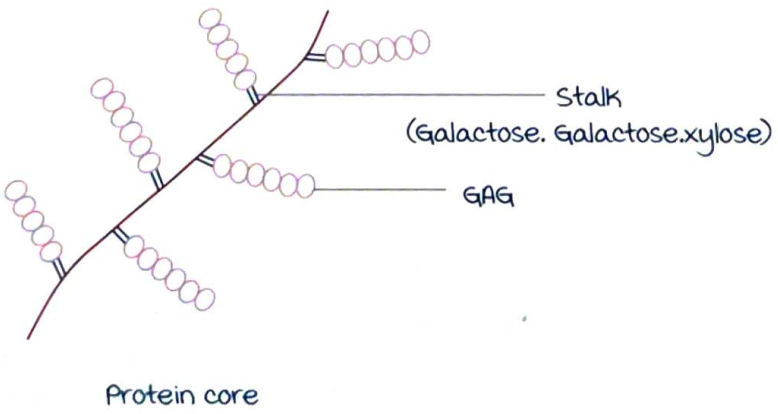
Heparin :

- The only intracellular GAG.
- Inj. Heparin dislodges lipoprotein lipase from its anchoring site.
- Naturally occurring anticoagulant which binds to Anti-Thrombin III.

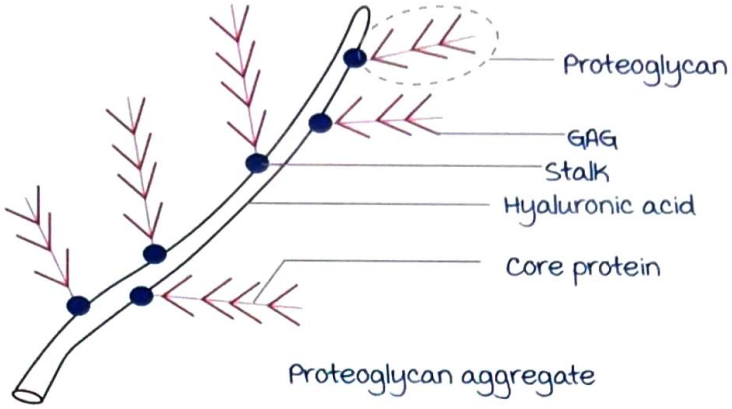
Hyaluronic Acid.

- Not covalently bound to protein.
- Polysaccharide.
- No sulfate group.
- Helps in cell migration (Wound Repair, Embryogenesis, Morphogenesis, Metastasis).

Proteoglycan Structure 00:21:14



Active space



GAG(95%) + Protein(5%) = Proteoglycan.
Bottle brush shape.

Mucopolysaccharidoses (MPS) 00:25:54

GAGs are synthesized in the Endoplasmic Reticulum and Golgi Apparatus.
They are degraded in the Lysosomes.
MPS are a group of disorders affecting the degradation of GAG in the Lysosomes, hence leading to intra lysosomal accumulation.

They are essentially, Lysosomal Storage Disorders.
Inheritance is Autosomal Recessive (Except Hunter's Disease).
Most common MPS : Sanfilippo> Hurler > Hunter .

General features of MPS :

- Coarse facial features.
- Frontal bossing.
- Depressed nasal bridge.
- Corneal clouding.
- Gingival hypertrophy.
- Macroglossia leading to recurrent URTIs and hearing loss.
- Claw Hand.
- Intellectual disability.
- Visceromegaly leading to umbilical or a inguinal hernia.
- Short stature.

Radiological findings :

- Dysostosis multiplex.

- Beaking of vertebrae.
- Tip of metacarpal degeneration leading to Bullet shaped phalanx.

Hurler's Disease/ MPS I-H :

- Mutation of IDUA Gene.
- Affecting α-L Iduronidase enzyme.

Scheie's Disease / MPS I-S :

- Mutation of IDUA Gene.
- Affecting α-L Iduronase enzyme but the enzyme activity is partially preserved.
- Normal intelligence.
- Presenting age is after 5 years.

Hunter Disease/ MPS II ;

- Mutation of IDS Gene.
- Affecting Iduronate Sulfatase enzyme.
- X-Linked Recessive condition, hence only males are
- affected.
- Clear Vision. (No corneal clouding)

Natowicz Syndrome/ MPS IX : Affecting Hyaluronidase enzyme.

Disease	Enzyme defect	mental Deficiency	Corneal Clouding
MPS I-H (Hurler)	L Iduronidase	Present	Present
MPS I-S (Scheie)	L Iduronidase	Absent	Present
MPS II (Hunter)	L Iduronate Sulfatase	Present	Absent
MPS III (Sanfilippo)	Enzyme that degrades Heparan Sulfate	Present	Absent
MPS IV (Morquio)	Galactosamine-6-Sulfatase, Beta-Galactosidase	Absent	Present
MPS VI (Moroteaux Lamy)	N-Acetyl Galactosamine-4-Sulfatase	Absent	Present

Active space

Disease	Visceromegaly	Short Stature	Dysostosis Multiplex	Leukocyte Inclusion (Reilly Body Inclusion)
MPS I-H (Hurler)	Present	Present	Present	Present
MPS I-S (Scheie)	Present	Present	Present	Present
MPS II (Hunter)	Present	Present	Present	Present
MPS III (Sanfilippo)- mc mps	Present	Present	Present	Present
MPS IV (morquio)	Absent	Present	Present	Absent
MPS VI (morateaux-Lamy)	Present	Present	Present	Present

Treatment Modalities

00:43:03

Stem cell Therapy :

MPS I-H and MPS I-S.

MPS VI.

Enzyme Replacement Therapy :

MPS I : Aldurazyme.

MPS II : Elaprase.

MPS VI : Naglazyme.

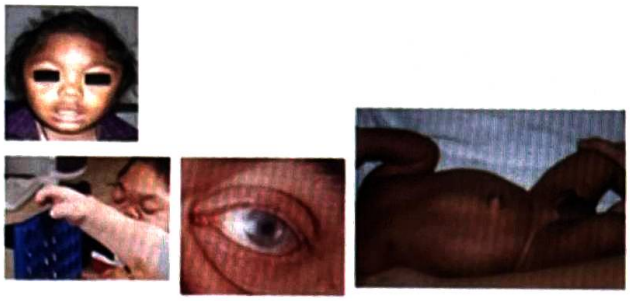
MPS IV : Recombinant Galactosamine Sulfatase (GALN) ; Still under trial.

Substrate Reduction Therapy :

MPS III : Flavinoids.

Clinical scenario 1 :

A 6 year old mentally retarded girl with protuberant abdomen, Umblical Hernia, short stature



Answer : Mucopolysaccharidoses.

Explanation : Mental Retardation/ Intellectual disability.

Umbilical Hernia.

Short stature.

Corneal Clouding.

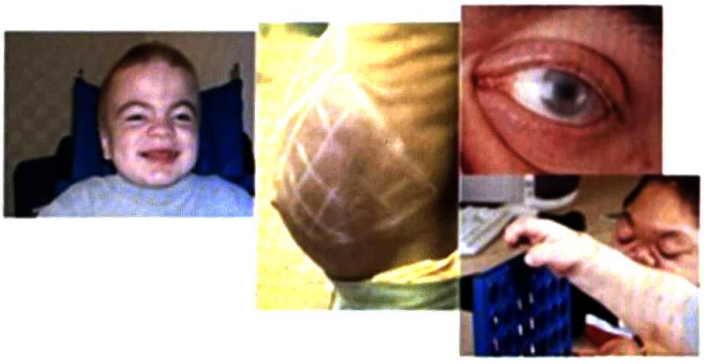
Coarse Facial features :Frontal bossing, depressed nasal bridge, Macroglossia.

Claw hand.

All of which are features of MPS.

Clinical scenario 2 :

A 16 year old boy with short stature, coarse facial feature, and hirsuitism and normal intelligence



Answer : Scheie's Disease

Explanation : 16 year old boy with Short Stature, Hirsuitism, Normal intelligence, Coarse facial features, Corneal clouding Abdominal protrusion Claw hand If L-Iduronidase enzyme is affected, we can conclude it to be Scheie's Disease.

Active space

Clinical scenario 3 :

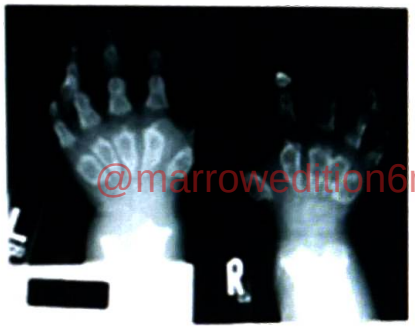
Q. 2 year old male presented with coarse facial features, intellectual disablility, prominent abdomen and clear vision. What is the diagnosis?

Answer : Hunter Syndrome (X-linked Recessive)

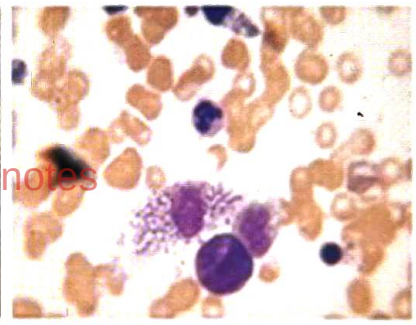
Explanation : its a male child with clear vision which indicates Hunter syndrome as it exclusivel spares cornea and is a X-linked recessive disease.

Clinical scenario 4 :

A 6 year old mentally retarded girl with protuberant abdomen, umbilical hernia, short stature along with following findings seen.



Bullet shaped middle phalanx



Reilly body inclusion

Answer : Not MPS IV

Explanation :

Leukocyte inclusion bodies called Reilly bodies are seen. Bullet shaped middle phalanx is also seen. These features are exclusively absent in MPS IV.

GLUCOSE TRANSPORTERS

Types of Glucose transporters 00:01:54

Sodium dependent Glucose transporters : SGLT.
Sodium independent Glucose transporters : GLUT.

Glucose is a hydrophilic compound. It must be transported through the hydrophobic plasma membrane. Hence Transporter is required.

SGLT 00:04:28

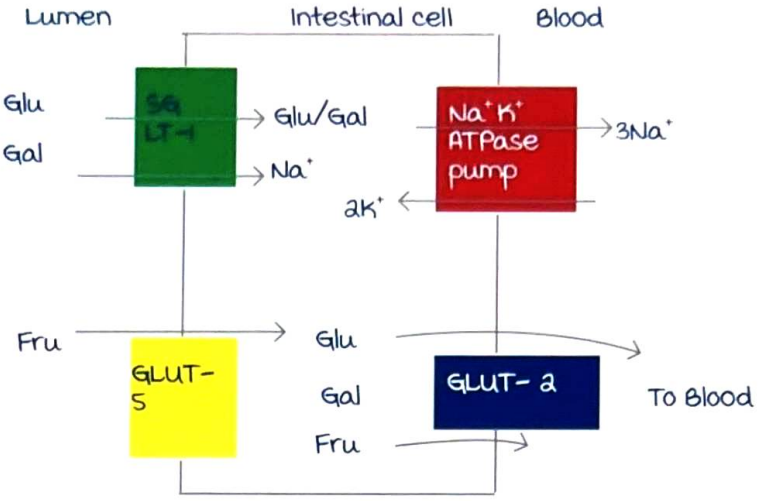
Types	Location	Function
SGLT-1	Intestine : Luminal side. Proximal Renal tubules.	Absorption of glucose.
SGLT-2	Proximal Renal tubules.	Absorption of glucose.

SGLT-1 : Characteristics

- Sodium - dependent.
- Unidirectional transport.
- Secondary active transport.
- Is a symport : Transports Glucose along with Sodium.

Na^+ K^+ ATPase pumps 3 Na^+ out and 2 K^+ in with the utilization of 1 ATP.
Hence in SGLT-1, Na^+ is transported along the concentration gradient while Glucose is transported against the concentration gradient.

~~Hence during SGLT-1 does not~~ require ATP but to pump out the Na^+ and to bring in K^+ (to maintain neutrality) the NA^+/K^+ ATPase pump utilizes ATP. (secondary active transport)



Clinical applications :

- In ORS, ~~Sodium and glucose are~~ supplemented together as they are absorbed together.
- Renal Glycosuria :
  Gene SLC5A2 codes for SGLT 2, mutation of this gene results in inhibition of SGLT2.
  Hence the reabsorption of glucose is inhibited.
  The renal threshold is lowered (normal- 180mg/dl).
  Glucose is eliminated in urine at a very less concentration of glucose.
  It is detected by benedicts test or urine dipsticks.
- SGLT-2 Inhibitors are used as oral hypoglycemic agents called Gliflozins.
  Renal glycosuria is seen in this condition.
  Side effect : Urinary tract infection.

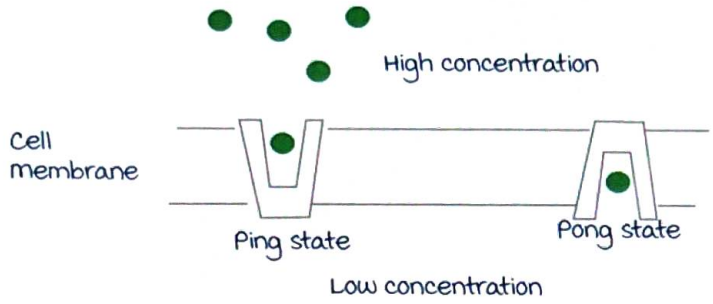
GLUT

00:17:22

Characteristics :

- Sodium independent.
- Facilitated carrier-mediated transport.
- Passive process.

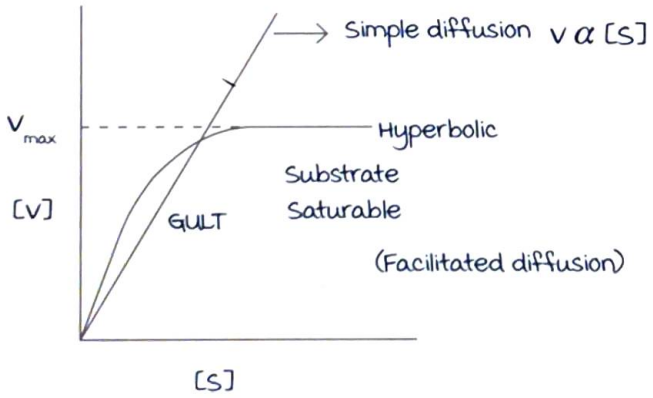
- Bidirectional.
- Along the concentration gradient.
- Ping Pong mechanism :



Ping state – Faces towards high concentration of glucose and attaches to it.

Pong state – Faces towards the interior of the plasma membrane. Ping state conformationally changes to Pong state and glucose is transported along the concentration gradient.

- A hyperbolic curve on velocity v/s substrate concentration graph.



S – Solute concentration.
V – Rate of absorption of solute.

Hyperbolic curve – The rate of absorption of solute raises initially as the solute concentration increases and remains the same after a particular concentration (Vmax).
Reason – all the carriers of glucose get saturated at V max.

In simple diffusion, S is directly proportional to V.

Active space

GLUT	Location	Concept/ features
GLUT-1	Brain, placenta, Kidney, RBC, colon, retina.	Widely distributed. High affinity & low K_m. Basal glucose uptake. Seen with Cell barrier mechanism.
GLUT-2	β cells of Pancreas. Sinusoidal cells of Liver. Basolateral/ serosal side of Intestine. Basolateral side of Proximal renal tubules.	Low affinity, K_m – high. Insulin secretion. Uptake glucose for Storage. Absorption of Glucose. Reabsorption of Glucose.
GLUT-3	Neurons, Kidneys, placenta.	Highest affinity to glucose -> low K_m:
GLUT-4	Heart, adipose, skeletal muscles	Insulin-dependent. Decrease blood glucose (maximum extraction of glucose in the postprandial state)
GLUT-5	Luminal side of the small intestine, spermatozoa.	Fructose transporter.
GLUT-6	Spleen, leucocytes.	Pseudogene. No transporter functions.
GLUT-7	Liver, Smooth endoplasmic reticulum (SER).	Transports glucose across the SER.

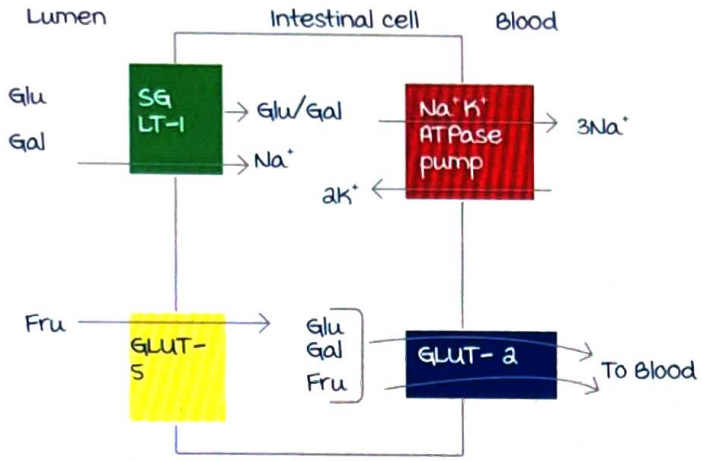
GLUT-8 : seen in Blastocysts.

GLUT-9 : Urate transporter. Mutation of GLUT-9 is a cause of Gout.

Active space

Absorption of Glucose in the intestine

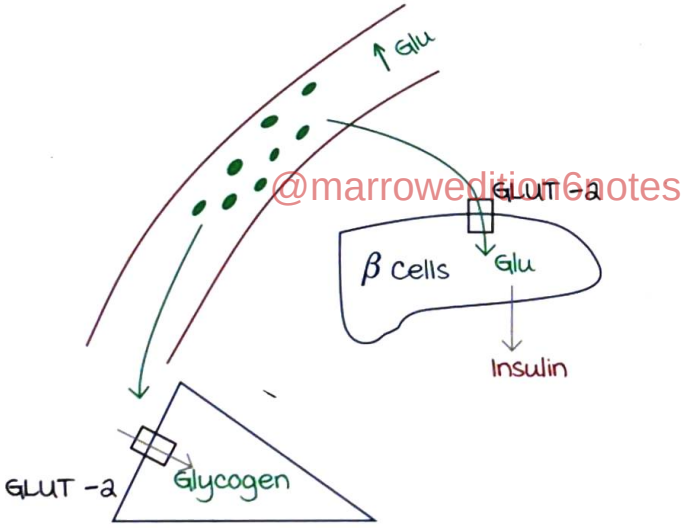
00:35:53



Luminal side of Intestine :

Absorption via SGLT-1 : Galactose > Glucose > Fructose.

GLUT-5 : Carrier mediated transport of Fructose along the concentration gradient. It is facilitated diffusion.



GLUT-2 : Transport of ~~Glucose~~, Galactose and Fructose from intestinal cells to the blood. It is Insulin Independent.

In β cells of the Pancreas and Liver, Glucose is transported into the cells via GLUT-2.

It has a low affinity to Glucose.

Hence it is transported when the concentration of Glucose is more in the Blood.

In the liver, Glucose is stored as Glycogen.

In Pancreas it helps in the secretion of Insulin.

In the Presence of Insulin, GLUT-4 is recruited to the plasma

Active space

membrane of Skeletal muscle, Adipose tissue, and heart.
The excess glucose in the blood is reduced.
Glucose is not the only source of energy in these organs.

Distribution of glucose transporters in various organs

00:41:38

Brain : GLUT-1 & GLUT-3 both have a high affinity to glucose, hence glucose is transported even if blood glucose levels are very low.
RBCs : GLUT-1.
Placenta : GLUT-1 & GLUT-3. With high fetal glucose demand, Glucose is transported to the fetus even if the blood glucose levels in maternal blood are low.

Neuronal Glucose Transporter : GLUT-3.
Widely distributed Glucose Transporter : GLUT-1.

Glucose has high Glycemic index than Fructose.
Reason : Glucose is absorbed via SGLT-1, against the concentration gradient, while Fructose via GLUT-5 which is facilitated diffusion.
Glucose, Maltose, Lactose, and Galactose have a Glycemic index of 1 or 100%.
Dietary fibres have a Glycemic index of 0.

Question : A 5-year-old boy after drinking contaminated water, resulted in acute watery diarrhea, later diagnosed to be cholera.

- What is the role of Glycolipids here?
  GM-1 Ganglioside acts as a receptor for cholera toxin.
- What's the role of ORS although electrolytes are lost in stools?
  Na^+ and Glucose are absorbed together via SGLT-1, which helps in rapid loss of electrolytes in stools.
- If we add amino acids, will it aggravate the situation?
  No, Because the transporter for amino acids is different.

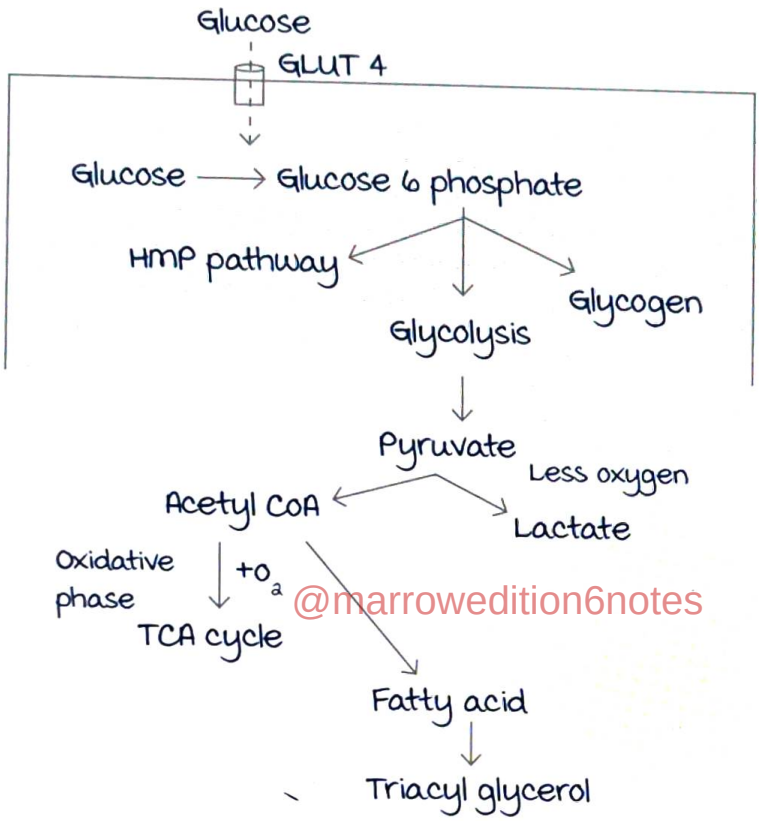
Active space

GLYCOLYSIS

Concept

00:02:17

Pathway taking place, when body is in a well-fed state (High insulin-glucagon ratio).



Glycolysis : Overview

00:05:40

Also known as Embden Meyerhof Parnas pathway (EMP Pathway).

Derived from Greek word 'Glykys' : Sugar.
'Lysis' : Splitting.

Site : All organs.

Organelle : Cytoplasm.

Significance :

- Only pathway that takes place aerobically and anaerobically.
- RBC : Only metabolic fuel in both fed and fasting state is entirely glucose.

Only anaerobic glycolysis occurs in RBC as RBC lack mitochondria.

Defect in glycolytic enzymes : RBC lysis.

- Skeletal muscles :

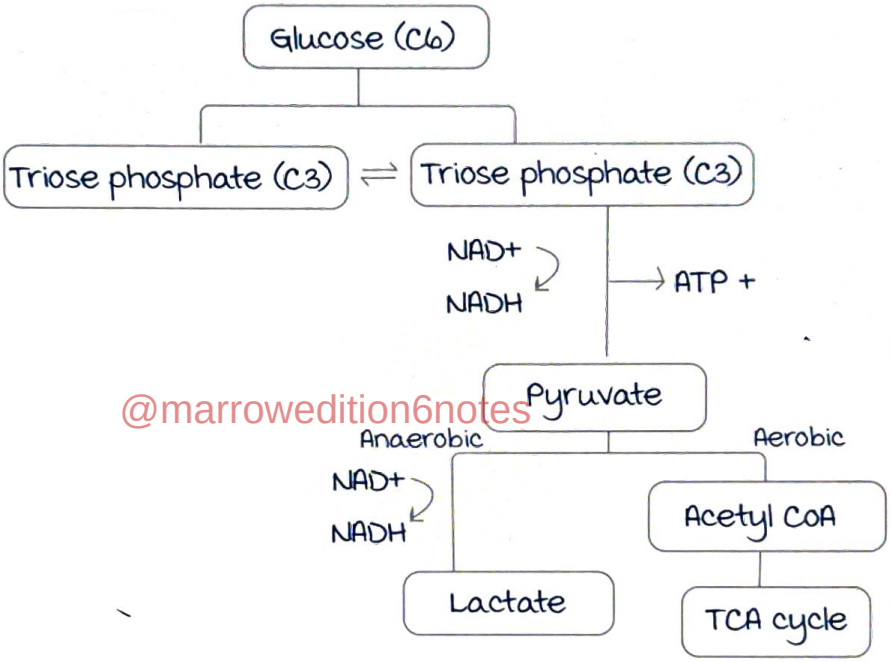
  Enormous capacity for glycolysis.

  Exercising muscle : Decreased oxygen supply and hence derive supply from anerobic glycolysis.

  Defect in glycolysis : muscle fatigue.

- Heart :

  Low glycolytic capacity : Cannot survive ischemia.



Steps of glycolysis

00:17:28

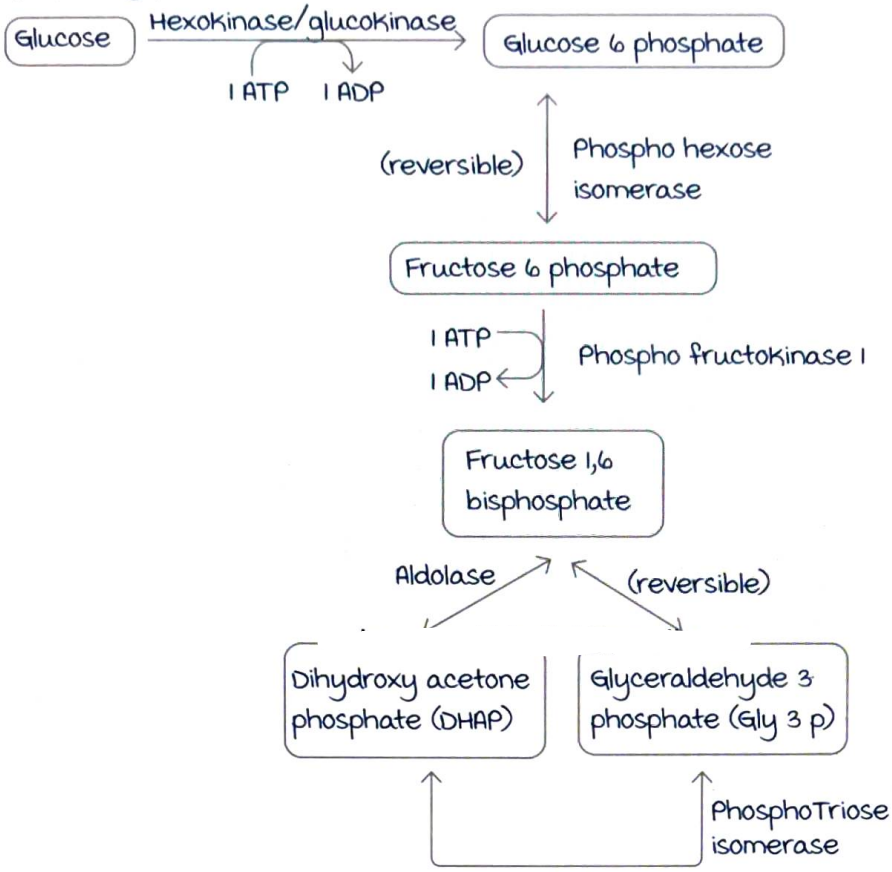
1. Preparatory phase :
   - Stages of phosphorylation.
   - Stage of splitting.

   This phase utilizes ATP.

2. Pay off phase :
   - Stage of oxidative phosphorylation.

   This phase generates ATP.

Preparatory phase :



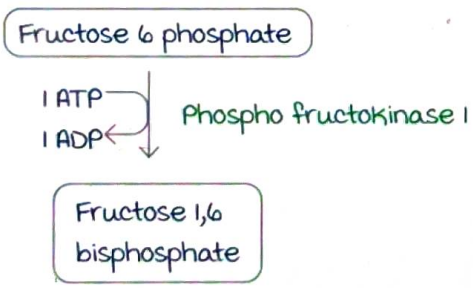
1)

Significance :

- 1st irreversible step.
- Regulatory step.
- Flux generating step.
- Phosphorylation by hexokinase traps glucose for cellular metabolism.

2) In isomerisation of G6 phosphate to fructose 6 phosphate, the C=O group is transferred to C2 so that a free hydroxyl group is available at C1.
It is a reversible step.

3)



Active space

Significance :

- Rate limiting step.
- 2nd irreversible step.
- Committed step of glycolysis as fructose 1,6bisphosphate formed can enter only glycolytic pathway.
- Bottle neck of the pathway.

4) Fructose 1,6 bisphosphate is split into two 3 carbon compounds, dihydroxy acetone phosphate (DHAP) and glyceraldehyde 3 phosphate by the enzyme, aldolase.
This is a reversible step.
DHAP gets converted to glyceraldehyde 3 phosphate by phosphotriose ismerase.
2 molecules of glyceralsehyde 3 phosphate enters the pay off phase.

Hexokinase	Glucokinase
• Has 4 isoforms. • High affinity. • Low K_m. • Not an inducible enzyme. • Housekeeping enzyme. • Inhibited by $G6PO_4$.	• Hexokinase IV. • Low affinity. • High K_m. • Induced by insulin. • Inducible enzyme. • Not inhibited by $G6PO_4$. • Regulates blood glucose in post prandial state. • Present in beta cells of pancreas & liver.

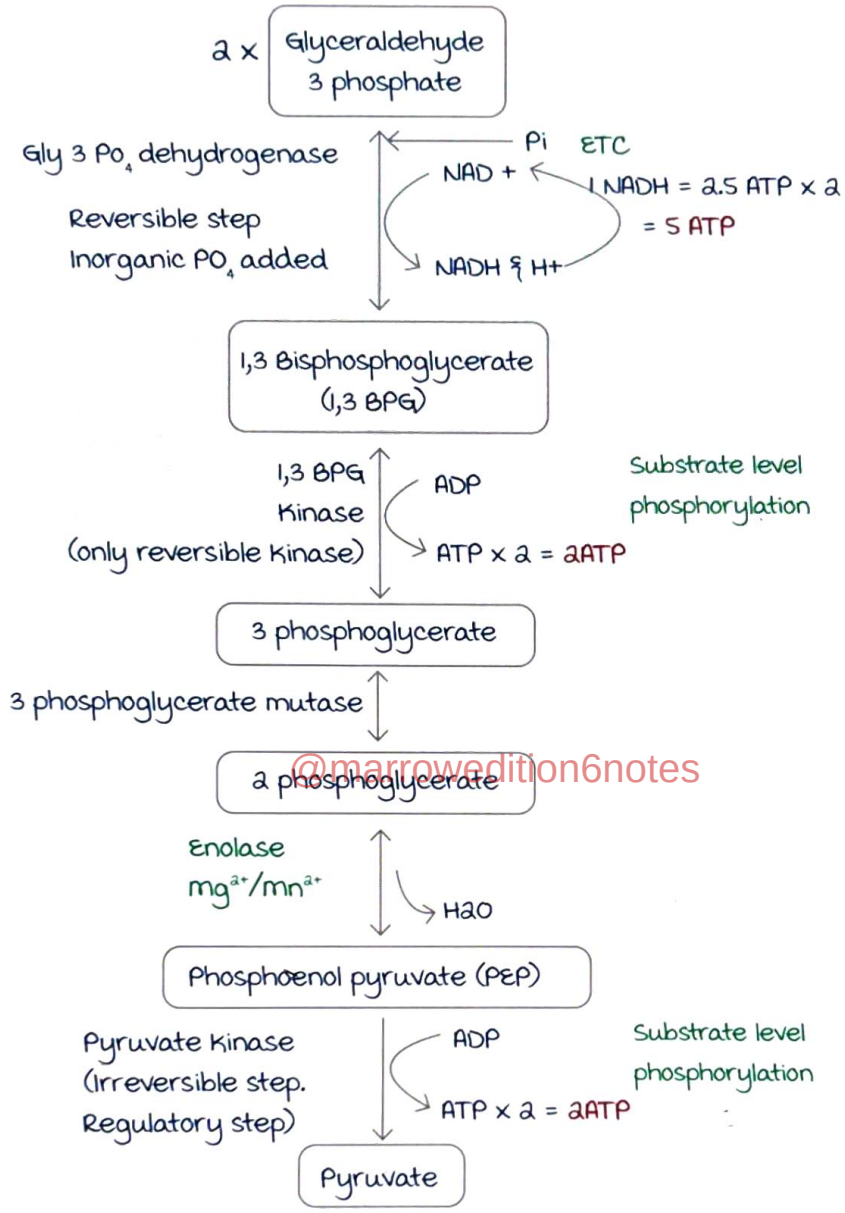
Phosphorylation by hexokinase traps the glucose for cellular metabolism.
Glucose enters the cell via GLUT 4 transporter which allows bidirectional transport of glucose.
However, by phosphorylating glucose, its transport outside the cell is prevented and glucose 6 phosphate now enters various metabolic pathways.

Phosphofructokinase I (PFK I) is an allosteric enzyme.
Allosteric activator of PFK I is fructose 2,6 bisphosphate.
Fructose 2,6 bisphosphate is formed from fructose 6 phosphate by action of the enzyme PFK 2.

PFK I is inducible enzyme.

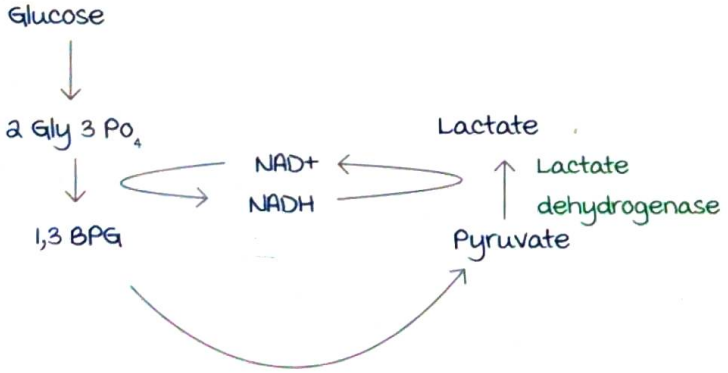
Pay off phase

00:37:06



Anaerobic glycolysis

00:46:19



Active space

No net generation of NADH.

Anaerobic glycolysis takes place either when there is no mitochondria or when there is lack of oxygen.

Significance of anaerobic glycolysis in RBC :

There is no mitochondria in mature RBC, hence regeneration of NAD+ will not occur.

Lactate dehydrogenase (LDH) in anaerobic glycolysis regenerates NAD+.

This also prevents accumulation of NADH (product) and ensures that glycolysis is not inhibited.

Energetics

00:50:35

1) Aerobic glycolysis :

Energy yielding steps	Number of ATP generated
Glyceraldehyde 3 phosphate	1 NADH X 2 = 2 NADH = 2.5 X 2 = 5 ATP
1,3 BPG Kinase	1 ATP X 2 = 2 ATP
Pyruvate Kinase	1 ATP X 2 = 2 ATP

Consumption of ATP :

Hexokinase = 1 ATP.

PFK 1 = 1 ATP.

So net ATP Generated : 9 - 2 = 7 ATP.

2) Anaerobic glycolysis :

Energy yielding steps	Number of ATP generated
1,3 BPG Kinase	1 ATP X 2= 2 ATP
Pyruvate Kinase	1 ATP X 2= 2 ATP

Consumption of ATP :

Hexokinase = 1 ATP.

PFK 1 = 1 ATP.

So net ATP Generated : 4 - 2= 2 ATP.

From 1 glucose by aerobic oxidation :

Active space

Aerobic glycolysis = 7 ATP.
PDH = 2.5 × 2 = 5 ATP
TCA cycle = 2 × 10 = 20 ATP
Total ATP generated : 32 ATP.

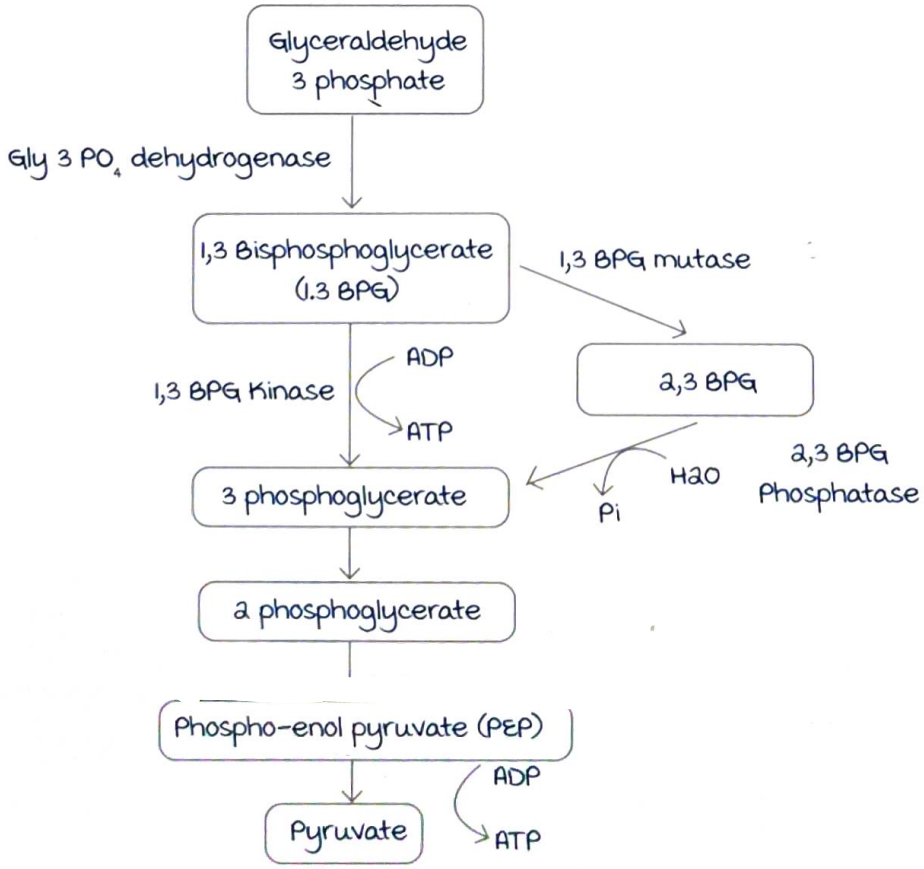
Inhibitors of glycolysis 00:55:02

1. Glyceraldehyde 3 phosphate dehydrogenase inhibited by
   - Iodoacetate.
   - Arsenate : Decreases the availability of Pi.
2. Enolase : Inhibited by fluoride by reducing the availability of Mg^{2+} or Mn^{2+}.
   Clinical significance : NaF oxalate mixture used in estimation of blood glucose.

Rapaport Leubering cycle 00:58:05

Takes place inside RBC.
Only 10% glucose enter this cycle.

Also called R-L Shunt / 2, 3 BPG Shunt.



Active space

There is production of 2,3 BPG by shunting the ATP generating step in glycolysis.

2,3 BPG :

- Shift Oxygen dissociation curve to right (low affinity state).
- Helps in unloading of oxygen from Hb.

No net generation of ATP.

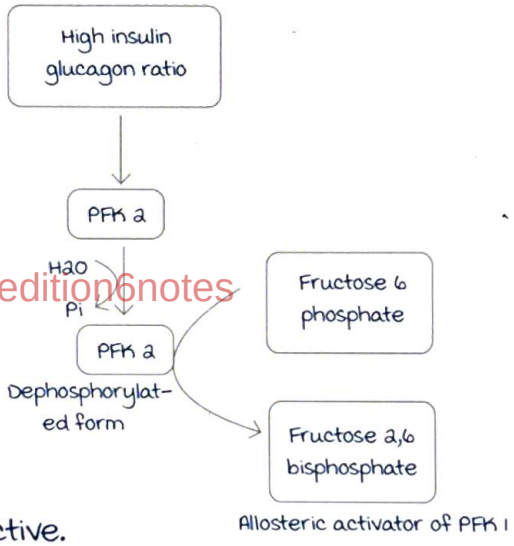
10% of glucose in RBCs enter this pathway.

Regulation of glycolysis 01:04:15

1. Hormonal regulation :

   By covalent modification.

   - Fed state :



PFK 1 is activated
hence glycolysis is active.

   - Fasting state :
     Low insulin glucagon ratio.
     Phosphorylation of PFK 2 occurs and fructose 2, 6 bisphosphate is not formed.
     Hence PFK 1 remains inactive which inhibits glycolysis.

2. Allosteric regulation :

Enzyme	Allosteric activator	Allosteric inhibitor
Hexokinase		ATP
PFK 1	F-6-P (substrate) F 2, 6 BP. 5' AMP (substrate).	ATP (product). Citrate (product). Low pH (product)
Pyruvate kinase		ATP

Active space

Clinical questions

01:12:09

Q. An elderly COPD patient admitted in emergency dept with loss of consciousness due to bleeding from a stomach ulcer. Her respiratory rate is rapid, cyanosis +, BP lower skin is cold and clammy. Emergency management started and blood send for lab investigation.
Why is there loss of consciousness?
Ans. Aerobic oxidation of glucose affected which is the primary metabolic fuel for brain.
Brain cannot survive if glucose is not going into oxidative phase, resulting of loss of consciousness.

Q. The hypoxia induces the gene transcription of enzymes of which metabolic pathway?
Ans. ~~Hypoxia induced transcription~~ factor 1 (HIF 1) will increase expression of enzymes of glycolysis.

Q. Lack of this pathway results in haemolysis. Why?
Ans. RBC solely depend on anaerobic pathway, which if disrupted will lead to swelling and haemolysis of RBC.

Q. RBC need ATP to maintain ion gradients in the membrane. The lack of it results in swelling of RBC and lysis. To maintain the ion gradient the major source of ATP is which pathway?
Ans. Anaerobic glycolysis.

Q. Enzyme defect in glycolysis cause muscle fatigue. Why?
Ans. Exercising muscle in hypoxic state depends on anaerobic glycolysis. Lack of ATP production results in muscle fatigue.

Q. Heart is susceptible to hypoxia but not skeletal muscle. Why?
Ans. Heart has very low glycolytic capacity and hence is susceptible to hypoxia. This when compared to skeletal muscle that has high glycoltic capacity and switches over to anaerobic pathway for ATP production.

Active space

APPLIED ASPECTS OF GLYCOLYSIS

Glycolysis and cancer :

Warburg hypothesis :
Given by Otto Warburg in 1924.
In cancer cells, glucose uptake occurs at a very high rate and ~~are converted~~ to lactate. This conversion produces far less ATP (2 ATP per cycle) as compared to glycolysis (32 ATP).
Inorder to compensate this ATP deficiency, cancer cells take up more glucose.

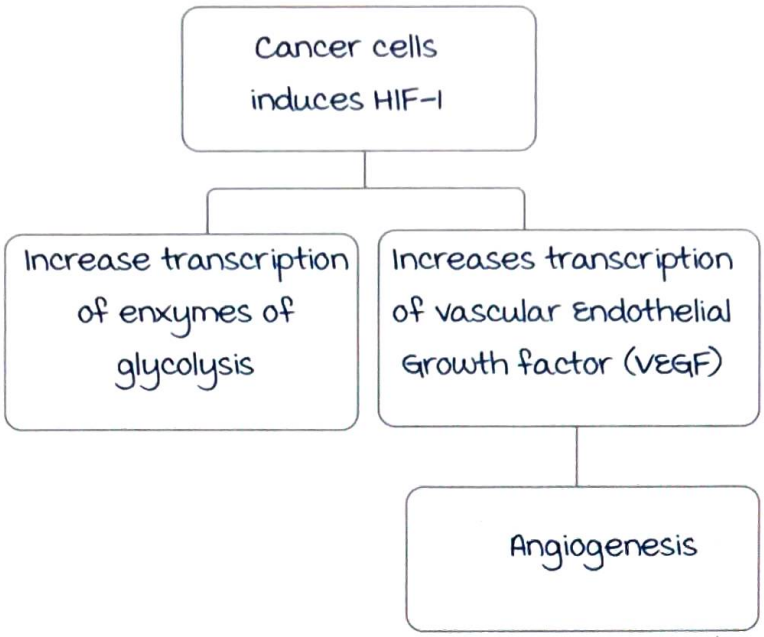
In cancer cells, there is lactate accumulation which creates an acidic environment.
Lactate needs to be regenerated to glucose through gluconeogenesis, needing more ATP.
Therefore, cancer cells go into hypermetabolic state.
This leads to cancer cachexia.

Metabolic reprogramming :

Normal cells	Cancer cells
Glucose → Glyceraldehyde 3 PO4 → PEP → Pyruvate → Acetyl CoA → CO_2 + H_2O → ATP.	Reprogramming : Glucose → Glyceraldehyde 3 PO4 → PEP → Pyruvate → Lactate
Pyruvate Kinase is in tetrameric state (high catalytic state) which is pyruvate kinase M1. When this enzyme is active, pyruvate will go in oxidative pathways.	Pyruvate Kinase (PKM2) Dimeric form (low catalytic state). Even in the presence of O_2, Lactate is produced (Aerobic glycolysis).

Cancer cells derive energy from aerobic glycolysis.

Cancer cells induce Hypoxia Induced Transcription Factor-1



Glycolysis & radiology 00:10:00

2 Fluorodeoxy glucose (FdG) (F^{18} labelled glucose) is given to locate the cancer.

FdG injected into the cell → FdG preferentially taken inside through GLUT transporters → Glucose concentrated inside cancer cell 10 times → Labelled glucose undergoes first step of glycolysis → Hexokinase step → Phosphofluorodeoxy glucose → Accumulation in cell → Decay → Emits positrons → Detected with PET scan.

Chemotherapeutic agents (under trial):

Inhibitors of glycolysis used to kill cancer cells.

- 2 deoxy glucose (2DG) inhibits glycolytic pathway at the level of hexokinase.
- Lonidamine.
- 3 bromopyruvate.

Active space

metabolic defects in glycolytic pathway :

1. Pyruvate Kinase deficiency :
   - 2nd m/c enzyme defect in humans.
   - Defective pyruvate Kinase → Anaerobic glycolysis affected → No ATPs → Ion channels not maintained → Ca^{2+} influx + K^{+} efflux → Swelling of RBCs → Hemolysis → Hemolytic anemia.
   - In RBCs, 2,3 BPG increased → Unloading of oxygen (compensatory mechanism).

2. Aldolase A defect :
   - Hemolysis.

3. Muscle PFK (Phosphofructokinase 1) defect :
   - Exercise intolerance.

Maturity Onset Diabetes of Young (MODY) 00:16:23

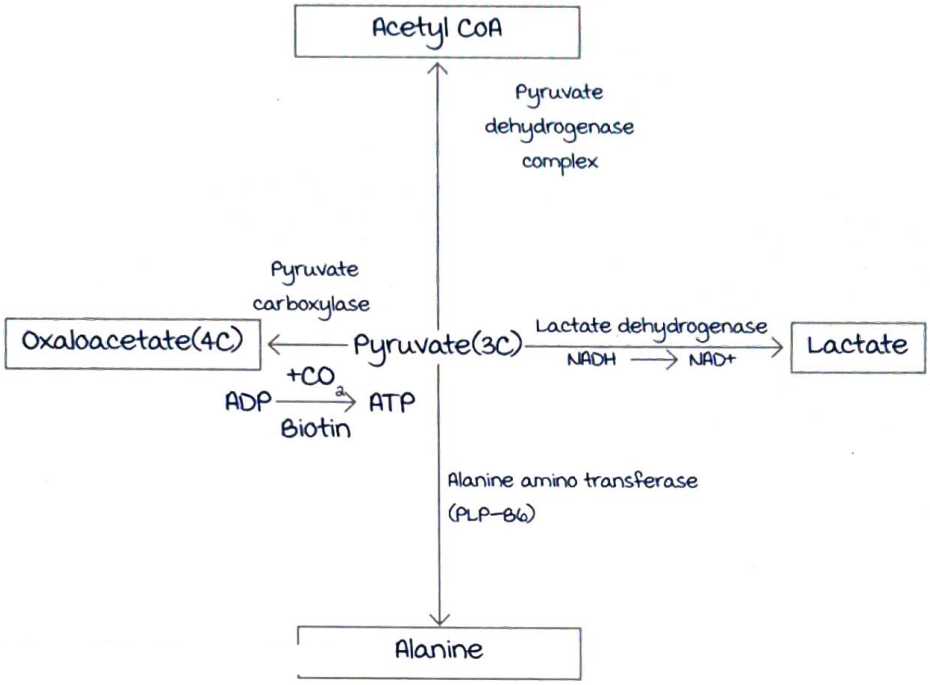
Glucokinase acts as glucose sensor as it has low affinity ans high Km value for substrate glucose.
Whenever there is high glucose, it is sensed by glucokinase, where the glucose enters the pancreas through GLUT-2, gets converted to G6 phosphate + ATP. This building up of ATP enables the release of insulin from beta cells.

In MODY 2 :
Glucokinase → Kinetic property altered → Building up of ATP not seen → Cannot secrete insulin even in hyperglycemic state.

PYRUVATE DEHYDROGENASE

Fates of pyruvate

00:01:56



Pyruvate Dehydrogenase (PDH) :

- Site : mitochondria.
- Formed as a result of glycolysis (in cytoplasm).
- Proton symporter transports pyruvate from cytoplasm to mitochondria.

PDH : multienzyme complex :

3 enzymes	5 coenzymes
• E1 pyruvate dehydrogenase	• Thiamine Pyrophosphate (TPP).
• E2 dihydrolipoyl transacetylase	• CoA • Lipomide
• E3 dihydrolipomide dehydrogenase	• FAD • NAD^+

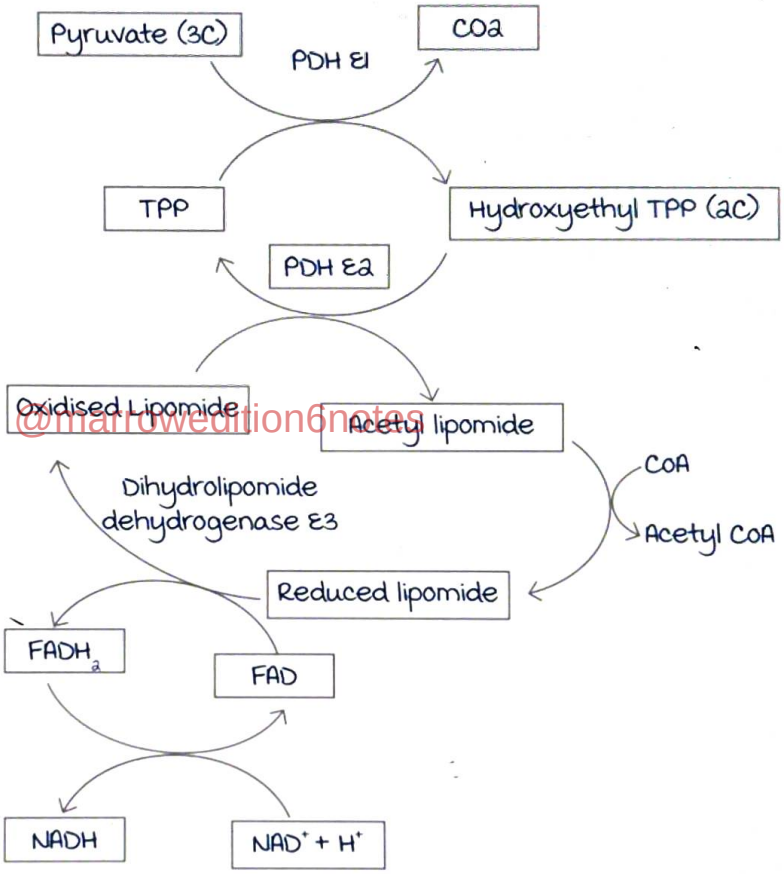
- This multienzyme complex is similar to other multienzyme complexes :
  1. α-Ketoglutarate dehydrogenase complex : α KDGH (TCA Cycle).
  2. Branched-chain keto acid dehydrogenase complex

Active space

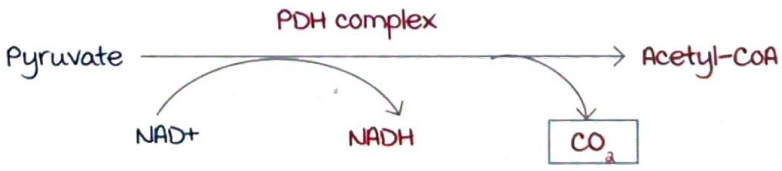
(BCKDH) (used for BCAA Catabolism)

- All require all the 5 Coenzymes.
- E_2 and E_3 are also required for α-KDGH and BCKDH.
- metabolic defect in E_2 or E_3 → PDH, α-KDGH and BCKDH will be affected.
- They catalyse Oxidative Carboxylation.

Pyruvate dehydrogenase reaction 00:09:45



Net reaction (oxidative decarboxylation):



Each NADH produces 2.5 ATPs and each cycle produces 2 NADH as glucose is a 6C compound. total ATP produced is 5 ATPs.

Active space

Regulation of pyruvate dehydrogenase 00:15:26

This enzyme is active in well fed state → under influence of high insulin-glucagon ratio → Dephosphorylated PDH (Active state).

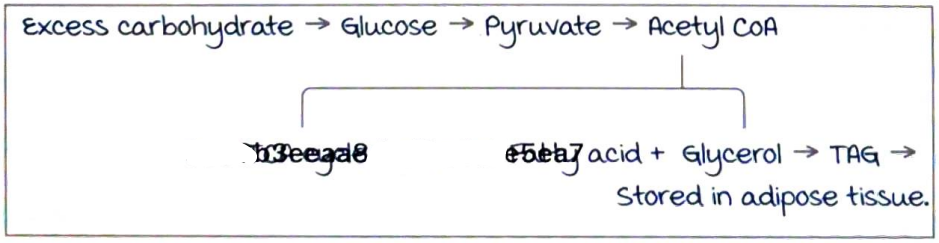
Increased ATP : ADP ratio.
Increased NADH : NAD^+ ratio
Increased acetyl CoA : CoA ratio
} PDH is inhibited

Significance of pyruvate dehydrogenase :

1. Pyruvate —(Irreversible reaction)→ Acetyl CoA
2. No enzyme in human body can circumvent this irreversible enzyme.
   Acetyl CoA cannot be converted into pyruvate.
   Acetyl CoA is never a substrate for gluconeogenesis.

Fat and glucose 00:21:26

3. Fat (Tri Acyl Glycerol) cannot be converted to glucose.
   - 2 exceptions :
     i. Glycerol.
     ii. Propionyl CoA (formed from odd chain fatty acid).
4. Excess carbohydrate is stored as fat in adipose tissue.



5. In chronic alcoholics → Energy depletion (less ATP production).
   - Reduced absorption of thiamine (co-factor) → Less ATP.
   - Nutritional deficiency (B complex vitamins low : B1, B2, B3, B5) → Less ATP.

Active space

6. Acetyl CoA enters TCA cycle.

TCA cycle ⟷ Glycolysis

Irreversible route
(PDH)
Link reaction.

Clinical significance

00:29:20

metabolic defect (PDH Complex : E1 MC affected)

- No production of Acetyl CoA → Increased pyruvate → Converts to lactate → Lactic acidosis.
- All oxidative pathways cut down → Brain affected (dependent on oxidative pathway) → Neurological manifestations (psychomotor disability).

Q. A male child presented with profound psychomotor disability, a metabolic disorder due to less utilisation of pyruvate, which resulted in accumulation of lactic acid.

- ATP production is stopped.
- Which enzyme complex is affected ?

Ans : PDH complex.

Q. Excess carbohydrate leads to excess fat position.

Ans : All excess carbohydrate are pushed through valve like PDH, irreversible reaction.

Q. Fatty acid cannot be converted to glucose.

Ans : Because of irreversible nature of PDH, and there is no enzyme to circumvent the PDH

Q.In chronic alcoholics, there is less ATP production.

Ans : There is energy depletion due to nutrition deficiency, and the B complex vitamins are required for PDH and α-ketoglutarate dehydrogenase.

GLYCOGEN METABOLISM

Structure of glycogen 00:01:13

Branched polymer of alpha D glucose.
Linear side : α 1, 4 glycosidic linkage.
Branches : α 1, 6 linkage.

Glycogen stored in cytoplasm as cytoplasmic granules, in the form of α rosettes.
Each alpha rosette → 20-40 β particles → Each is 21 nm in diameter + 55000 residues + 200 non-reducing ends (no free functional group).
Free functional group present only at 1st glucose residue linked to polypeptide : Glycogenin (only 1 reducing end).

Reasons why glucose is stored as glycogen :

- Compact.
- Multiple non-reducing ends (glucose can be released at a faster rate).
- Low molarity of glycogen.

If glucose is stored as it is, high molarity will be seen, which attracts more water, leading to cell swelling and death.

Classification of glycogen metabolism 00:07:18

Glycogen synthesis :
Process happens in fed state (4-6 hours post prandial state).
Glucose → Glycolysis/glycogen synthesis.
There is high insulin-glucagon ratio.
In a basal metabolic rate, if excess glucose if given, it is stored as glycogen.

Glycogen synthesis :
Site : Liver (10% by weight), muscle (1-2% by weight).
Highest content of glycogen present in muscle.
Highest % by tissue weight : Liver.

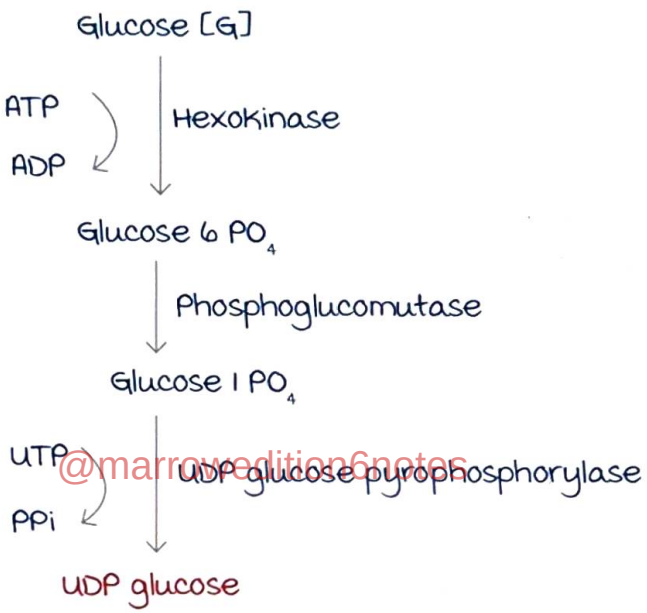
Organelle of glycogen synthesis : Cytoplasm.

Steps :

Glycogen synthase (rate limiting enzyme).

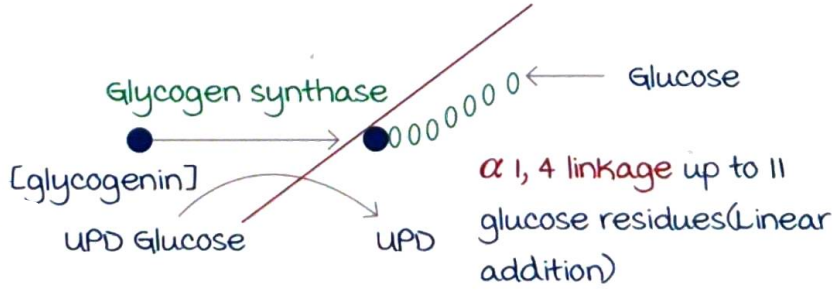
Branching.

1. Synthesis of UDP glucose.
   - UDP glucose : Active glucose donor.
   - Nucleotide involved : UTP (Uridine triphosphate).



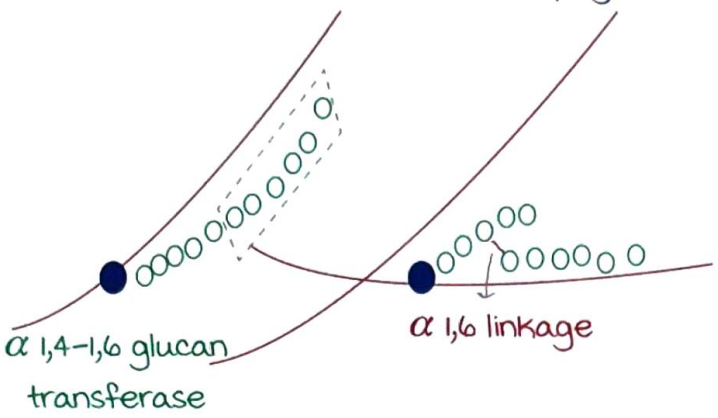
2. Glycogen synthase (rate limiting enzyme).
   - Primer : Glycogenin (tyrosine residues – polypeptide).
   - 7-8 glycogenin residues added (without enzyme).
   - Synthesis of linear chain glycogen.
   - No enzyme required for initial addition of chains.

> DNA polymerase and
> glycogen synthase both need primers.



Active space

3. Branching.
   - Branching enzyme (alpha 1,4 1,6 glucan transferase).
   - Hexasaccharide residue is cut from end and translocated to another location in the polymer.

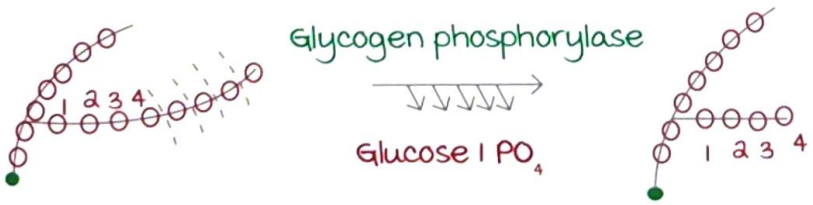


Glycogenolysis 00:19:58

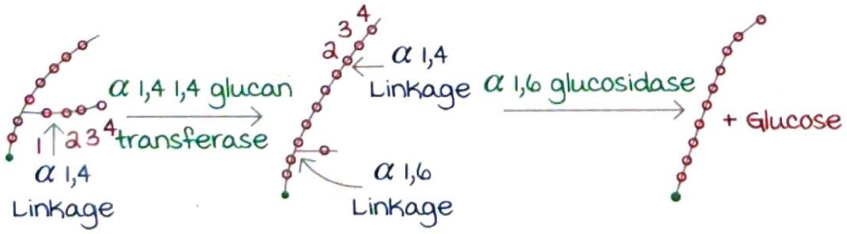
Occurs in fasting state (post absorptive state).
In early fasting state (4–16 hours after food) :
Source of blood glucose : Hepatic glycogenolysis.

Hormone : Glucagon.
Low insulin-glucagon ratio.
Site : Liver, skeletal muscle.
In cytoplasm, lysosomes (1–2%), SER plays a role in liver.

Steps :
1. Glycogen phosphorylase (rate limiting enzyme) : Needs PLP (Vitamin B6)



2. Debranching enzyme.
   - Alpha 1,4 1,4 glucan transferase & alpha 1,6 glucosidase.



Active space

By the action of enzyme alpha 1,4 1,4 glucan transferase, the terminal 3 glucose molecules are cut and relocated to non reducing linear end.
By the action of enzyme alpha 1,6 glucosidase, it cuts the terminalglucose of the branch from 1,6 linkage and releases it as free glucose.

3. Conversion of glucose-1-PO4 to glucose.

In liver :

G1 PO_4

↓ Phosphoglucomutase [PGM]

G 6 PO_4 present in cytosol and transported to SER via T1

H_2O ↘ ↓ G6 phosphate [G6p] present in SER

P ↙

Glucose (formed in SER & released into cytosol via T1 and T2)

In muscle :

G1PO4 $\xrightarrow{PGM}$ G6PO_4 → Pyruvate → Lactate

↓

Anaerobic glycolysis (due to absence of G6p in muscle) ATP is released. Net : 3 ATP (hexokinase step is bypassed).

The role of glycogen in muscle is to provide ATP for the muscle itself. In stressful situation when epinephrine acts, or during exercise, glycogen converts into G1PO_4, then G6PO_4 which undergoes glycolysis

Glucose 6 phosphatase :

- Glucose-6-phosphatase activity seen within the smooth endoplasmic reticulum,
- Enters SER through T1 transporter and exits into cytoplasm through T2 and T3 transporters.
- Glucose 6 phosphatase not present in skeletal muscle and adipose tissue.

Active space

Regulation of glycogen metabolism :

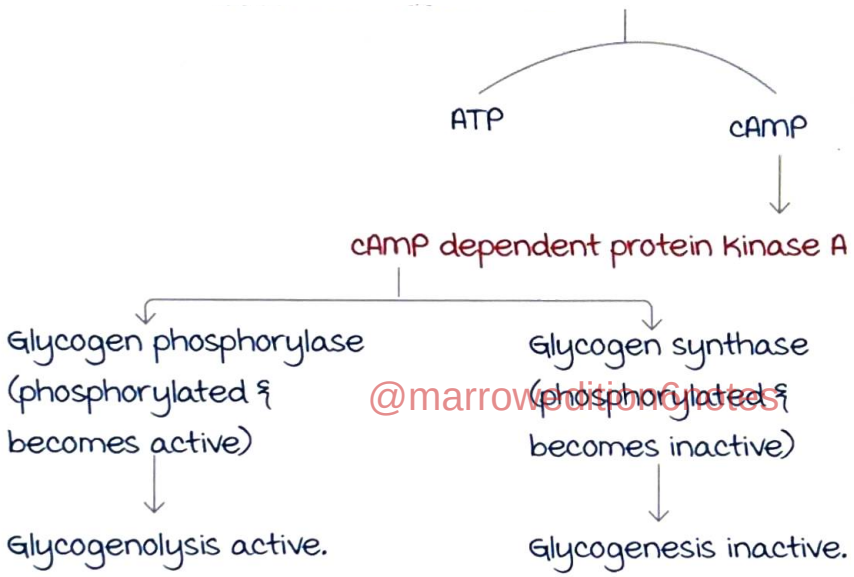
1. Hormonal regulation.
2. Allosteric regulation.

Hormonal regulation :

Fasting state/stress/exercise :

- Active hormones : Glucagon (liver).
  Epinephrine (liver, muscle).
- more glycogenolysis, less glycogenesis.

Glucagon & epinephrine activates G protein coupled receptors which activeates G protein → Increased adenylyl cyclase.

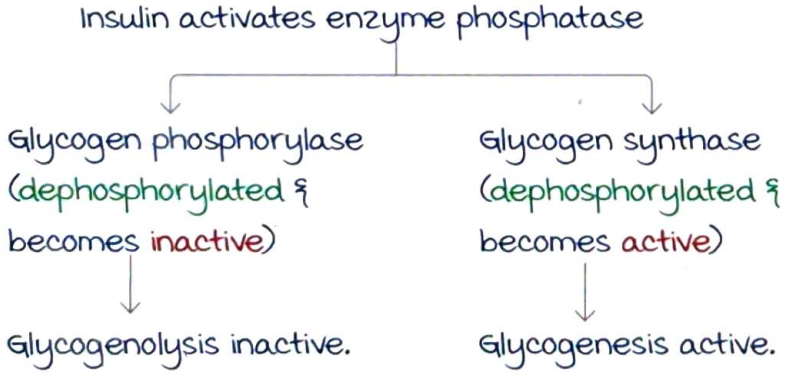


Fed state :

- Active hormones : Insulin.
- more glycogenesis, less glycogenolysis.
- In fed state, insulin activates phosphodiesterase enzyme, which converts cAMP to 5' AMP.

Hormone : Insulin ATP ⟶✕ cAMP ↓ ⟶ ↓ cAMP dependent protein kinase A

Insulin activates enzyme phosphatase



Active space

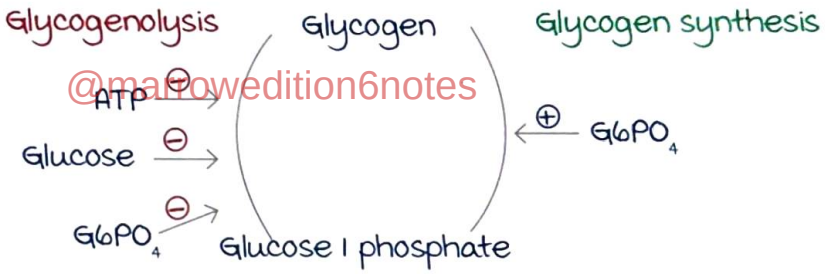
In muscle

00:43:48

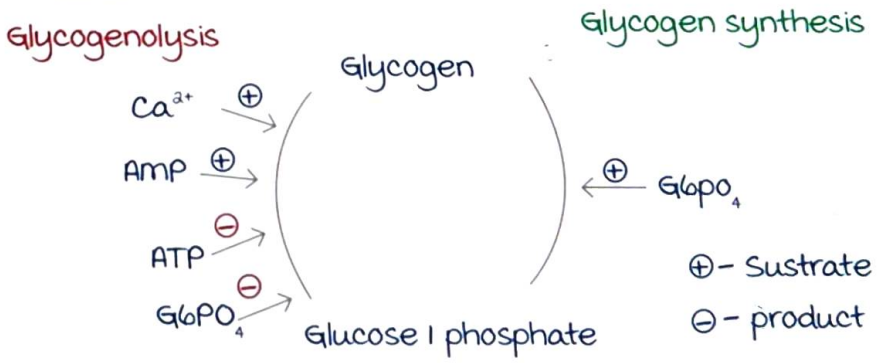
1. Epinephrine → cAMP dependent protein kinase A active.
2. Nerve impulse (stress/exercise) → Calcium released from sarcoplasmic reticulum → Increased activity of calcium calmodulin dependent protein kinase → Glycogen phosphorylase active → Glycogenolysis.
3. Extreme anoxia (strenuous exercise) → myosin ATPase release 5'AMP from ATP → Binds to site in muscle glycogen phosphorylase → Enzyme becomes active without any active phosphorylation
   5'AMP is allosteric activator of glycogen phosphorylase.

Allosteric regulation :

In liver :



In muscle :



Glucose in not a product of glycogenolysis in the muscle, hence it is not an allosteric activator in muscle.

Active space

Questions :

Q. If steady state of blood glucose is not maintained, the person will become hypoglycaemic and experience coma and even seizures.
Which one of the following maintain blood glucose and why ?
A. Glycogen in heart.
B. Glycogen in brain.
C. Glycogen in muscle.
D. Glycogen in liver.

Glycogen in the glucose maintains blood glucose for the first 4–16 hours of fasting state. By 16–18 hours, glycogen stores are depleted.

Q. If a glucose load is given to a normal person ,in a basal metabolic state large glucose is ingested. What will this result in?
Ans. Excess glucose first undergoes glycolysis, then goes for glycogen synthesis and then fatty acid synthesis.

Active space

GLYCOGEN STORAGE DISORDERS

Group of disorders associated with defective metabolism of glycogen. Glycogen storage disorders (GSD) can be divided into two :

- Liver GSD.
- Muscle GSD.

Liver GSD :

Fasting hypoglycemia is seen. Liver is responsible for glucose levels during early fasting (4-16hrs). No exercise intolerance.

Muscle GSD :

Normoglycemia is seen. This is because muscle is not a source of blood glucose, it only supplies glycogen for exercises. Hence exercise intolerance is seen.

Liver GSD	Muscle GSD	
Type Ia (Von Gierke's disease : most common GSD)	With hypertrophic cardiomyopathy	Without hypertrophic cardiomyopathy
Type Ib GSD	Type II GSD (Pompe's disease/Danon disease)	Type V GSD (McArdle's disease)
Type III GSD (Cori's disease/ Fob's disease/Limit dextrinosis)		Type VII GSD (Tarui's disease)
Type IV GSD (Amylopectinosis/ Andersen disease)		
Type VI GSD (Her's disease)		

Von Gierke's disease

00:05:03

Type Ia disease also known as Von Gierke's disease.

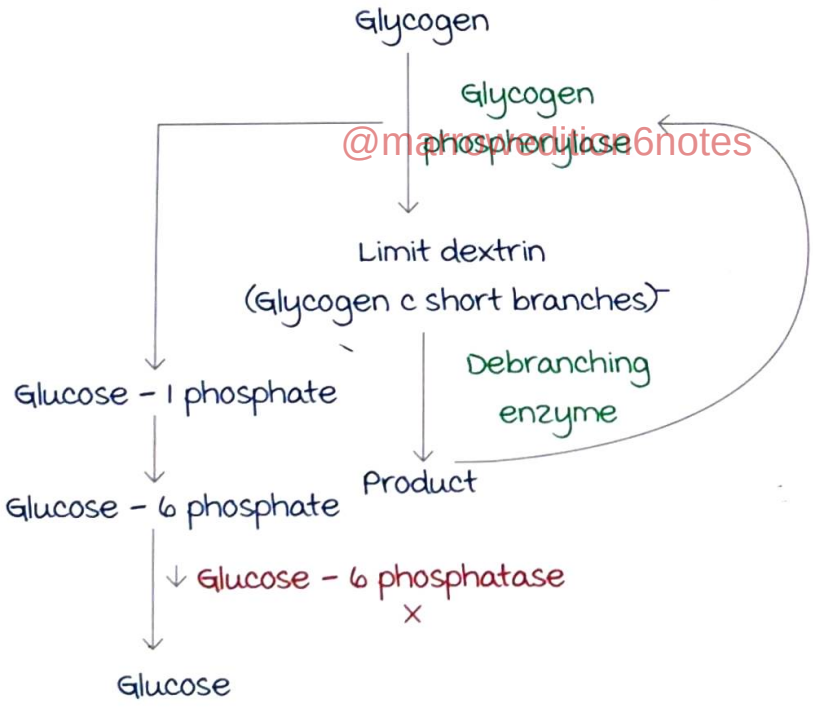
Biochemical defect :

Glucose-6-Phosphatase enzyme defect. This enzyme is needed for glycogenolysis in the liver and gluconeogenesis.

Biochemical hallmarks :

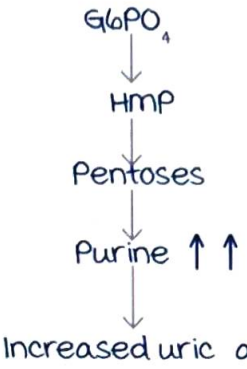
- Fasting hypoglycemia.
- Lactic acidosis.
- Ketosis.
- Hyperuricemia.
- Hyperlipidemia.

In Normal individuals :



In patients of Von Gierke's disease, the glucose-6-phosphatase enzyme is deficient. Therefore to produce glucose, the body shifts to gluconeogenesis. (non-carbohydrate substrates to get glucose) but again fails to produce and release glucose because of the glucose-6-phosphatase enzyme deficiency.
Therefore, gluconeogenesis also fails and the patient ends up with fasting hypoglycemia.

Active space

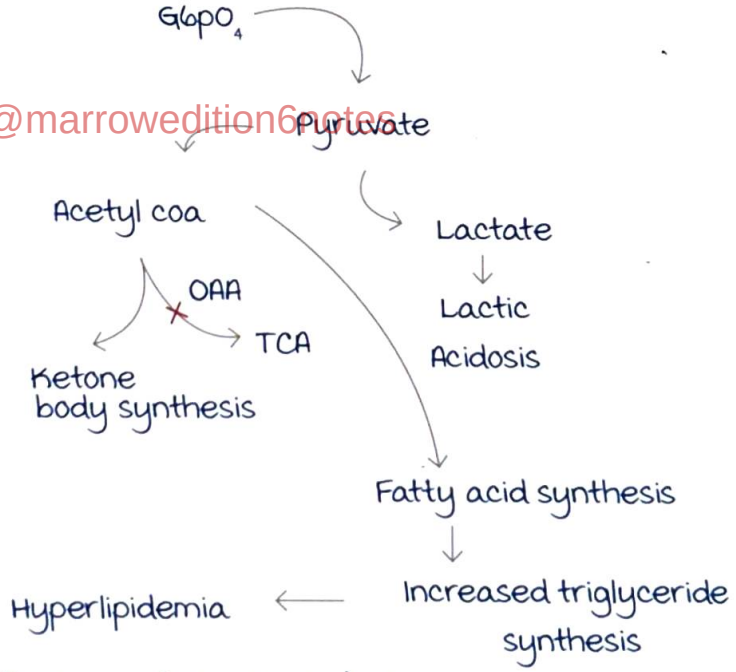


Excess G-6-PO4 now enters HMP pathway to form purines and cause hyperuricemia.

$G6PO_4$ can also form pyruvate and then Acetyl CoA.

Acetyl CoA can then be used in TCA cycle (but fails to do so because of depleted oxaloacetate) or for Ketone body synthesis and results in Ketosis.

Acetyl CoA can also be used for the synthesis of fatty acids, resulting in excess triacylglycerol and eventually hyperlipidemia.



Clinical features of Von Gierke's disease :

1. Chubby cheeks
2. Thin extremities. — doll like facies.
3. Protruding abdomen.
   a. Hepatomegaly.
   b. Renomegaly (due to increased fat deposition).
   c. No splenomegaly.
4. Hypoglycemia.

Investigation :

1. Increased lactate.
2. Increased uric acid.
3. Increased Ketone bodies.
4. Decreased blood glucose.
5. Increased triacylglycerol.
6. IV Glucagon challenge

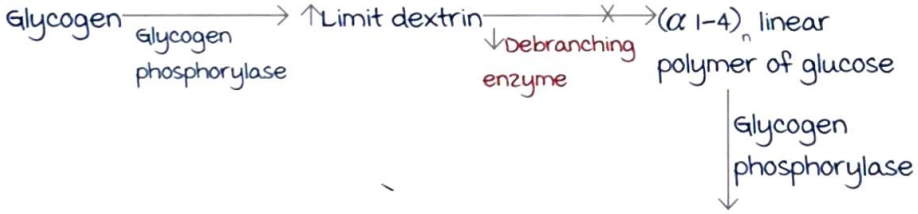
Type Ib GSD :

Involves all features of type Ia GSD + Neutropenia is also seen, resulting in recurrent bacterial infections.

Type III GSD

00:16:22

Also called as limit dextrinosis or Forbe's disease or Cori's disease. Here the debranching enzyme is deficient and hence, limit dextrin accumulates in the liver. This results in deposition of abnormal glycogen in liver.



Clinical features of Type III :

1. Fasting hypoglycemia.
2. Protruding abdomen :
   a. Hepatomegaly (+++).
   b. Splenomegaly (+).
   c. No renomegaly.
3. Micronodular liver cirrhosis : Reversible after puberty.

Investigation :

1. No increase in Lactate and uric acid
2. Ketosis.
3. Liver enzymes are elevated.
4. IV glucagon challenge can be done.

Type IV GSD

00:20:29

Also known as Andersen disease or amylopectinosis.

Biochemical defect :

Glycogenin ——(UDP glucose)n——→ (Alpha 1-4)n glucose polymer ——Branching enzyme——→ Glycogen

There is deficiency of branching enzyme in type IV GSD and abnormal glycogen (amylopectin like glycogen) accumulates.

Clinical features :

This is a fatal condition :

- Hypoglycemia.
- Ketosis.
- Progressive micronodular liver cirrhosis which can cause :

1. Portal hypertension.
2. Esophageal varices.
3. Liver failure.
4. Death within 5 years.

Diagnosis :

- By hypoglycemia and ketosis. Note that in type IV GSD, there is no lactate acidosis or uric acid increase.
- Increased elevation of liver enzymes (transaminase).
- Abnormal glycogen which is amylopectin can be viewed on electron microscope.

Type VI GSD :

Also known as Her's disease. The defect is in hepatic glycogen phosphorylase which converts glycogen to limit dextrin. But gluconeogenesis is intact. Therefore, hypoglycemia is seen but not severe.

Glycogen —✕→ Limit dextrin
↓Glycogen phosphorylase

Muscle Glycogen Storage Disorder 00:26:48

With hypertrophic cardiomyopathy	Without hypertrophic cardiomyopathy
Type II GSD	Type V GSD
Danon disease (defect in lysosomal associated membrane protein-2)	Type VII GSD

Type II GSD :

Also called as Pompe's disease.

Lysosomal storage disorder. Glycogen metabolism is affected in the lysosome.

α 1,4 1,6 glucosidase (acid maltase) is affected.

Clinical features :

- Feeding difficulties.
- Failure to thrive.
- Hypotonia (floppy infants).
- Hypertrophic cardiomyopathy causing cardiomegaly and death (around 2 years due to cardiac failure).

Investigations :

- X-Ray to view cardiomegaly.
- Elevated serum creatinine Kinase.
- Elevated serum AST.
- Elevated serum LDH.

Type V GSD :

Also known as McArdle's disease. Defect in the enzyme muscle glycogen phosphorylase.

Clinical features :

- Exercise intolerance.
- Second wind phenomenon : Pain during exercise subsides with rest and then continue with exercise after sometime.

- Rhabdomyolysis :
  1. Myoglobinuria.
  2. Burgundy colored urine.

Type VII GSD :
Also called as Tarui disease. The defect is seen in muscle & erythrocyte PFK-1.
Muscle : Exercise intolerance, myoglobinuria, no second wind phenomenon.
Erythrocyte : Hemolysis.

Most common muscle glycogen storage disorder in adolescents : Type V GSD.

Type 0 GSD :
Defect in glycogen synthase. These patients die at very young age as glycogen is not synthesized.

Recently added glycogen storage disorders :

Fanconi Bickel syndrome : GLUT-2 defect.
Causes proximal renal tubular acidosis and impaired glucose utilization.

Liver GSD with myopathy : Type III and Type IV.

Liver GSD with neurological manifestation : Andersens disease (the brain and anterior horn cells are affected).

Identifying GSD

00:37:20

If fasting hypoglycemia is present then : Liver GSD.
If exercise intolerance is present : Muscle GSD.

Fasting hypoglycemia → Liver GSD → Abnormal glycogen present → Type III & IV GSD.
Fasting hypoglycemia → Liver GSD → Abnormal glycogen absent → Type I & VI GSD.

Exercise Intolerance → muscle GSD → Second wind phenomenon present → Type V GSD.

Exercise Intolerance → muscle GSD → Second wind phenomenon absent → Type VII GSD.

Exercise intolerance → Hypertrophic cardiomyopathy → Type II GSD.

If a patient presents with hypoglycemia and ketosis, the diagnosis can be between type I & III.

IV glucagon challenge is used to differentiate.

IV glucagon is given during 2 states :

- Well fed state.
- Overnight fast.

In Von Gierke's disease, since G-6 phosphatase (which is required for release of glucose) is absent, there is no increase in blood glucose in both the states.

In Cory's disease, some amount of glucose synthesis occurs via production of glucose-1-phoshphate by glycogen phosphorylase. This along with dietary glucose leads to increase in glucose in well fed state. This is not seen in overnight fasting as the effective glycogenolysis does not produce enough glucose to cause a surge in levels.

GSD	Well-fed state	Overnight Fast
Von Gierkes	No increase in blood glucose	No increase in blood glucose
Cory's	Increase in blood glucose	No increase in blood glucose

Type of GSD	Defect in
Type 0 GSD	Glycogen synthase
Type I GSD	Glucose 6 phosphatase
Type II GSD	Acid maltase
Type III GSD	Debranching enzyme
Type IV GSD	Branching enzyme
Type V GSD	Muscle phosphorylase
Type VI GSD	Hepatic phosphorylase
Type VII GSD	Phosphofructokinase
Fanconi Beckel syndrome	Glucose transporter 2

Active space

GLUCONEOGENESIS

Concept of gluconeogenesis 00:01:46

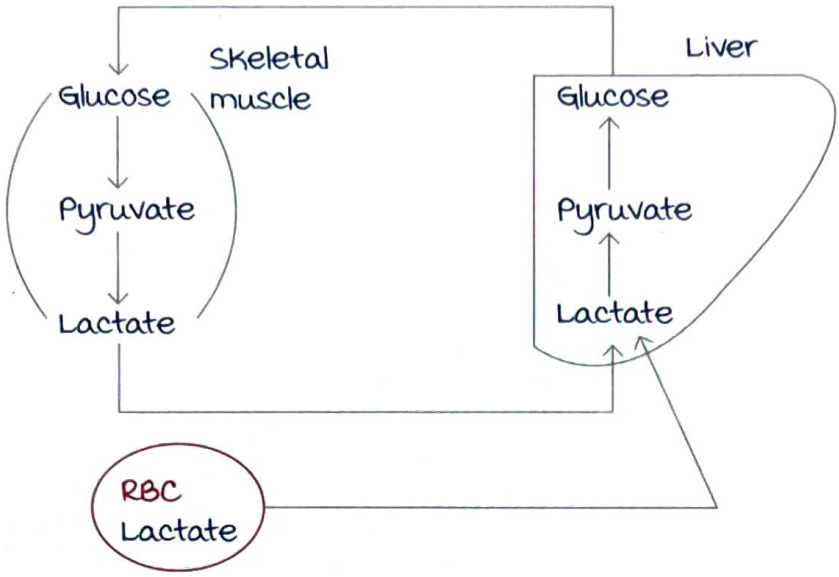
In different stages of fasting, the body gets glucose by
4 to 11 hrs (Early fasting) : Glycogenolysis (hepatic).
In 16 to 18 hrs : All glycogen will be depleted.
16–48 hrs (Fasting) : Gluconeogenesis.
Supplier of ATP for gluconeogenesis : Fatty acid oxidation (by β oxidation).

Definition of gluconeogenesis :

- The process by which glucose is synthesized from non carbohydrate substrates.
- Non-carbohydrate substrates :
  1. Glucogenic amino acids : Alanine is the principle gluconeogenic amino acid.
  2. Lactate.
  3. Glycerol part of fat.
  4. Propionyl coA : From odd chain fatty acid oxidation.
- Note : Acetyl coA is never a substrate for gluconeogenesis.

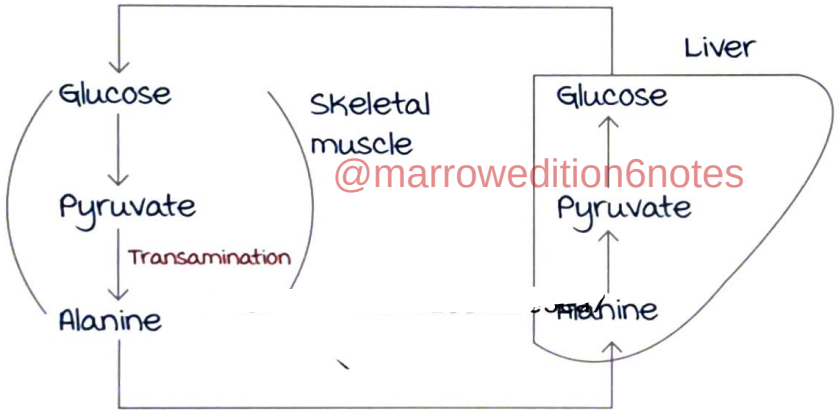
Site : Liver (majorly) & Kidney.
Organelle : Cytoplasm & Mitochondria, Smooth endoplasmic reticulum (SER) also has a role.

1. Cori's cycle / Glucose Lactate cycle :
   - Organs involved : Skeletal muscle, Liver, RBC.
   - It prevents lactic acidosis inside the muscle.
   - In RBCs, lactate is the end product of glycolysis.
   - It happens when muscles are involved in exercise.

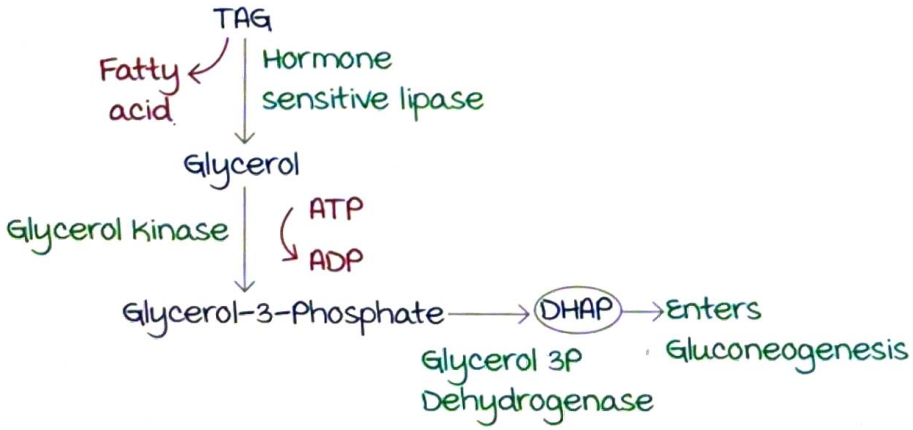


2. Cahill Cycle/ Glucose Alanine cycle :

- It is involved in the 'stages of fasting'.



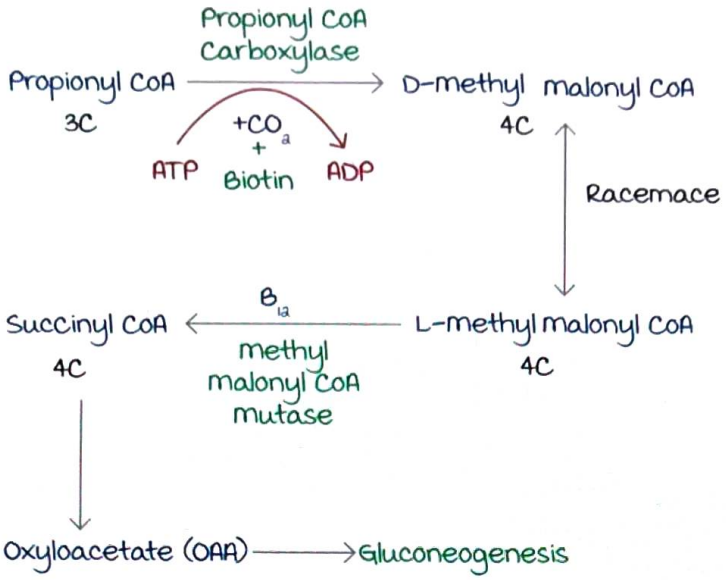
3. Using Glycerol :



DHAP : Dihydroxy Acetone Phosphate.

Active space

4. Using Propionyl CoA :



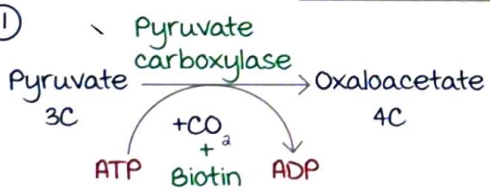
- In case of Vitamin B12 deficiency : There is accumulation of serum methylmalonic acid.

Key enzymes of gluconeogenesis 00:16:15

1. Pyruvate Kinase :

To convert pyruvate back to PEP 2 enzymes and one shuttle are required.
1. Pyruvate carboxylase.
2. Malate aspartate shunt.
3. PEP. carboxy kinase.

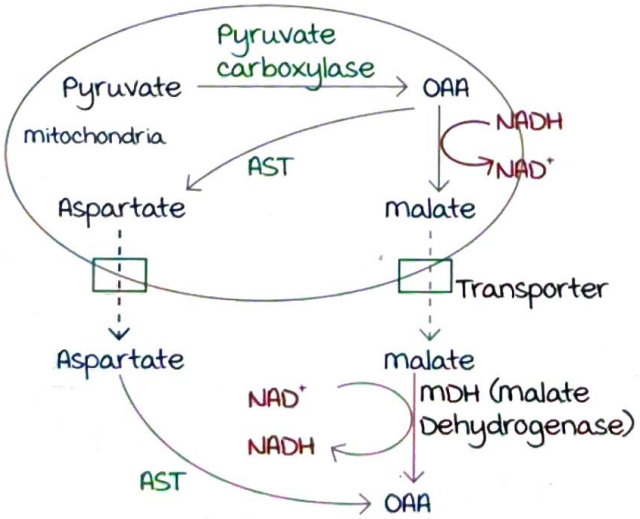
① Pyruvate carboxylase



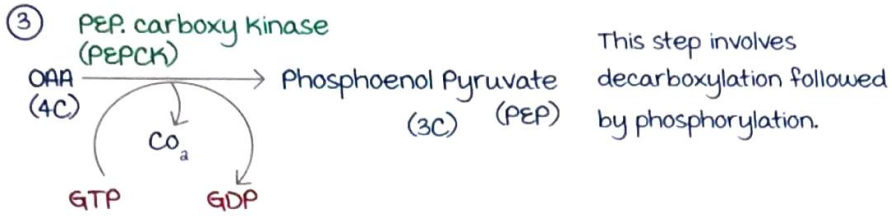
This step takes place in mitochondria and OAA is produced there & cannot cross into cytoplasm.

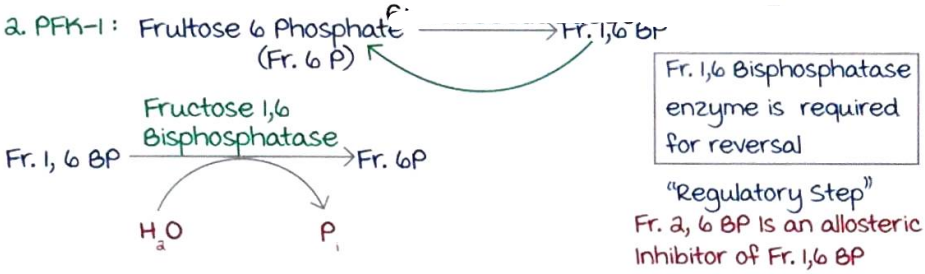
"1st regulatary step" : (Mitochondrial)

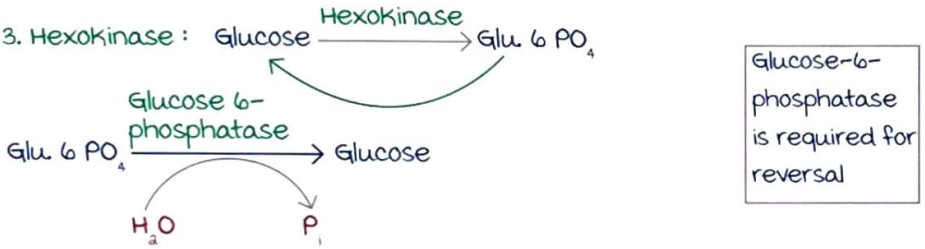
② Malate aspartate shuttle : To transport OAA across mitochondrial membrane.



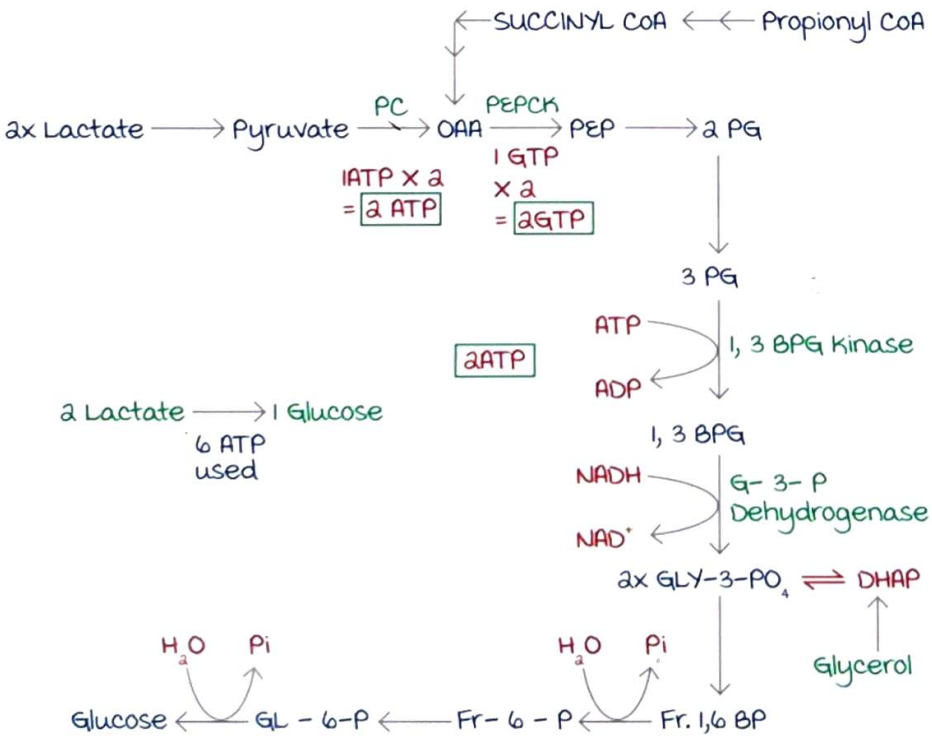
Active space







Summary of gluconeogenic pathway 00:27:53



Regulation of gluconeogenesis 00:33:32

Hormonal regulation :

Active space

- Active in low insulin glucagon ratio : Enzymes are in phosphorylated state (active).

Allosteric regulation :

- Acetal CoA : allosteric activator of 1st regulatory step of gluconeogenesis (Pyruvate carboxylase).
- Fructose 2,6 bisphosphate : allosteric inhibitor of Fructose 1,6 bisphosphatase.

Applied aspect of gluconeogenesis

00:36:03

Raw egg ⟶ Avidin

↓

⊖ Biotin

Raw egg is inhibiting gluconeogenesis by Avidin as it inhibits Biotin required steps.

Biotin is required for

1. Pyruvate ⟶ (⊖, Pyruvate carboxylase) OAA
2. ⊖ Propionyl CoA Carboxylase

As a result, ↓ ↓ Gluconeogenesis

Question 1 : Biguanides are used as oral hypoglycemic agent .A theoretical concern about this drug is lactic acidosis. What is the reason for this concern?

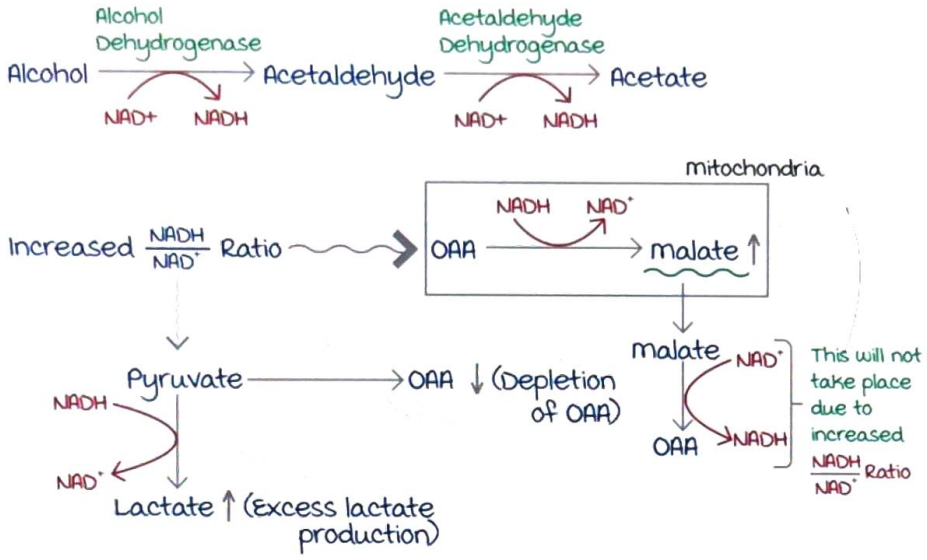
Answer :

- It is because Biguanides inhibit pyruvate carboxylase & decrease gluconeogenesis.
- If pyruvate is not converted to oxaloacetate, it may convert into lactate.
- When there is no OAA, TCA cycle is also inhibited.

Question 2 : A chronic alcoholic brought to casualty, as he was drinking heavily for the past 1 week and he had not eaten any food for past 3 days. He was confused and was sweating profusely. He developed seizure in the casualty. His blood glucose drawn just before seizure was 30 mg/dl and blood ethanol was very high. What are the metabolic changes in him which resulted in hypoglycemia?

Answer :

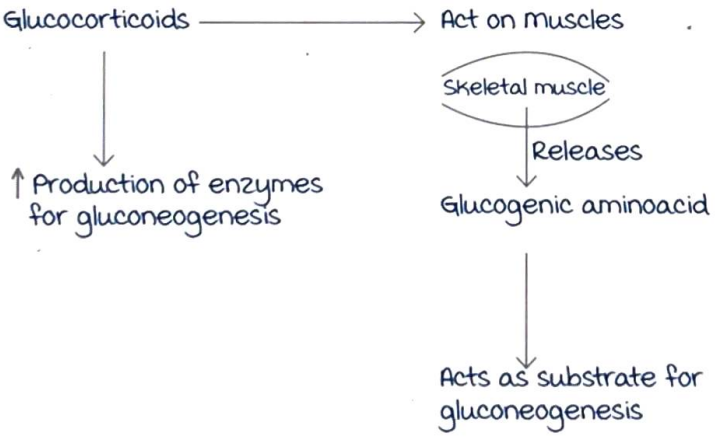
Active space



As a result : ↓ Gluconogenesis

Question 3 : A patient with acute exacerbation of chronic bronchial asthma treated with high dose IV methyl prednisolone. On discharge she was discharged by switching over to oral prednisolone. After 5 days she presented with polyuria, polydipsia, and muscle weakness. Substantiate the above clinical history biochemically?

Answer :



As a result : Increased Gluconeogenesis leading to development of given symptoms.

Active space

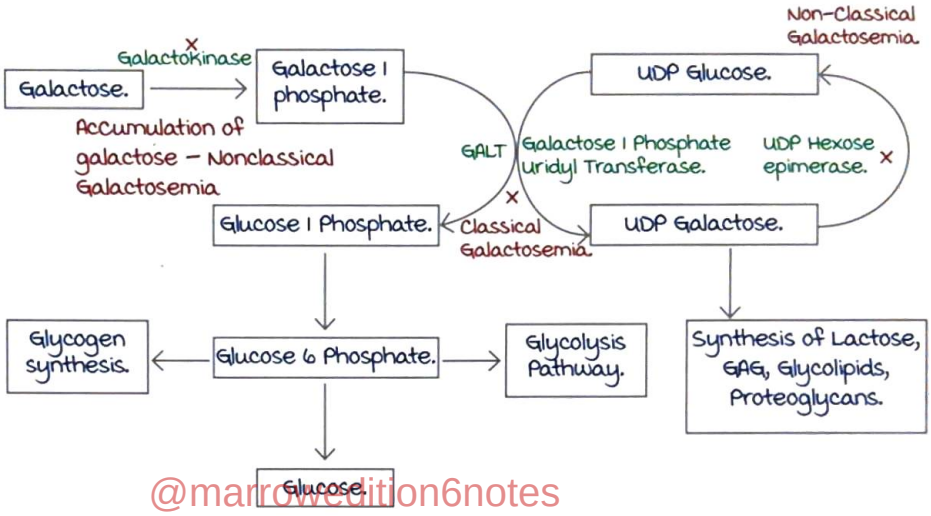
MINOR METABOLIC PATHWAYS

Galactose metabolism 00:02:20

Source : Lactose, Milk Sugar.

Function : Convert to Glucose and used in synthesis of lactose, Glycosaminoglycans.

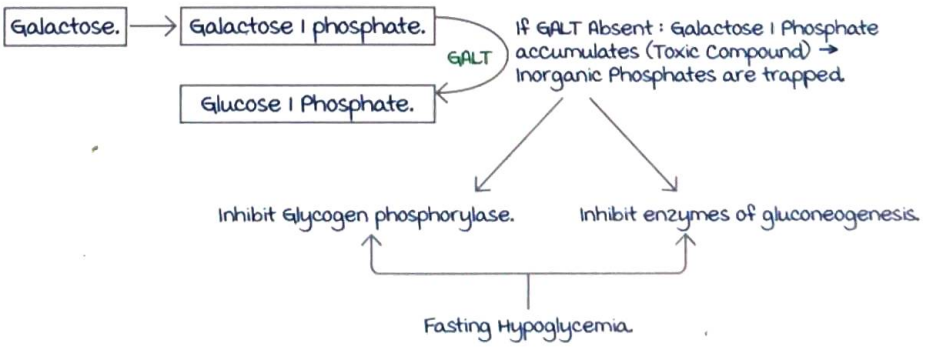
Site : Liver (major), Erythrocytes, Fibroblasts.



Galactosemia 00:07:36

Classic Galactosemia.	Non-Classic Galactosemia.
GALT deficiency.	Galactokinase deficiency : Only Clinical feature: Cataract. UDP Hexose epimerase deficiency : Clinical feature: Varies.

Biochemical Defect :



Clinical features :

Age of onset : "First 2 weeks of life" – Breast milk (Galactose present).

Feeding difficulties, Vomiting, Jaundice, Failure to regain birth weight.

Hepatomegaly, Liver failure, Mental retardation.

"Oil drop Cataract".

Neonatal Sepsis due to E.coli bacteria.

Lab diagnosis :

- Urine : Reducing substance positive (Benedict's test) (Non specific).
- Glucose oxidase test : Negative.
- Mucic Acid test : White precipitate (Specific).
- Enzyme studies.
- Genetic mutation studies.

Treatment :

Restrict Lactose : Till 4 years of age → C/I for Breast feeding.

Galactose 1 phosphate pyro-phosphorylase activated.

Galactose. → Galactose.

Galactokinase and Epimerase deficiency are Benign conditions manifests as Cataract only.

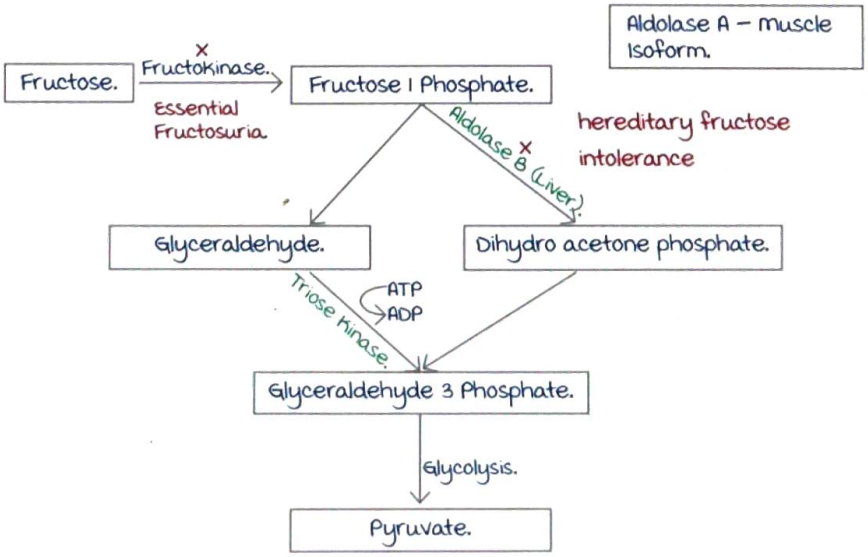
Fructose metabolism

Source : Dietary sucrose,

Dietary free fructose : Honey and fruits,

Polyol Pathway : Glucose → Fructose.

Site : Liver.



Active space

Hereditary Fructose Intolerance :

Deficiency of Aldolase B → Fructose 1 Phosphate Accumulation → Toxic.

Similar to Galactosemia.

Traps inorganic Phosphates → Inhibits glycogen Phosphorylase and Enzymes of Gluconeogenesis → Fasting Hypoglycemia.

C/F : Similar to galactosemia except,

Age of onset : Around 6 months (age of supplementary feeding).

Feeding difficulties, Vomiting, Jaundice, Failure to regain birth weight.

Hepatomegaly, Liver failure, Mental retardation.

No Cataract.

Lab diagnosis :

- Benedict's test : Positive.
- Test for Ketoses : Positive.
  - Rapid Furfural test : Positive.
  - Seliwanoff's test : Positive.
- Glucose oxidase test : Negative.
- Enzyme studies.
- Mutation studies.

Treatment:

Restrict Sucrose.

Essential Fructosuria 00:26:29

Deficiency of fructokinase.

Benign Condition.

Fructose has no renal threshold → Fructose excreted in urine.

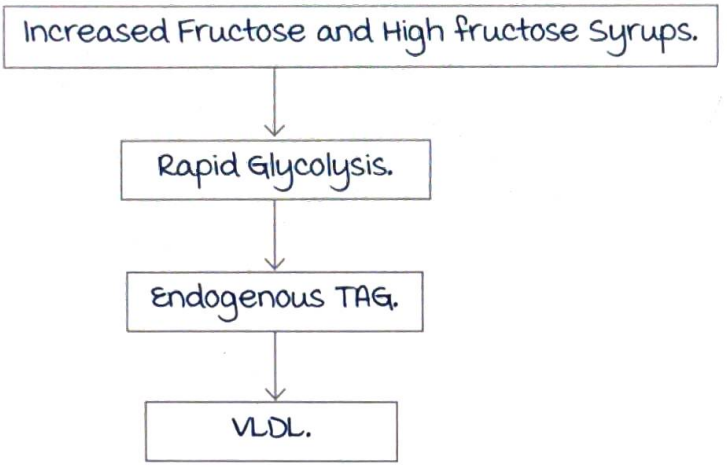
Fructose is harmful because :

- Fructokinase is not dependent on insulin and converts to fructose-1-phosphate.
- Tightly regulated step of glycolysis : PFK 1 is not seen in

Fructose metabolism.

- Easily convert Pyruvate → Acetyl Co A → Fatty acid → TAG → VLDL & LDL.

Diets :



Hexose Monophosphate Pathway 00:30:59

Other Names : Pentose Phosphate Pathway, Dickens Horecker pathway, Phosphogluconate pathway.

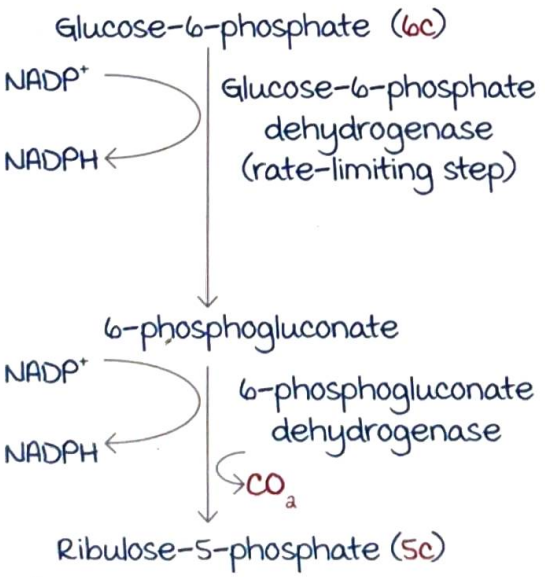
Sites : Cytoplasm.

Phases : Oxidative is Irreversible & Nonoxidative is Reversible.

Do not produce ATP.

CO_a is produced.

Oxidative phase : Irreversible.



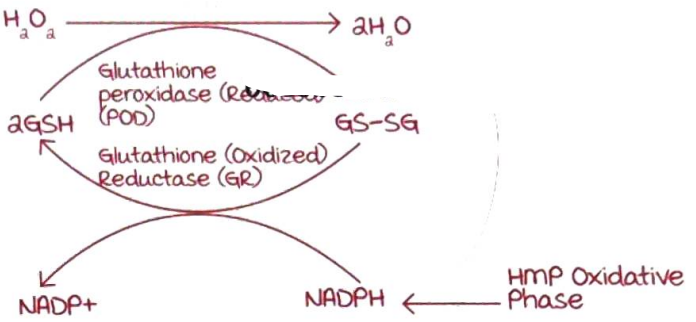
Biochemical Significance :

1. Generation of NADPH.

Active space

Functions of NADPH :

1. Free radical scavenging : Important in RBC and Lens.



2. Keep iron in reduced state in Hb (Fe^{2+}) (Prevent methemoglobinemia).
3. Reductive Biosynthesis of fatty acids and steroid hormones.

Organs of HMP Shunt : Liver, Adipose tissue, Adrenal cortex, Gonads.

Non-Oxidative phase

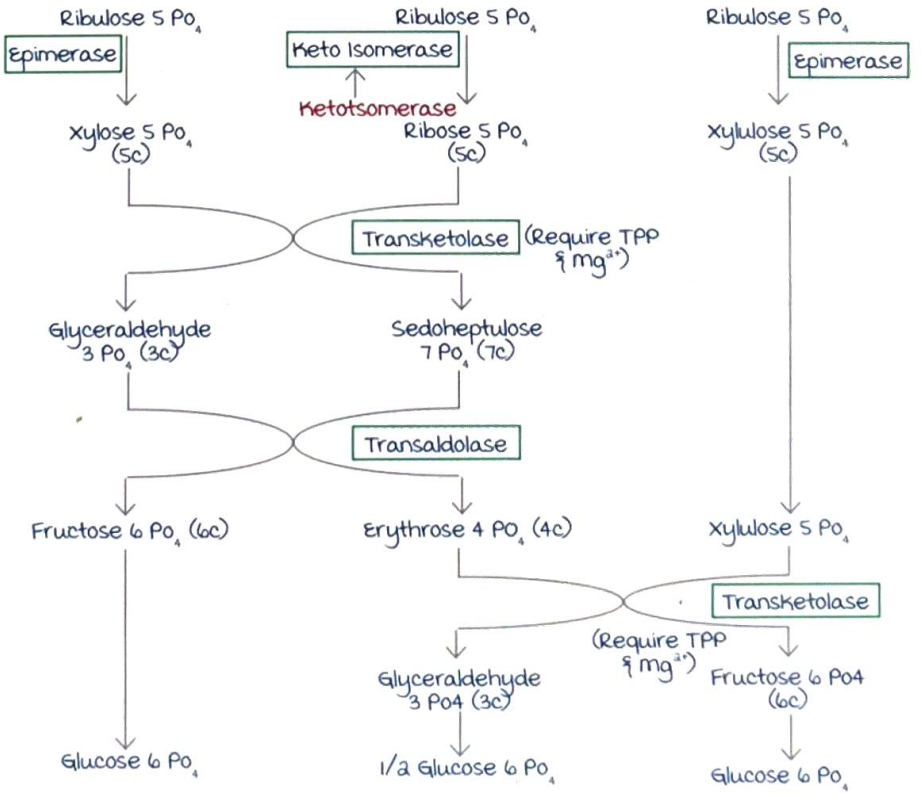
00:39:08

Produce pentoses.

All steps are reversible.

Biochemical Significance :

Organs : Rapid cell turn over like Bone marrow, Intestine, Skin.



Active space

Clinical significance :

1. G6PD Deficiency.
   - m/c enzyme deficiency in Humans.
   - X linked recessive disorder.
   - Only males are affected.

Aggravating factors :

- Fava bean ingestion : Favism.
- Drugs : Sulfa drugs, Primaquine.
- Prevalent in Mediterranean and middle east regions (Due to prevalence of Plasmodium falciparum).

C/F :

Decreased G6PD → Decreased NADPH → Free radical scavenging → RBC membrane integrity lost → Hemolysis → (I) Hemolytic anemia (II) Jaundice.

NADPH required to keep iron in Fe^{2+} state. In NADPH defecient state it gets converted to Fe^{3+} → methemoglobinemia

Uronic Acid pathway 00:52:54

Oxidative pathway of glucose.

Site : Liver.

Organelle : Cytoplasm.

Functions : a) Produces Uronic acid:

- Conjugation of Bilirubin (Glucuronic acid).
- Synthesis of GAG and Proteoglycans.

b) Produce pentoses.

c) Ascorbic acid : can't be synthesized in humans and higher primates (Gluconolactone oxidase).

Essential Pentosuria :

Benign.

L – Xylulose is excreted in urine.

Enzyme: Xylitol dehydrogenase / Xylulose reductase.

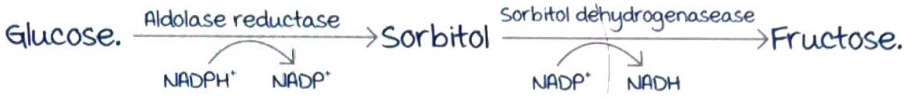
Benedict's test : Positive.

Test for pentoses : Positive.

Polyol Pathway

00:56:53

To convert Glucose → Fructose.



Lens, Nerves and Kidneys	Liver, Ovary and Seminal Vesicles
Activaty of Sorbitol dehydrogenase is less. Glucose → Sorbitol (In case of accumulation of Glucose).	Glucose → Fructose.

Q. Why Cataract in Galactosemia and DM?

Lens ← GLUT I

GLUT I : Independent of insulin , present in Lens.

Excess Glucose → To lens (Aldolase reductase present here) → Aldose reductase Activated → Glucose converted to Sorbitol and Galactose converted to Galactitol or Dulcitol.

Both Sorbitol and Galactitol are osmotically active and attracts H_2O → Cataract.

Clinical questions :

Q. A person had a road traffic accident,his legs were badly injured . Later it got infected .Pus culture revealed methicillin resistant S aureus . A comination of Trimethoprim and Sulfamethoxazole was started. On the third day of therapy she developed jaundice and the Hb level fall down to 4g/dl. Suspected an apparent acute hemolysis on exposure to infection and sulfa drugs?

1. Which enzyme defect might have aggravated the attack of hemolytic jaundice and anaemia in this patient.
2. Substantiate the reason for hemolysis.

Ans : 1) G6PD deficiency.

2) G6PD and NADPH deficiency.

Active space

Q. A patient with recently diagnosed Diabetes mellitus started to avoid cane sugar from her diet .But she continued to consume lots of fruits which resulted in poor diabetic control.

1. Which sugar is responsible for the poor diabetic control?
2. What is the reason ?

Ans : High fructose may lead to hypertriglyceridemia due to increased glycolysis.

Q. A neonate soon after birth started vomiting, jaundice and distended abdomen. The consultant paediatrician noticed that her liver is enlarged. Sooner an ophthalmology consultation advised. Certain laboratory investigations done.

1. What is the probable diagnosis?
2. Which enzyme deficiency is suspected?
3. What are the investigations done?

Ans : 1) Galactosemia.
2) Galactose-1-phosphate uridyl transferase.
3) Mucic acid test (Specific) and urine reducing sugars.

Active space

CHEMISTRY OF LIPIDS

Definition and classification of lipids 00:00:52

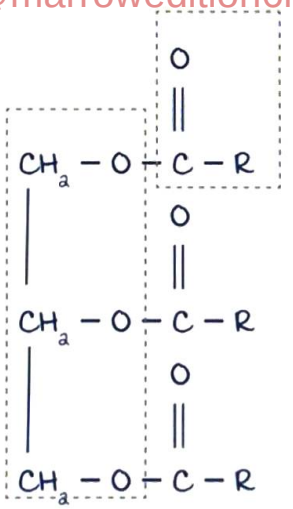
Definition : Heterogeneous group of compounds which are insoluble in water but are soluble in non-polar solvents.

Lipids are related to each other more physically than chemically.

Bloor's classification of lipids :

- Simple lipids : Ester of alcohol (glycerol) + acid (fatty acid).
  E.g. Fats, oils, waxes.
- Compound lipids : Ester of alcohol + acid + other component.
  E.g. Phospholipids, lipoproteins, glycolipids.
- Derived lipids : Derived from simple or compound lipids.
  E.g Glycerol, fatty acid.
- Miscellaneous : Steroid hormones, fat soluble vitamins.

Neutral fat : Triacylglycerol (TAG) is a simple lipid.



Fatty acids 00:06:52

General formula : R – COOH.

R represents a hydrophobic hydrocarbon chain.

Classification of fatty acids :

Based on number of carbon atoms :

1. Short chain fatty acid (SCFA) : $C_2 - C_6$

2. Medium chain fatty acid (MCFA) : $C_8 - C_{14}$
3. Long chain fatty acid (LCFA) : $> C_{16}$.
4. Very long chain fatty acid (VLCFA) : $> C_{20/22}$.

Based on the presence of double bond :

1. Saturated fatty acid : No double bonds.
2. Unsaturated fatty acid : [illegible]
   MUFA (Monounsaturated FA) : 1 double bond.
   PUFA (Polyunsaturated FA) : > 1 double bond.

Saturated fatty acids :

SCFA	Source
Acetic acid	Vinegar
Propionic acid (3C) Butyric acid (4C) Valeric acid (5C) Capric acid (10C)	Butter
MCFA	
Lauric acid (12C) myristic acid (14C)	Coconut oil (richest)
LCFA	
Palmitic acid (16C) Stearic acid (18C)	Animal fat

Unsaturated fatty acids :

MUFA	Source
Palmitoleic acid Oleic acid Elaidic acid	mustard oil/grapeseed oil (richest source)
PUFA	
Linoleic acid (18 C : 2 double bonds)	Safflower oil (richest)
GLA : γ linolenic acid (18:3)	Oil of evening primrose
α linolenic acid (18:3)	Flaxseed oil (richest)
Arachidonic acid (20:4)	Animal fat
Timnodonic acid (20:5) EPA : Eicosapentaenoic acid Cervonic acid (22:6) DHA : Docosahexaenoic acid	Fish oils, algal oils, breast milk

Active space

Highest concentration of PUFA is present in safflower oil.

- 2nd highest is in sunflower oil.
- Least concentration : Coconut oil.

Essential fatty acids : Cannot be synthesised in the body.

- Linoleic acid.
- α Linolenic acid.

Semi essential fatty acid : Can be synthesised from essential acids.

- Arachidonic acid (from linoleic acid).
- γ Linolenic acid (from lioleic acid).

Omega (ω) classification of fatty acids 00:17:42

Carbon atoms are numbered from the terminal methyl group. The fatty acid is classified based on the position of the 1st double bond from terminal methyl group.

Example : ω_4 fatty acid :

ω 1 2 3 4 5 6 7 8

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

Δ 8 7 6 5 4 3 2 1

Δnumbering system : Numbering starts from functional group.

ω_3 fatty acid	ω_6 fatty acid
Alpha linolenic acid	GLA
Timnodonic acid	Linoleic acid
Cervonic acid	Arachidonic acid (20 C)

ω_6 fatty acids is more harmful than ω_3 fatty acids :

- Arachidonic acid is a source of eicosanoids (prostaglandins, leukotrienes).
- These inflammatory mediators increase cardiovascular risk & degenerative disorders.

Significance of ω_3 fatty acids :

Decreased cardiovascular risk.
Decreased platelet aggregation.
Decreased inflammation.
Infant development (DHA for brain development).
Decreased chance of mental illness.

Decreased chance of degenerative disorders.
(rheumatoid arthritis, alzheimer's).

Docosahexaenoic acid :

DHA is an ω_3 fatty acid.

Source : Breast milk, algal oils, fish oils.

Uses :

- Infant and foetal brain development.
- Retinal development.

Low DHA is associated with retinitis pigmentosa.

Transplacental transport is possible for DHA. So, taking DHA tablets during pregnancy may be beneficial.

Cis and trans fatty acids 00:26:38

Fatty acids with a double bond exists in 2 isomeric forms.

- Cis form : The structure has a bend (angle : 120°).
  It increases fluidity of plasma membrane.
- Trans form : The structure is straight (extended).

Sources of trans fatty acids :

Vanaspati is the richest source.
Partially hydrogenated fat (margarine, dalda, vanaspati, cake butter) used in bakery products to improve shelf life.
Deep frying e.g french fries.
Reheating of vegetable oil.

Heat converts cis form to harmful trans form.

Daily allowance of trans fatty acids : 2-7 g/day.

Disadvantages of trans fatty acids :

- Essential fatty acid deficiency.
- Lipid fractions : Increased TAG & LDL, decreased HDL.
- Increased cardiovascular risk.
- Increased inflammatory response.
- Causes insulin resistance.

Coconut oil can be better than sunflower oil as :

- Coconut oil is saturated FA, trans-isomerism occurs in UFA.
- MCFA is directly absorbed from portal vein.

Active space

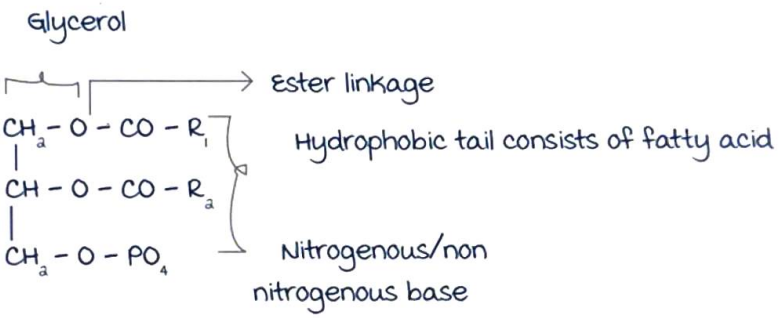
SPHINGOLIPIDOSES

Phospholipids 00:00:42

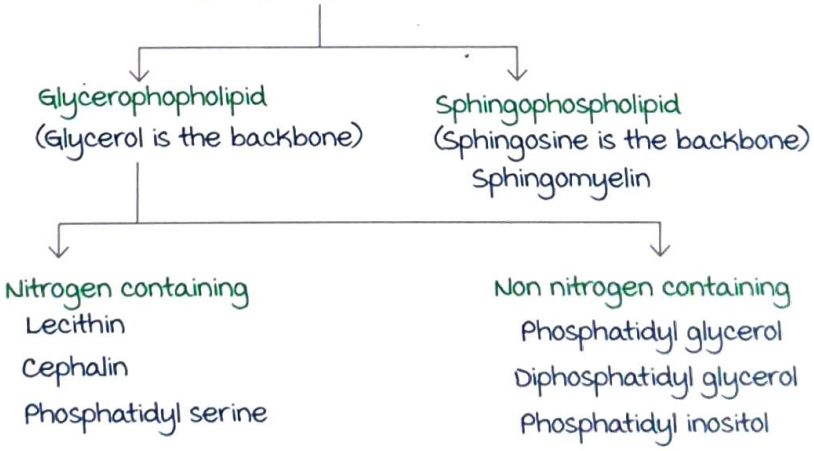
Phospholipids are compound lipids that consist of :

1. Fatty acid.
2. Alcohol.
3. Phosphoric acid.
4. Base : Nitrogen containing and non-nitrogen containing.

General structure of phospholipid :



Classification of phospholipids :



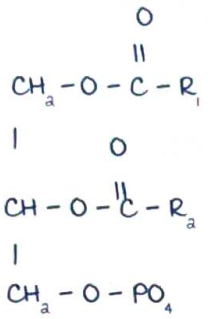
Phosphatidic acid, lecithin, cephalin 00:03:30

Phosphatidic acid :

Simplest glycerophospholipid.

Glycerol + 2 fatty acid residues (Diacyl glycerol) + PO_4.

No nitrogenous base :



All glycerophopholipids are derived from phosphatidic acid.
Diacyl glycerol + PO_4 + nitrogenous base = Lecithin (phosphotidyl choline).

Significance :
Most abdundant phospholipid in cell membrane.
Major constituent of lung surfactant.
Store house of choline.

Cephalin :
Phosphatidic acid + Nitrogenous base = Cephalin (ethanolamine) (phosphatidyl ethanolamine).

Significance : Blood coagulation.

Cardiolipin, phosphatidyl serine & phosphatidyl inositol

00:08:06

Cardiolipin : Diphosphatidyl glycerol (phosphatidic acid + Glycerol + phosphatidic acid).
Significance :
Isolated first from cardiac muscle, hence the name.
Present in the inner mitochondrial membrane.
Only antigenic phospholipid.

Disorders associated with defect in cardiolipin are :
1. Cardioskeletal myopathy (Barth syndrome).
2. Ageing.
3. Hypothyroidism.
4. Heart failure.

Phosphatidyl serine :
Phosphatidic acid + Nitrogenous base (serine).
Significance : mediator of programmed cell death/apoptosis.

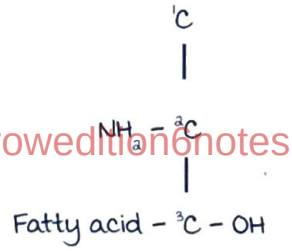
Phosphatidyl inositol :
Significance :
Cell signalling.
Mediator of second messengers in hormonal pathways.

Sphingomyelin

00:11:26

Only phospholipid with sphingosine as backbone.
Amino alcohol.
Derived from serine.

Sphingosine :



Sphingosine + Fatty Acid + PO_4 + Nitrogenous base.

Ceramide : Sphingosine + Fatty Acid.

Sphingomyelin : Sphingosine + 2 Fatty Acid + PO_4 + Choline.
Significance :
1. Outer membrane of plasma membrane.
2. Specialised structures in plasma membrane : Lipid rafts.
3. Myelin sheath of nervous tissue.

Glycolipids

00:15:23

Complex lipid that contain carbohydrate but no phosphate group.
Contain sphingosine.
A/K/a glycosphingolipids.

Phospholipid	Glycosphingolipid
Glycerol or sphingosine	Sphingosine
PO_4 present	No phosphate group

Structure of glycolipids :
Sphingosine + Fatty Acid + Carbohydrate.
Three types :
1. Cerebroside.
2. Globoside.
3. Ganglioside.

Cerebroside, globoside & ganglioside 00:15:23

Cerebroside : Ceramide + Monosaccharide.
If the monosaccharide is :
Glucose : Glucocerebroside.
Galactose : Galactocerebroside.

Glucocerebroside : Present in non-neural tissue.
Galactocerebroside : Present in neural tissue.

Globoside :
Ceramide + Disaccharide/oligosaccharide.
Eg. Lactosyl ceramide.

Ganglioside :
Ceramide + Oligosaccharide (NANA).
NANA : N-Acetyl Neuraminic Acid = Sialic acid.
Named as GM_n
Ganglioside | Unique number : Assigned based on chromatography
Monosialo containing

GM1 : Ganglioside that acts as receptor for cholera toxin in human Intestine.
GM3 : Simplest ganglioside.

Active space

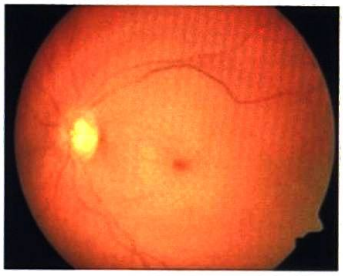
Sphingolipidoses

00:22:53

Definition : Sphingolipidoses is a group of lysosomal storage disorders charcterised by an inherited deficiency of lysosomal hydrolase, leading to an intralysosomal accumulation of sphingosine containing lipid substrates.

GM_1 gangliosidosis :

Case : 4 month old infant presented with frontal bossing, depressed nasal bridge, long philtrum, low set ears. Fundoscopy shows cherry red spot.



Biochemical defect : Defect in β-galactosidase enzyme results in accumulation of GM_1 ganglioside.

$$GM_1 \text{ ganglioside} \xrightarrow{\beta - \text{galactosidase}} GM_a \text{ ganglioside}$$

Clinical features :

Blindness.

Typical facies : Frontal bossing, long philtrum, depressed nasal bridge, low set ears.

macular cherry red spot (seen in approximately 50%).

Angiokeratoma.

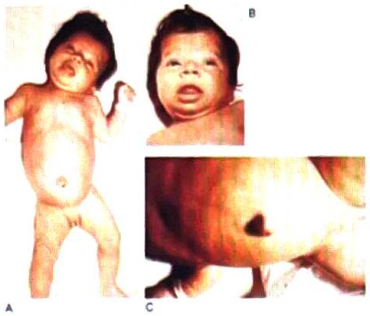
Hepatosplenomegaly.

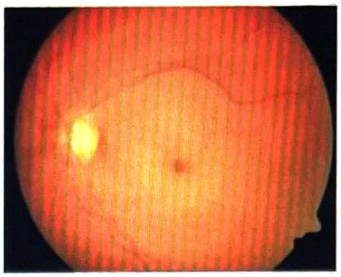
mental retardation.

GM_a gangliosidosis :

Case : 2 year old boy who is hypotonic, decerebrate and blind. Fundus examination reveals cherry red macular spot. What is the defect in this child?

Ans. Defect in hexosaminidase enzyme





Biochemical defect : Defect in β-hexosaminidase enzyme results in accumulation of GM_a ganglioside.

$$GM_a \text{ ganglioside} \xrightarrow{\beta\text{-hexosaminidase}} GM_3 \text{ ganglioside}$$

β- hexosaminidase isoforms :

A → 1α and 1β

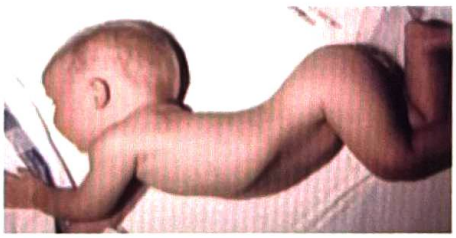
B → 2β

Types of GM_a gangliosidosis :

Tay Sach's disease	Sandhoff's disease
β- hexosaminidase A (defect in α subunit). Clinical features : Cherry red spot in macula and retina. Hyperacusis. Neurological deficits. Macrocephaly.	β- hexosaminidase A and B (defect in β subunit) Clinical features : All features of Tay Sach's disease along with hepatosplenomegaly, cardiac abnormalities and bony deformities.

Krabbe's disease & Gaucher's disease 00:09:41

Q. 5 month old girl presented with developmental delay. At 9 months the same childs condition rapidly deteriorated. Now she is in ophiothotonus posture with clenched fist.



Krabbe's disease :

Biochemical defect : defect in β-galactocerebrosidase/ galactosidase enzyme results in accumulation of β-galactocerebroside.

β-galactocerebroside $\xrightarrow{\text{β-galactocerebrosidase}}$ Cerebroside

Major location of β-galactocerebroside in neural tissues.

Clinical features :

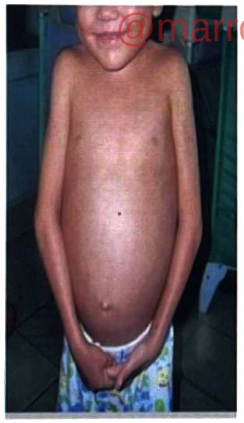
Severe neurological deficit.

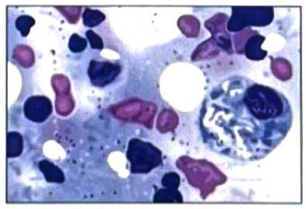
No hepatosplenomegaly (as enzyme not present here)

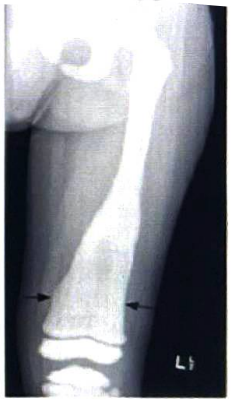
Cherry red spot + enlarged macrophages causing globoid cell inclusions (in white matter).

Gaucher's disease :

Case : 3 year old child presents with abdominal distension & bone pain. Xray of femur shows peculiar deformity. BMA shows cells with crumpled tissue paper appearance.







Most common lysosomal storage disorder.

Biochemical defect : Defect in β-glucocerebrosidase/ β-glucosidase enzyme results in accumulation of β-glucocerebroside.

β-glucocerebroside $\xrightarrow{\text{β-glucocerebrosidase}}$ Cerebroside

Location of β-glucocerebroside : Extra neural tissues.

Clinical features :

Pain in long bones, and pathological fractures.

Haematological features include decreased thrombocytes &

pancytopenia. This can lead to bleeding manifestation & anemia. No intellectual deficit.
No cherry red spot (exception : Type II Gaucher's disease - pseudo-cherry red spot present).
X-ray features : Erlenmeyer flask deformity.
Bone marrow shows Gaucher cells (crumpled tissue paper/ wrinkled paper appearance).

Treatment :

1. ERT (Enzyme Replacement Therapy) :
   Recombinant acid β glucosidase (imiglucerase).
   Velaglucerase α.
   Taliglucerase α.
2. Oral substrate reduction therapy :
   Miglustat : Inhibits glucosyl ceramide synthase.
3. Bone marrow transplantation.

Gaucher's disease	Krabbe's disease
β-glucocerebrosidase accumulation in non-neural tissues	β-galactocerebrosidase accumulation in neural tissues
No mental retardation or neurological deficit	Severe neurological deficit
Hepatosplenomegaly is present	No hepatosplenomegaly

Niemann Pick disease, Farber's disease & Fabry's disease

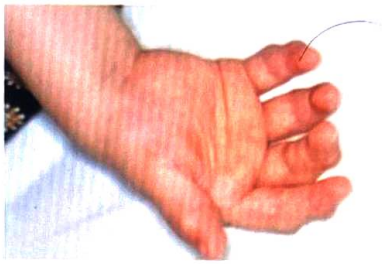
00:42:00

Niemann pick disease :
Biochemical defect : Defect in sphingomyelinase enzyme, results in accumulation of sphingomyelin.
Clinical features :
Cherry red spot in macula.
Zebra body inclusions.

Farber's disease :
Case : 18 month old child presented with painful joint swelling & nodule.

Biochemical defect of acid ceramidase enzyme.

Clinical feature : Painful joint swelling (resembles rheumatoid arthritis).



Fabry's disease :

X-linked recessive disorder.

Males are affected.

Biochemical defect of α-galactosidase enzyme, leading to accumulation of globotriaosylceramide.

Clinical features :

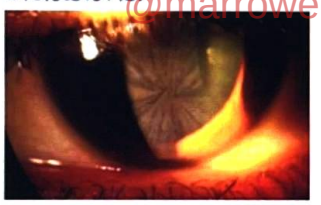
Angiokeratoma.

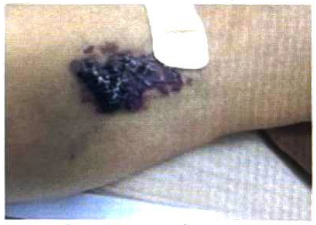
Corneal & lenticular opacity.

Fabry's crisis (agonising pain in the proximal joints).

Hypohydrosis.

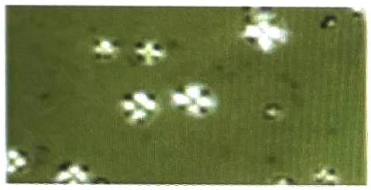
Urinary sediments show maltese appearance, due to lipid inclusions.





Angiokeratoma

maltese cross



Treatment :

Enzyme Replacement Therapy (ERT) : Recombinant α-galactosidase :

1. Agalsidase β (fabrazyme).
2. Agalzidase α (replagal).

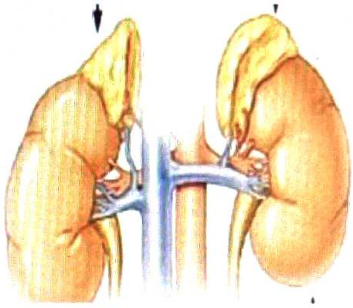
Wolman's disease & general features of all sphingolipidoses

00:25:51

Wolman's disease :

Also known as cholesterol ester storage disease (CESD).

Biochemical defect of acid lipase enzyme, leading to accumulation of cholesterol ester & triacylglycerol, in histiocytic foam cell. Not type of sphingolidoses.



Clinical features :
Watery green diarrhoea.
Failure to thrive.
Relentless vomiting.
Hepatosplenomegaly.
Calcification of adrenals (pathognomonic feature).

General features of all sphingolipidoses :
All are autosomal recessive, except Fabry's disease.
All have mental retardation, except Gaucher's disease.
All have cherry red spots, except Gaucher's & Fabry's disease.
Sphingolipidoses with corneal clouding : Fabry's disease & GM$_1$ gangliosidosis.

Inclusion bodies :

Globoid cell : Seen in Krabbe's disease.
Zebra cell : Seen in Niemann Pick disease.
Maltese cross in urinary sediment : Seen in Fabry's disease.

Lysosomal storage disorders	
Disease	Enzyme defect
GM1 gangliosidosis	β-galactosidase
Tay Sach's disease	β-hexosaminidase A
Sandhoff's disease	β-hexosaminidase A & B
Krabbe's disease	β-galactocerebrosidase
Niemann Pick Type-I	Sphingomyelinase
Gaucher's disease	Glucocerebrosidase
metachromatic leukodystrophy	Aryl sulfatase A
Farber's disease	Ceramidase
Wolman's disease	Acid lipase
Fabry's disease	α-galactosidase

OXIDATION OF FATTY ACID

Concept :

- Early fasting (4-16 hrs) : Glycogenolysis.
- Fasting (16-48 hrs) : Gluconeogenesis.
  Fatty acid oxidation (provide acetyl CoA and ATP to activate gluconeogenesis).
- Prolonged fasting (>2 days) : Fatty acid oxidation (ketone bodies synthesis).

Types of fatty acids oxidation 00:03:58

m/c oxidation : β oxidation.

m/c fatty acid to undergo fatty acid oxidation : Saturated fatty acid (palmitic acid : C 16).

Other : VLCFA (Very long-chain fatty acid).
UFA (Unsaturated fatty acid).
Odd chain FA.

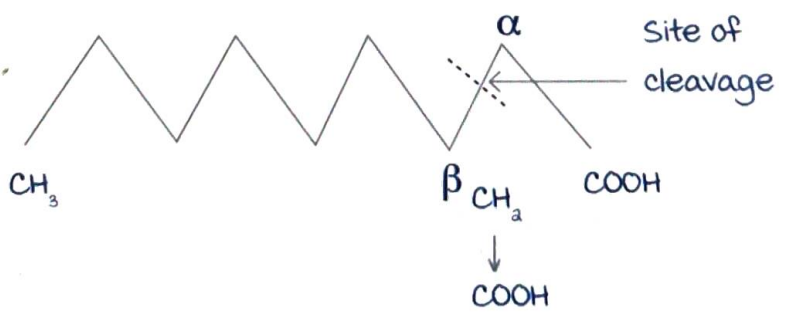
Minor oxidation pathway :

α oxidation.

ω oxidation.

β oxidation 00:05:03

A process by which fatty acids are successively cleaved to 2C acetyl CoA and release energy.



The process is known as β oxidation as β carbon (CH_2) group gets oxidised to COOH.

Site of cleavage : Between α & β.

Liver, muscle, and adipose tissue.

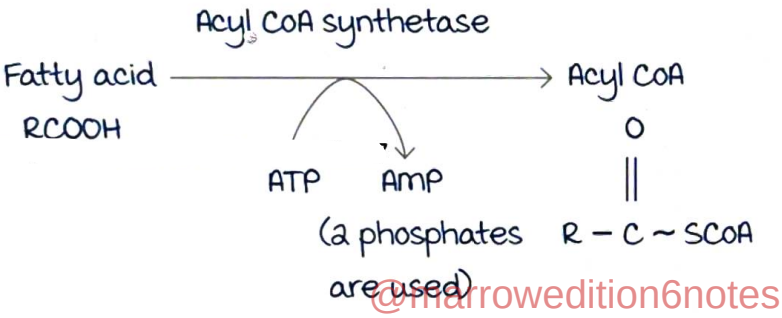
In mitochondria.

Steps of fatty acid activation :

1) Activation of fatty acids.
2) Transport of activated fatty acid from cytoplasm to mitochondria.
3) β oxidation.

Activation of fatty acid :

Site : Cytoplasm.



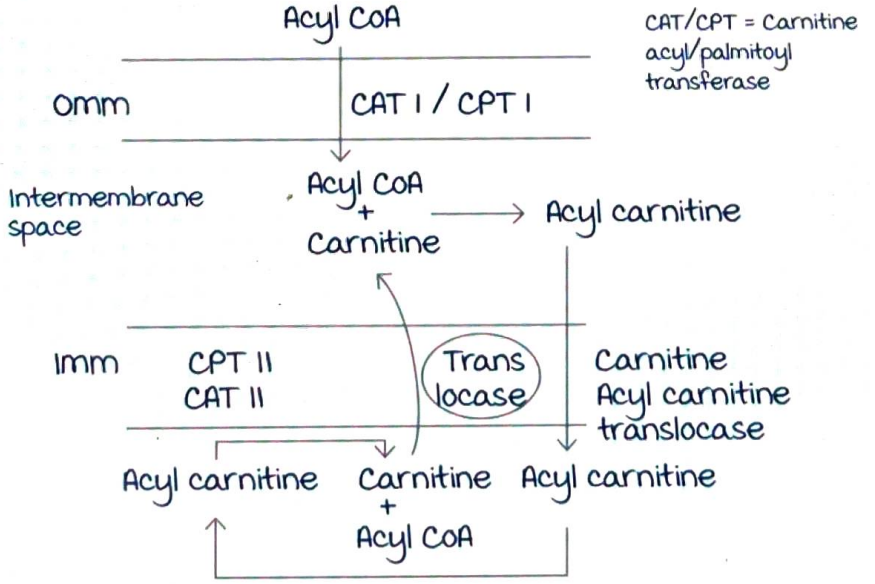
The only place that requires energy.

The enzyme (acyl CoA synthetase) is situated in the outer mitochondrial membrane.

The reaction is happening in the cytosol.

The enzyme belongs to the ligase.

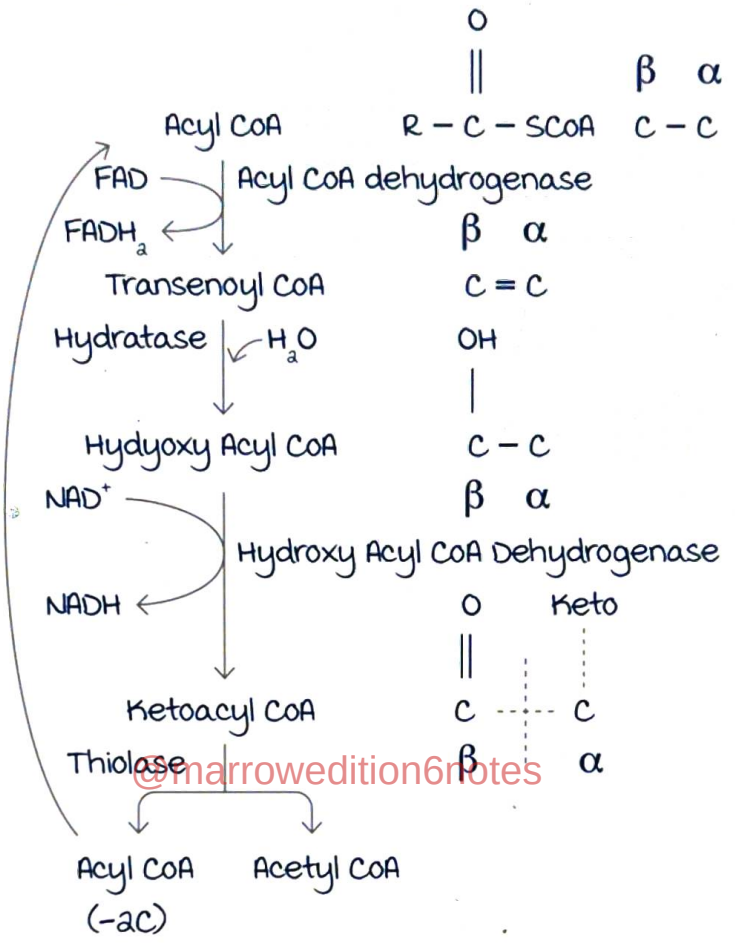
Transport of fatty acid to mitochondria : Occurs with help of carnitine (FA < 14C does not require carnitine).



Active space

CAT I (Carnitine Acyltransferase) : Transfer carnitine to the activated fatty acid.

Reactions of beta-oxidation :



Carnitine (β hydroxy gamma methyl ammonium butyrate) :

Lysine & S adenosyl methionine is involved in the synthesis of carnitine.

Vitamin C is also involved.

FA < 14C doesn't require carnitine for transport.

Energetics

00:23:48

$$\text{No of beta oxidation} = \frac{\text{No of C atom}}{2} - 1$$

$$\text{No of acetyl CoA} = \frac{\text{No of C atom}}{2}$$

Example : Palmitic acid (16C).

$$\text{No of beta oxidation} = \frac{16}{2} - 1 = 7$$

Active space

No of acetyl CoA = 16/2 = 8.

1 beta oxidation produce 1 $FADH_2$ (1.5 ATP) & 1 NADH (2.5 ATP)

= 4 ATP.

Total = 7x4 = 28.

8 acetyl CoA :

1 acetyl CoA produces 10 ATP in TCA.

8 acetyl CoA = 80 ATP.

Total ATP = 80 + 28 = 108.

For activation of acyl CoA synthetase, 2 ATP are used.

Net ATP = 108−2 = 106.

Based on old calculation :

1 $FADH_2$ = 2 ATP.

1 NADH = 3 ATP.

1 TCA = 12 ATP.

The number of ATPs produced by beta oxidation of stearic acid is 120.

The number of ATPs produced by beta oxidation of palmitic acid is 129.

Regulation of beta-oxidation 00:29:39

Rate Limiting Enzyme (RLE) : CPT 1 (a/k/a gateway of beta oxidation).

In well-fed state :

Insulin : Glucagon ratio is high.

Increased cetyl CoA carboxylase (active).

Malonyl CoA inhibits CPT 1 ⟶ FA oxidation will not occur

In fasting state :

Insulin : Glucagon ratio is low.

Decreased acetyl CoA carboxylase (inactive).

Malonyl CoA level will be less ⟶ FA oxidation occurs

Oxidation of various fatty acids

VLCFA :

Modified beta-oxidation.

For > C_{ao-aa}.

Site : Peroxisome.

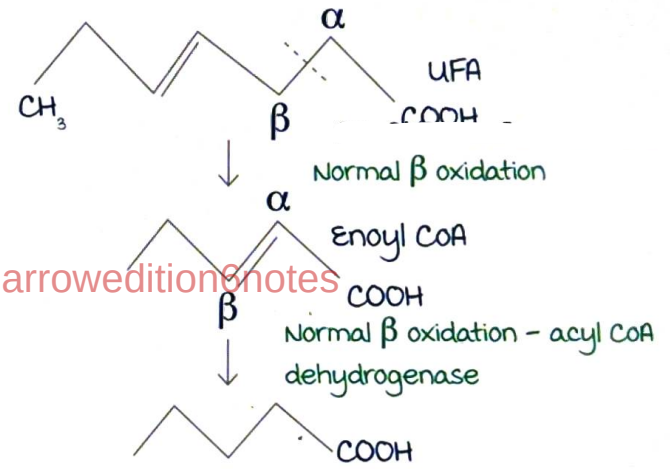
mitochondria also have some role.

Products : Acetyl CoA, H_aO_a, octanoyl CoA.

Octanoyl CoA enters mitochondria and goes through the same as beta-oxidation.

UFA (unsaturated fatty acid) :

Site : Mitochondria.

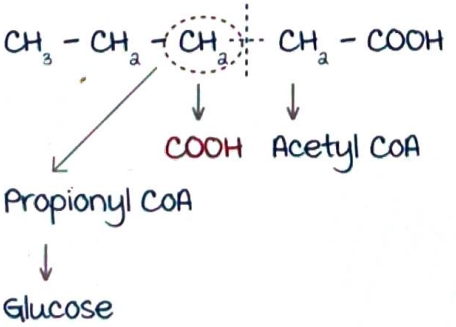


FAD acyl CoA dehydrogenase is bypassed.

ATP generated : 1.5 less for every double bond in an even position.

Odd chain fatty acid :

Site : Mitochondria.



Acetyl CoA + Propionyl CoA is produced.

Alpha oxidation :

Site : Endoplasmic reticulum & peroxisome.

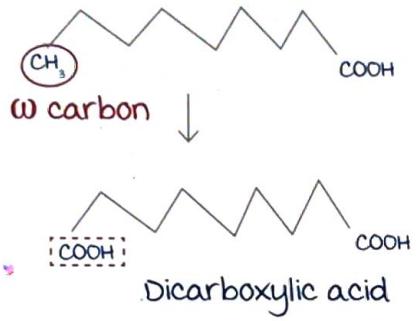
Process : Remove 1 C at a time.

For branched-chain FA with a branch at β carbon.

m/C : Phytanoyl CoA (dairy products, green leafy vegetables).

ω oxidation of fatty acid :

Site : SER (microsome). No ATP is generated.

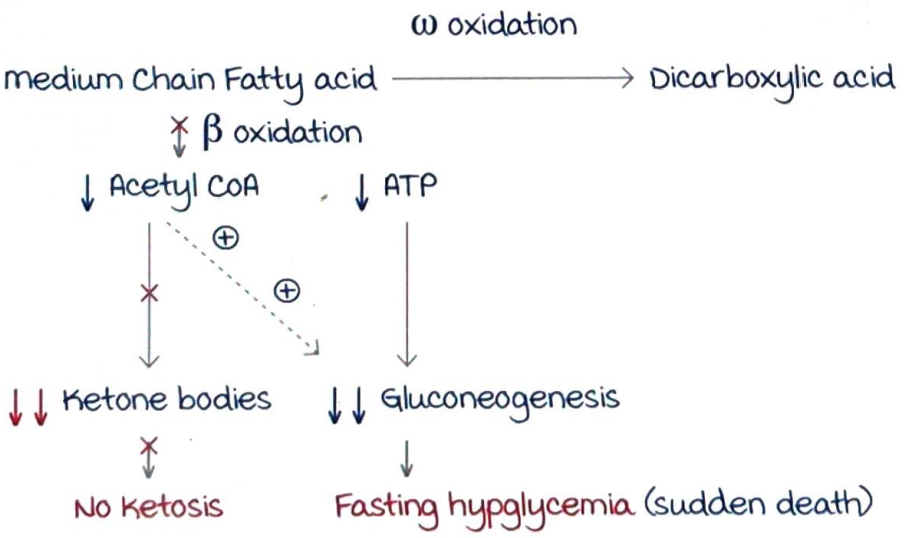


Medium-chain acyl CoA dehydrogenase deficiency (MCAD defect)

00:41:20

Q. This is a 1 year old child. Her sibling died in the 1st days of life of SIDS. At 7 months she had a life-threatening episode of seizure and hypoglycemia. Her blood examination revealed C_{8-10} dicarboxylic acids. No Ketone bodies. Her doctor advised her mother to give her frequent meals with high carbohydrates and low fat.

Answer : MCAD defect.



Active space

Jamaican vomiting sickness

00:45:48

Q. After ingestion of this fruit, man develops sudden onset vomiting 2-6 hours later, followed by convulsion, coma death.

A. Jamaican vomiting sickness.

Unripe ackee fruit → Hypoglyein

↓

⊖ Acyl CoA Dehydrogenase (β oxidation)

↓ Acetyl CoA → ↓ Ketoacid synthesis

↓ ATP → ↓ Gluconeogenesis

Ackee fruit is found in West Africa (Jamaica).

Clinical features :

Sudden onset vomiting, convulsions, coma and death.

Death can occur as there is deficiency of ketone bodies along with glucose, causing lack of substrates for brain.

Refsum's disease

00:50:44

Defect : α oxidation.

Phytanoyl CoA hydroxylase (phytanoyl CoA oxidase) is defective.

Clinical features :

- Retinitis pigmentosa.
- Ichthyosis.
- Peripheral neuropathy.
- Cardiac arrhythmia.

Treatment : Restrict dairy products and green leafy vegetables.

Active space

Zell weber syndrome/cerebro hepatorenal disease

00:50:07

Peroxisomal targeting disorder.

The synthesized peroxisomal enzyme in the endoplasmic reticulum reaches the peroxisome with the help of PTS (peroxisomal targeting sequence).

Mutation in PTS will lead to no enzyme in the peroxisome.

VLCFA oxidation and α oxidation is affected.

Clinical features :

- Mongoloid facies.
- Hypertelorism.
- Unslanting palpebral fissure.
- Frontal bossing, high forehead
- Brushfield spot in iris
- Epicanthal fold.
- Resembles down syndrome.

Diagnosis :

- Peroxisome ghost.
- Accumulation of VLCFA in the peroxisome.
- Accumulation of phytanic acid.

Clinical problems :

Reason for fasting hypoglycaemia in fatty acid oxidation disorders ?

Role of fatty acid oxidation is to provide ATPs are gluconeogenesis. Once 16 hours of fating is over, body gets glucose from gluconeogenesis. Hence when the fatty oxidation is blocked, gluconeogenesis does not occur leading to hypoglycemia.

Why drugs that reduce fatty acid oxidation are oral hypoglycemic agents ?

Example : Sulphonylureas.

In DM, there will be excess gluconeogenesis. So oral hypoglycemic agents blocks this excess gluconeogenesis by inhibiting fatty acid oxidation.

Active space

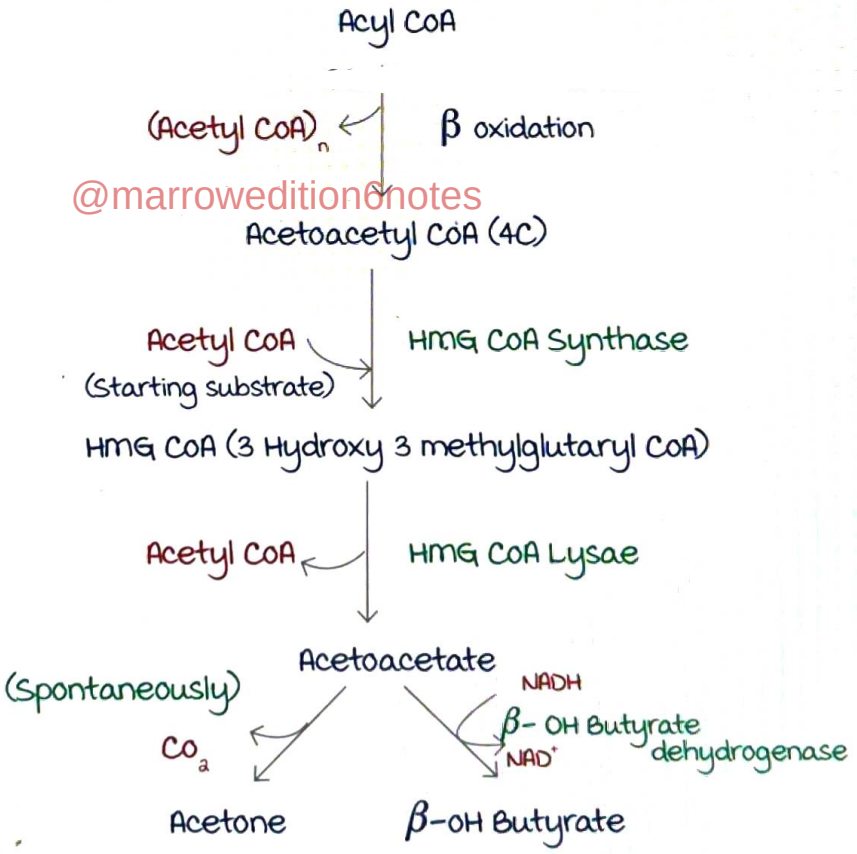
KETONE BODIES

Early fasting (4–16 hrs) → Glycogenolysis.
Fasting (16–48 hrs) → Gluconeogenesis.
Prolonged fasting (starvation) [> 2 days] → Fatty acid oxidation → Ketone bodies.

Provide fuel for vital organs after depletion of glucose.
The brain can derive only 20% of energy from Ketone bodies.

Ketone body synthesis 00:02:55

Site : Occurs only in the liver, inside mitochondria.



Starting substrate : Acetoacetyl CoA.
Rate limiting enzyme : HMG CoA synthase (mitochondria)
Cytosolic HMG CoA synthase for cholesterol synthesis.

Acetoacetate : 1° Ketone body.
Acetone, β- OH Butyrate — 2° Ketone bodies

Active space

Ketone body utilisation 00:08:10

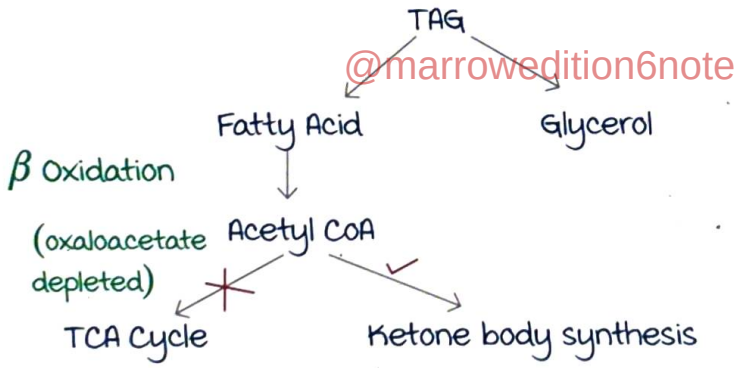
Occurs in extrahepatic tissues as liver lacks thiophorase.
Enzyme for Ketone body utilization :
Succinyl CoA acetoacetate CoA transferase/Thiophorase.

Organs that never utilise Ketone bodies : Liver.
RBC.

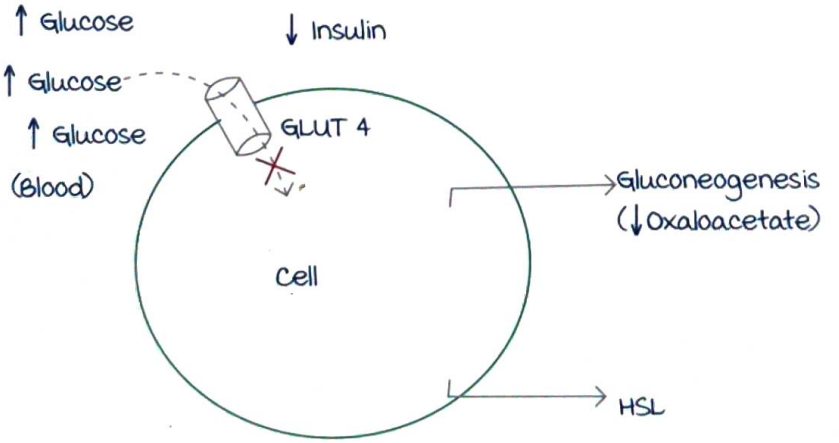
Acetone :

- Volatile, neutral.
- Excreted through the lungs. It has a fruity smell, which is seen in Diabetic Ketoacidosis.

Starvation Ketosis :
After utilisation of glycogen and failure of gluconeogenesis, TAG becomes the next source.



Diabetic Ketoacidosis :



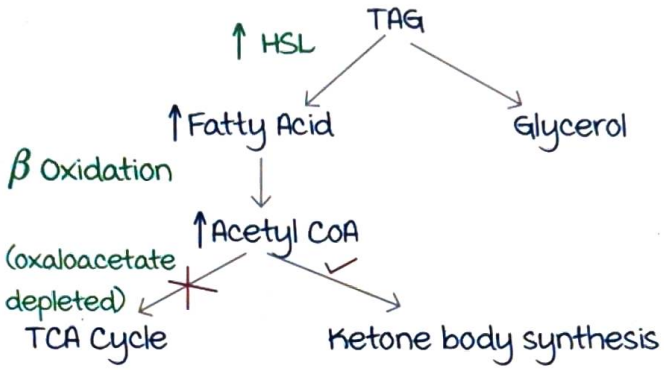
Increased glucose in the blood but cannot enter cell as Insulin dependent GLUT 4 transporter is reduced.

Active space

Cell synthesizes new glucose in response (gluconeogensis – depletion of oxaloacetic acid).

Hormone sensitive lipase (HSL) helps in conversion of TAG to fatty acids and glycerol.

Insulin generally inhibits HSL. But in case of diabetes there is excess of HSL :



This is the reason for Ketoacidosis in uncontrolled diabetes.

Tests for ketone bodies

00:14:17

1. Rothera's test :

Purple ring at the junction of 2 liquids.

Positive in : Acetone and acetoacetate.

2. Gerhardt's test :

Positive in : Acetoacetate.

3. Ketostix :

Dipstick test to detect Ketone bodies.

Positive in : Acetone and acetoacetate.

4. Enzymatic assay for β-OH Butyrate.

Predominant Ketone body synthesised in Ketosis : β-OH Butyrate.

β-OH Butyrate : Acetoacetate = 6 : 1

Ketone body predominantly synthesized in normal condition :

β-OH Butyrate : Acetoacetate = 1 : 1

FATTY ACID SYNTHESIS

Steps of fatty acid synthesis 00:01:25

Fatty acid synthesis occurs in a well fed state under the influence of insulin.

Also known as de novo synthesis/Lynen's spiral.

Discovered by Feodor Lynen.

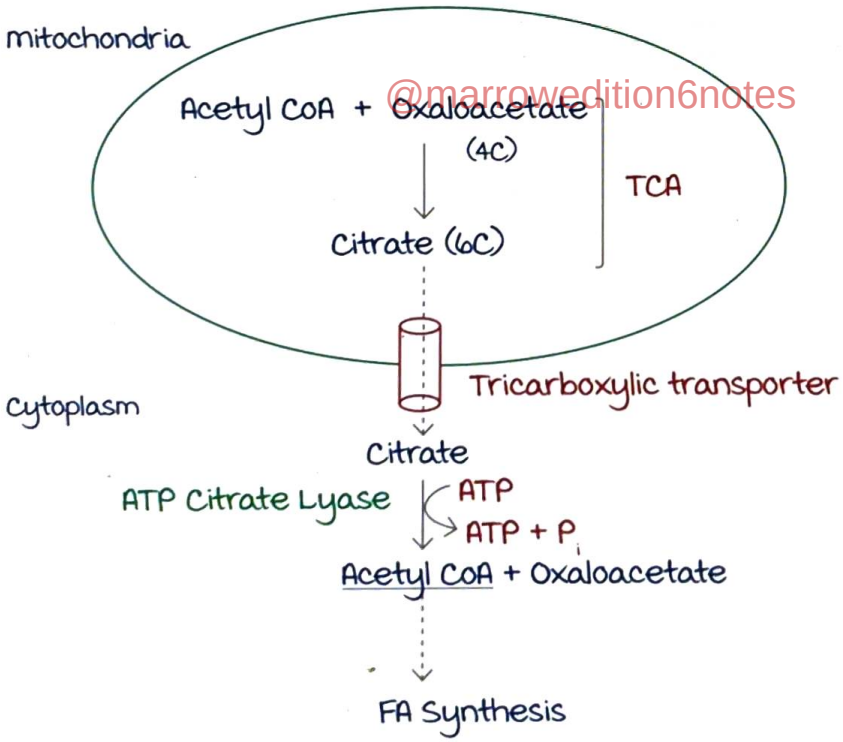
Site : Liver, Kidney, brain, lung, lactating mammary gland.

Organelle : Extramitochondrial fatty acid synthase system (cytosol).

Starting substrate : Acetyl CoA.

Source : Pyruvate dehydrogenase (fed state) [mitochondria].

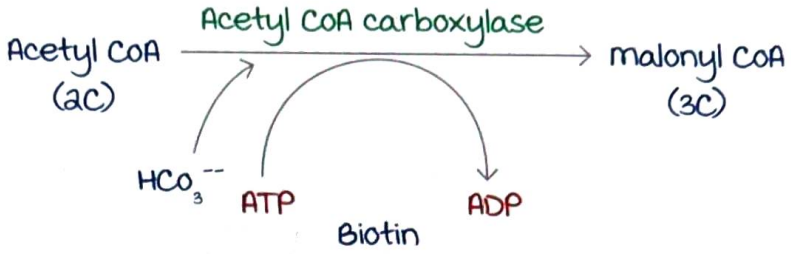
Transport of acetyl CoA :



Enzyme systems

00:06:58

1. Acetyl CoA carboxylase system :



Rate limiting enzyme.

2. Fatty acid synthase complex :

Multifunctional enzyme.

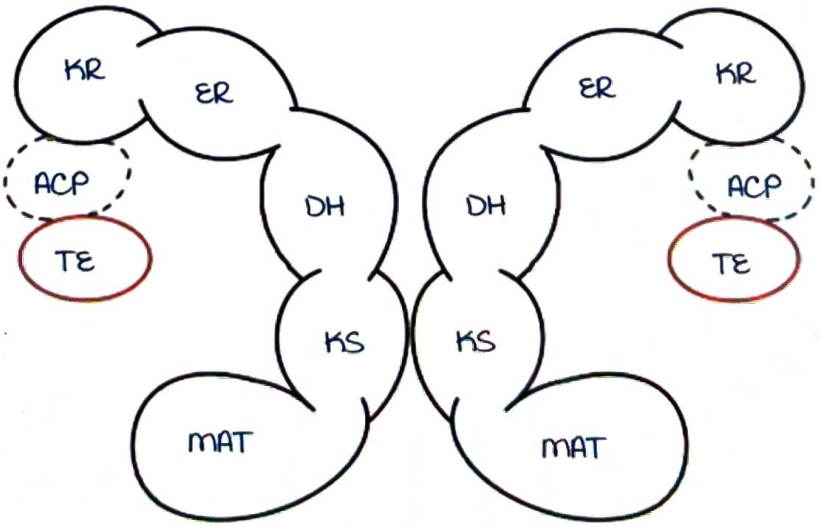
It is a homodimer and is X shaped.

Each monomer is divided into 3 units/domains :

1. Condensing unit.
2. Reduction unit.
3. Releasing unit.

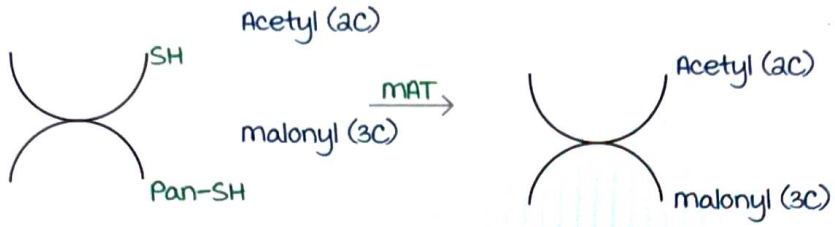
Condensing unit	Reduction unit	Releasing unit
• Malonyl Acetyl Transacylase (MAT). • Ketoacyl Synthase (KS).	• Ketoacyl Reductase. • Enoyl Reductase. • Dehydratase.	• Thioesterase. (Deacylase)

It has 6 enzyme activities.

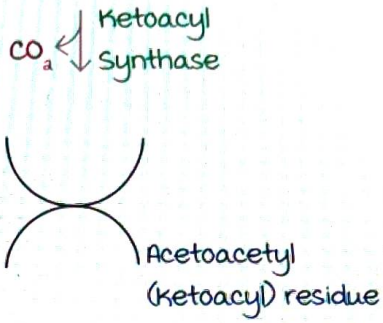


Active space

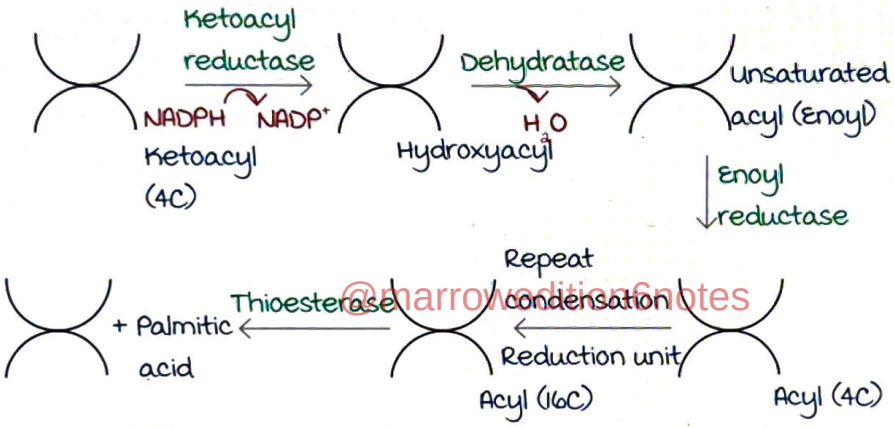
Condensation unit :



After realease of CO_2 two 2C compounds formed at the ends of each subunit combine to form acetoacetyl residue (4C).



Reduction unit :



Acyl group (4C) combines with another malonyl group (3C), forms a 6C compound after release of CO_2. This compound then undergoes repeat condensation & reduction to give rise to a 16 C acyl compound attached to FA synthase complex.

Releasing unit :

Thioesterase (deacylase) enables release of completely formed fatty acid from the fatty acid synthase complex.

Cofactor requirement :

- NADPH : KR, ER.
- Mn^{2+}
- Biotin] Acetyl CoA carboxylase
- HCO_3^{--}] (donor of C atom for acetyl CoA carboxylase)

Sources of NADPH are : HMP (oxidative).
Malic enzyme.
Cytosolic ICDH.

Active space

Regulation

00:21:19

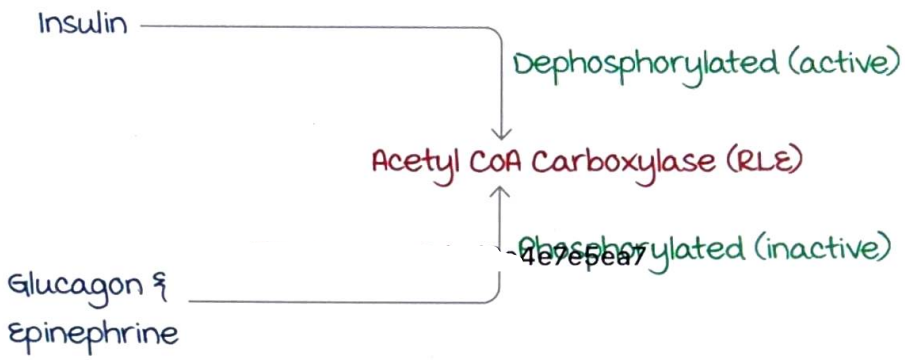
Rate limiting enzyme : Acetyl CoA Carboxylase.

Allosteric activator of acetyl CoA carboxylase : Citrate.

Citrate converts the inactive dimer form to an active tetramer.

Allosteric inhibitor of TCA transporter : Long chain fatty acid.

Hormonal regulation :



CHOLESTEROL AND BILE ACIDS

Significance of cholesterol :
Exclusively animal sterol.
Cholesterol elimination is difficult.

Chemistry :
27 carbon compound (cyclopentano perhydro phenanthrene ring).
Amphipathic (has both hydrophilic & hydrophobic ends).

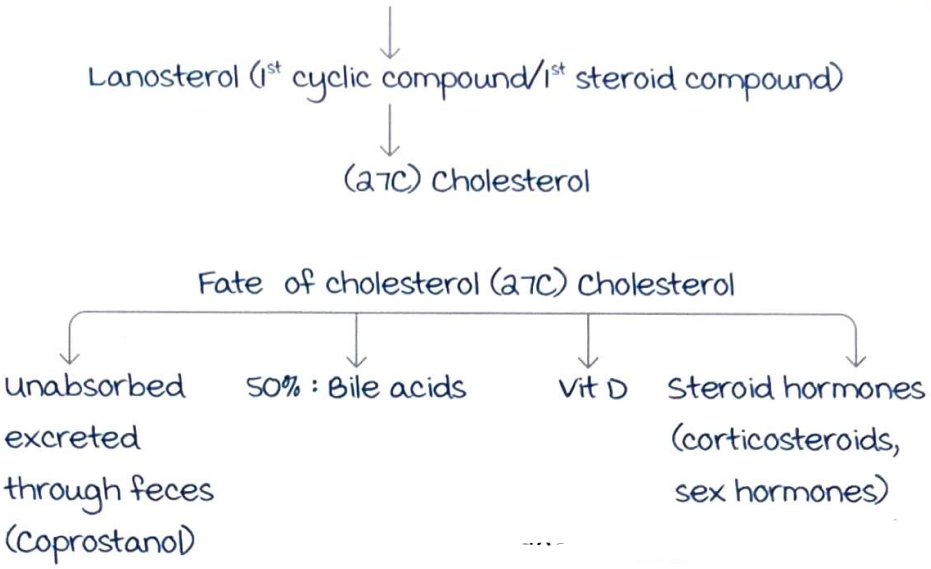
Cholesterol synthesis 00:04:45

Site : All nucleated cells.
Liver, adrenal cortex, testis, ovary, intestine.
Organelle : Cytoplasm & SER.
Starting material : Acetyl CoA.

Steps :

2 Acetyl CoA
↓ Thiolase
Acetoacetyl CoA
+ Acetyl CoA
↓ HMG CoA synthase (cytosolic)
HMG CoA
NADPH NADP⁺ ↓ HMG CoA reductase
(In endoplasmic reticulum)
Rate limiting step
↓
Mevalonate
↓
2 x Isoprenoid (Isopentanyl units)
↓
Geranyl ppi (10C)
+ [5C]
↓
(15C) Farnesyl ppi x 2
↓
(30C) Squalene

Active space



Regulation of cholesterol synthesis 00:12:11

Tightly regulated pathway.
Rate limiting enzyme : HMG CoA reductase.

Feedback regulation :
Dietary cholesterol decrease the synthesis of enzymes for cholesterol synthesis with help of SREBP (Sterol Regulator Element-Binding Protein).

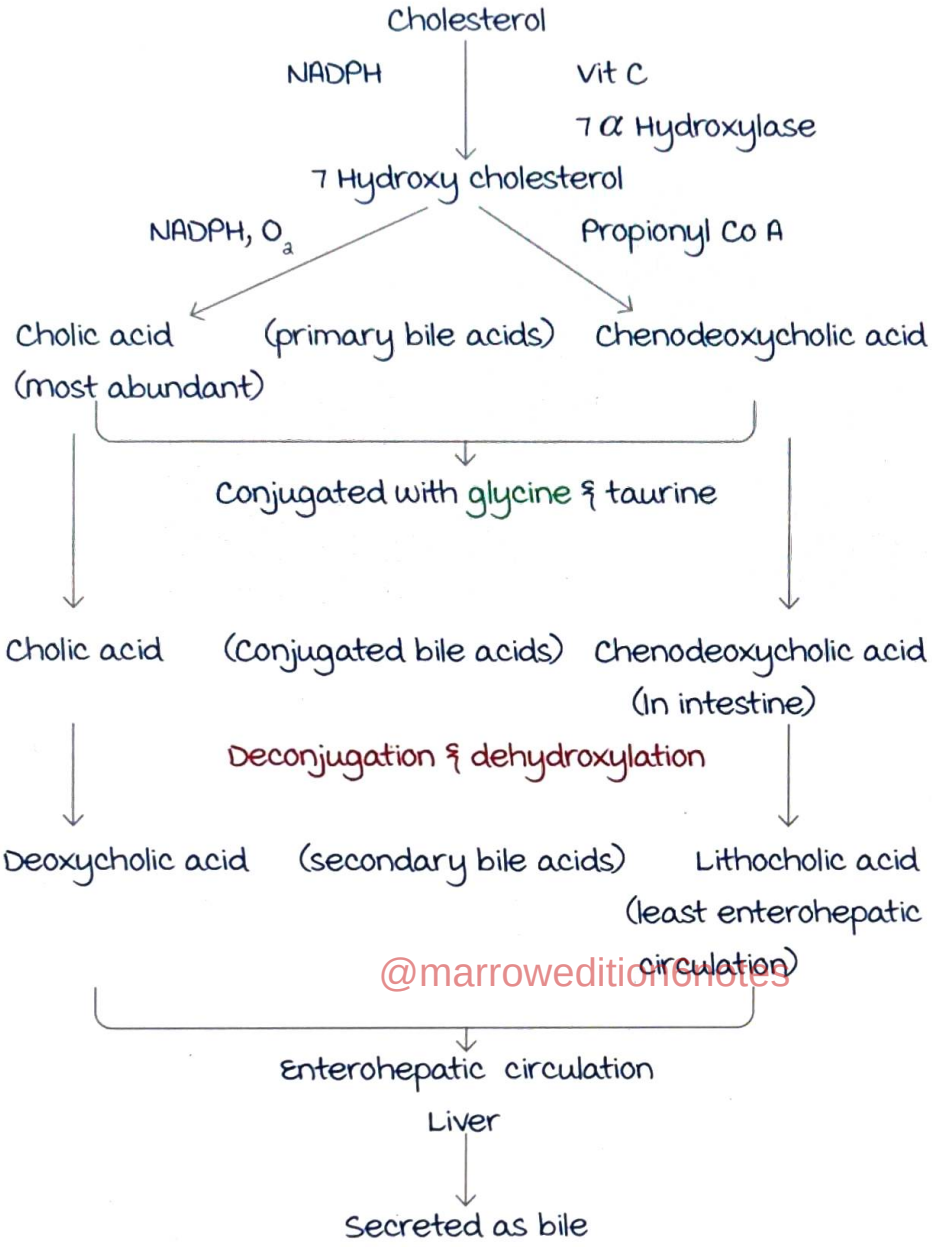
Feedback inhibition :
Cholesterol inhibits HMG CoA reductase.

Hormonal regulation :
Insulin & thyroxine favor HMG CoA reductase.
Glucagon & glucocorticoids inhibit HMG Co A reductase.

Bile acid 00:15:21

Starting substrate : Cholesterol.
Rate limiting enzyme : 7 α Hydroxylase.

Steps :



Cholic acid and chenodeoxycholic acid are primary bile acids and formed in the liver.

Bile salt :
It is the form in which the bile acid exists in the bile.

Bile acid sequestrant :
It sequesters the bile acid and excrete it in feces and avoids enterohepatic circulation.
So, cholesterol will be excreted in bile form.
Therefore, it is used as a hypocholesterolemic drug.

Active space

LIPOPROTEINS

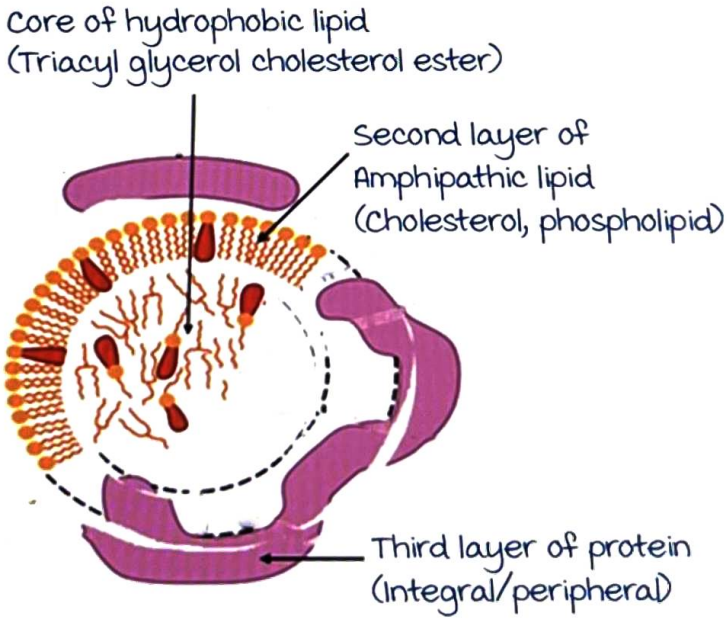
Definition : Compound lipids complexed with proteins.

Concept :
Lipids are hydrophobic.
To be carried in the blood it is complexed with proteins.
The proteins are called apolipoproteins/ apoproteins.

Major classes of lipoproteins 00:03:20

1. Chylomicron
2. LDL (Low-density lipoprotein)
3. HDL (High-density lipoprotein)
4. IDL (Intermediate density lipoprotein)
5. VLDL (Very low-density lipoprotein)

Structure of lipoprotein 00:04:10



Active space

Characters of lipoproteins

00:05:19

	Chylomicron	VLDL	LDL	HDL
Formed from	Intestine	Liver	From VLDL by Lipoprotein cascade pathway. VLDL → IDL → LDL	Intestine & liver
Function	Carry exogenous/ dietary TAG to the liver and peripheral organs	Carry endogenous TAG to the peripheral organs	Carry cholesterol to extrahepatic tissue & liver	Reverse cholesterol transport
Size	Maximum			
Density	Least			Highest (Protein content is highest)
Lipid content	Maximum TAG (Tri-Acyl Glycerol)		Maximum TAG	Least. (Phospholipid & apolipo protein content is maximum)
Apolipo protein	Apo B_{48}: Unique to chylomicron. Apo C_{II} Apo E	Apo B_{100} Apo C_{II} Apo E	Apo B_{100}	Apo A_I Apo C Apo D Apo E

Enzymes in HDL :

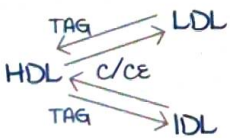
1. Lecithin cholesterol acyltransferase (LCAT)

   Lecithin + cholesterol (amphipathic)

   ↓ LCAT

   Lysolecithin + cholesterol ester (hydrophobic)

2. Cholesterol ester transfer protein (CETP)



CETP transfers cholesterol/ cholesterol ester to other lipoproteins like IDL, LDL etc in exchange of TAG.

Active space

Lipoprotein (a) [Lp (a)]

Similar to LDL.

Apo (a) bound to apo B_{100} by a disulfide bond.

Apo (a) is a plasminogen analogue, which may get acted upon by activator (which converts plasminogen to plasmin).

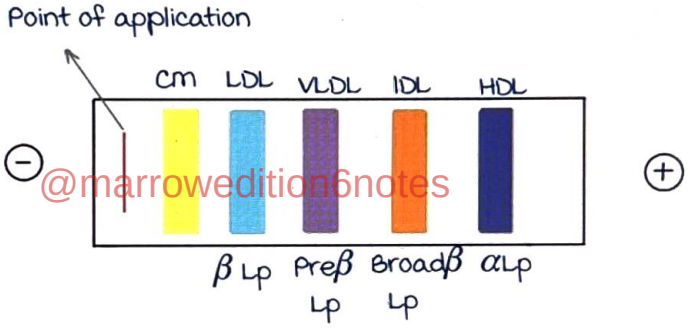
This can inhibit clot lysis ⟶ Risk factor for thrombosis.

Lipoprotein (x) [Lp(x)]

If there is a block in bile flow, the cholesterol will be accumulated in the liver.

This cholesterol will combine with phospholipid and form Lp (x). Lp(x) is an indicator of cholestasis.

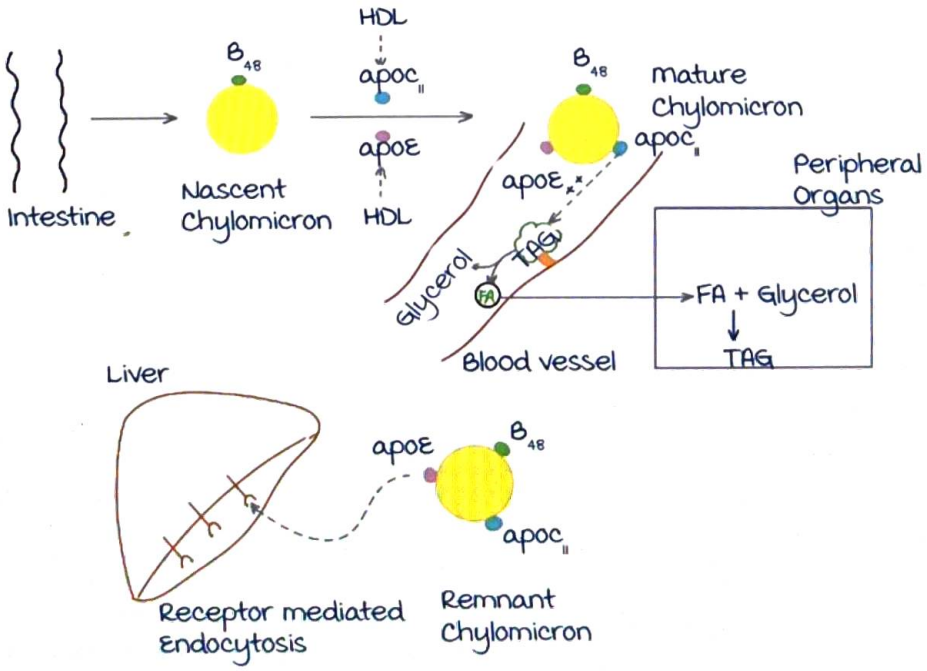
Electrophoretic mobility of lipoproteins :



Lipoprotein metabolism

Chylomicron metabolism

00:19:15



Active space

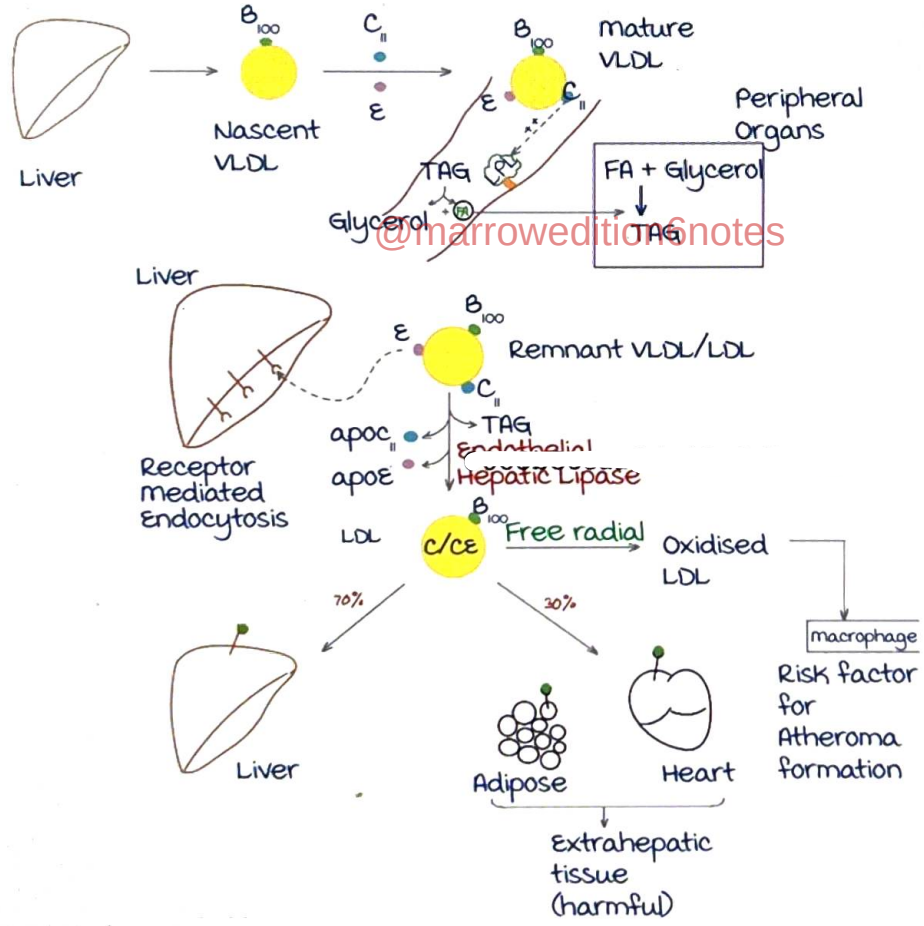
Nascent chylomicron contains apolipoprotein B_{48} that helps in its assembly. Apo C_{II} & Apo E (repository : HDL) gets attatched to chylomicron forming mature chylomicron, which on passing through capillaries gets acted on by lipoprotein lipase (L_pL) present in endothelium. L_pL converts TAG to fatty acid & glycerol.

Apo C_{II} present in mature chylomicron is the activator of L_pL. The subsequently released fatty acid gets deposited in peripheral tissues and TAG content of chylicron reduces, forming remnant chylomicron.

Apo E acts as ligand for uptake of remnant chylomicron by liver via receptor mediated endocytosis.

LDL and VLDL metabolism

00:24:43

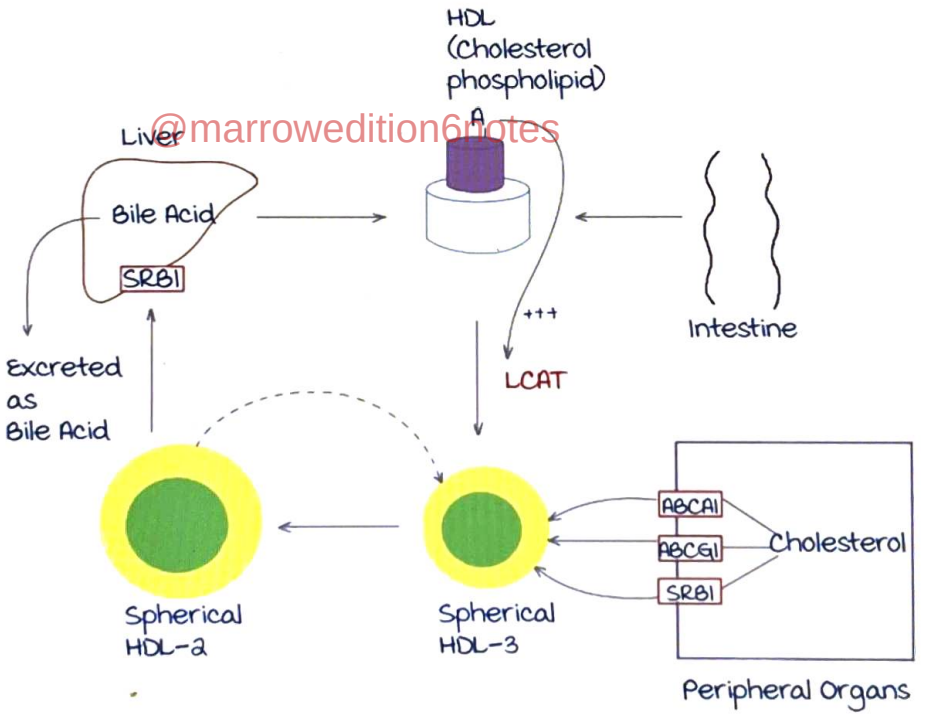


VLDL is formed from the liver. B 100 helps in its assembly to form nascent VLDL. Mature VLDL is formed after it acquires C_{II} and E. C_{II} activates L_pL which converts TAG to fatty acid and glycerol. Fatty acid then gets deposited to peripheral

organs.
VLDL with reduced TAG is known as remnant VLDL/ IDL.
Fate of remnant VLDL :
1) Receptor mediated endocytosis by ligand apo E into liver.
2)Converted to LDL (cholesterol/cholesterol ester rich) by the action of endothelial/ hepatic lipase.
70% of LDL undergoes receptor mediated endocytosis into liver catalysed by ligand apo B_{100}.
30% of LDL taken up by extrahepatic tissues that have LDL receptors like heart or adipose tissues.
In the presence of free radicals, LDL gets oxidised and is taken up by macrophages which can be a risk factor for atheroma formation.

HDL metabolism

00:32:25



HDL is formed from liver and intestine.
Lipid content of newly formed HDL consists of amphipathic lipids like cholesterol and phospholipid which gives it a discoidal structure (aka discoidal HDL).
Apo A_1 activates LCAT which converts cholesterol to cholesterol ester, a hydrophobic lipid.
Hydrophobic lipids gets internalised giving HDL a spherical

shape (aka spherical HDL-3). This HDL accepts cholesterol from peripheral organs with help of certain transporters.

Transporters in HDL metabolism :

1. ATP binding cassette protein A1 (ABCA 1)
2. ATP binding cassette protein G1 (ABCG 1)
3. Scavenger receptor B1 (SRB 1)

Function : Transport cholesterol to HDL from the peripheral organ.

With uptake of cholesterol, spherical HDL-3 swells up and forms HDL-2, which releases the cholesterol into liver via SRB 1 transporter. The cholesterol then goes for bile acid synthesis. After release, HDL-2 becomes HDL-3. This is known as HDL cycle.

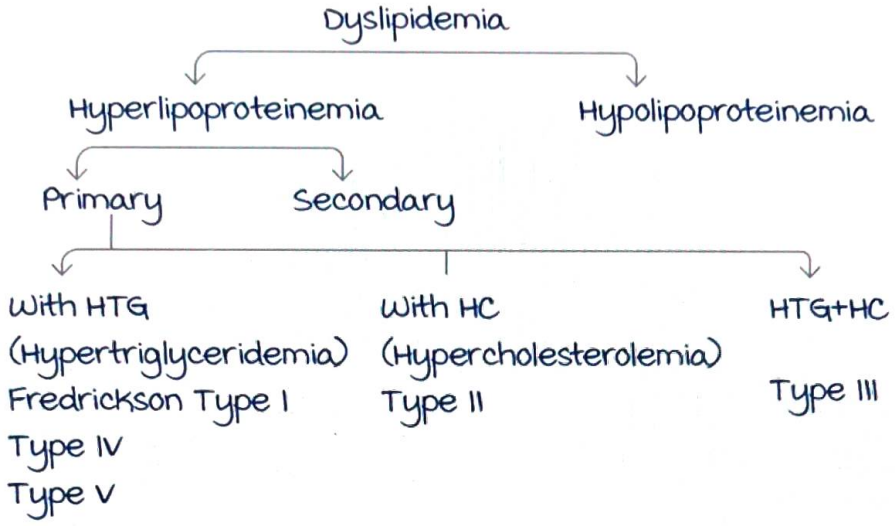
Apolipoproteins 00:41:34

Apo A_I : Activate LCAT.
Apo A_{II} : Inhibit $L_p L$.
Apo A_V : Promote $L_p L$
Apo B_{100} : Assemble VDRL in liver.
Act as a ligand for LDL.
Apo B_{48} : Assemble chylomicron.
Apo C_I : Inhibit CETP.
Apo C_{II} : Activate $L_p L$
Apo C_{III} : Inhibit $L_p L$
Apo E : Ligand for remnant Lipoprotein (VDRL & chylomicron)
Arginine rich.
Apo E_4 : Late-onset Alzheimer's disease
Apo D : Associated with a human degenerative disorder.

LDL is dangerous because it has tendency to getdeposited in extra hepatic tissues. Also, oxidised LDL can predispose to atheroma formation.

HDL is heart friendly as it transfers cholesterol from extrahepatic tissues to the liver where it undergoes bile acid synthesis.

DYSLIPIDEMIA



Type 1 Hyperlipoproteinemia 00:04:40

A/K/A Familial chylomicronemia syndrome.

Defect in lipoprotein lipase (LpL)/ apo C2 (activator of LpL) → Accumulation of mature chylomicron and mature VLDL.

Mature chylomicron $\xrightarrow{\text{LpL/ apo C2}}$ Remnant chylomicron.

Mature very low density lipid (VLDL) $\xrightarrow{\text{LpL/ apo C2}}$ Remnant VLDL.

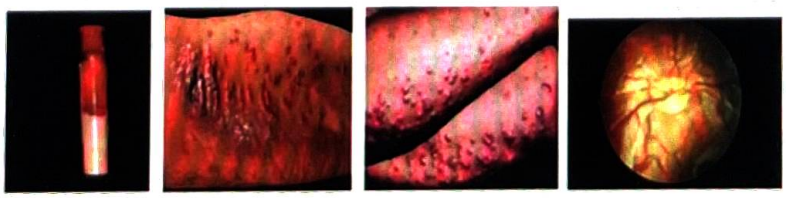
Lipoprotein elevated : Chylomicron >> VLDL.

Lipid elevated : Triacylglycerol (TAG).

Cholesterol levels will be normal.

Clinical features :

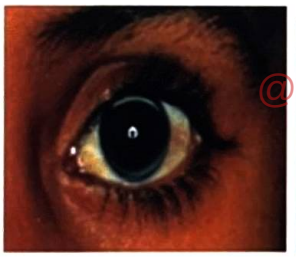
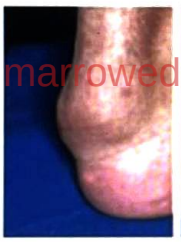
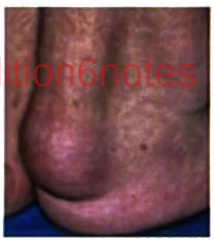
1. Recurrent abdominal pain : Hypertriglyceridemia → Pancreatitis → Acute abdomen.
2. Lactescent (milky white) plasma due to raised triacylglycerol glycerol.
3. Eruptive xanthoma.
4. Fundoscopy : Lipemia retinalis.



There is no increased risk of coronary artery disease (CAD).

Type 2 Hyperlipoproteinemia 00:10:40

1. Familial hypercholesterolemia :
   A/K/A autosomal dominant hypercholesterolemia type I (ADH Type I).
   m/c primary hyperlipoproteinemia.
   Defect : Mutation of LDL receptor (Uptake of LDL in liver and extrahepatic tissues.
   Lipoprotein elevated : LDL.
   Lipid elevated : Cholesterol.
   Triacylglycerol level is normal.
   Clinical features :
   1. Presents with premature CAD
   2. Corneal arcus.
   3. Clear plasma.
   4. Tendon xanthoma/ Tuberous xanthoma. (m/c site : Achilles tendon).

2. ADH Type 2 :
   A/K/A familial defective Apo B100 (FDB).
   mutation in gene encoding apo B100.
3. ADH Type 3 :
   Gain of function mutation to PCSK9 protein.
   PCSK9 binds to LDL receptor → Lysosomes :
   Accelerated degradation of LDL receptor.
   This leads to raised LDL and cholesterol levels.
4. Autosomal recessive hypercholesterolemia (ARH) :
   Mutation in LDL receptor adapter protein (Clears LDL from body).
5. Sitosterolemia :
   Sitosterol is a plant sterol.
   ATP binding cassette : ABCG5 and ABCG8 :
   - Intestinal cells : Actively secrete absorbed plant sterols back to the lumen.

Active space

- In liver : Excrete plant sterol from hepatic cells into the bile.

Mutation of ABCG5 and ABCG8 → Raised cellular sterol
↓
Decreased transcription of LDL receptor
↓
Decreased LDL receptor
↓
Raised LDL

Type 3 Hyperlipoproteinemia 00:23:59

A/K/A Familial dysbetalipoproteinemia, Remnant removal disease, Familial broad beta disease.

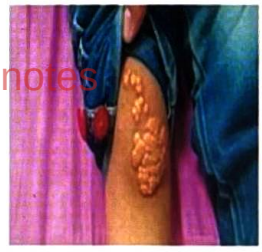
Defect : Mutation of apoE : Acts as ligand for uptake of :

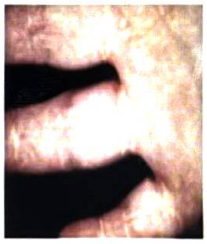
- Remnant chylomicron.
- Remnant VLDL.

Lipoprotein elevated : Remnant chylomicron and VLDL.

Lipid elevated : TAG and cholesterol.

Clinical features :
Tuberoeruptive xanthoma.
Palmar xanthoma.
Slight increased risk of CAD.





Recent modalities of treatment for hyperlipoproteinemia 00:28:23

Type 1 : Lipogene Tiparvovec.
Gain of function LpL variant.

Type 2 (Familial homozygous hypercholesterolemia) :

1. Lomitapide : Inhibits microsomal triglyceride transfer protein (MTP).
   MTP carries TAG to VLDL and chylomicron.
   Results in decreased LDL.
2. Mipomersen : Antisense oligonucleotide therapy.

Hypolipoproteinemia 00:31:23

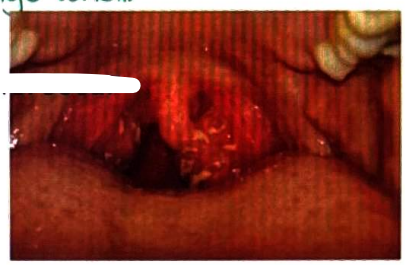
Tangier's Disease :

Mutation of ABCA1 gene → Decreased HDL formation.

ABCA1 transfers cholesterol from peripheral organs to HDL

Clinical features :

Enlarged greyish tonsil/orange tonsil.



Peripheral neuropathy.

Hepatosplenomegaly.

Abetalipoproteinemia :

Defect in MTP :

Decreased VLDL and Chylomicron :

- Decreased Intermediate density lipoprotein (IDL) → Decreased LDL.
- Deficiency of fat soluble vitamins (A,D,E,K).

Clinical features :

Diarrhoea.

Failure to thrive.

Neurological manifestations.

Progressive pigmentary retinopathy.

Acanthocytes.

Bleeding manifestations.

LCAT deficiency :

$$\text{Cholesterol} + \text{Lecithin} \xrightarrow{\text{LCAT}} \text{Cholesterol ester} + \text{Lysolecithin.}$$

Types :

Complete: Norum's disease.

Partial : Fish eye disease.

Norum's disease : Complete LCAT deficiency :

Raised cholesterol and lecithin.

Decreased cholesterol ester and lysolecithin.

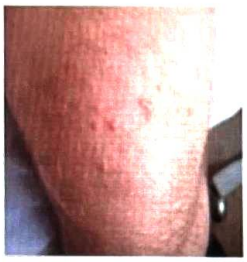
Clinical features :

Progressive corneal opacification.

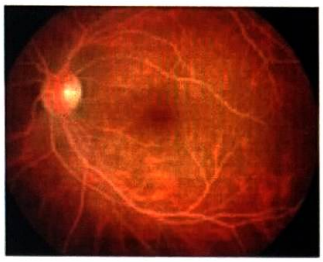
Progressive end stage renal disease.

Active space

Question : A 15 year old boy presented with recurrent episodes of abdominal pain.His blood was drawn for investigation and it looks like a cream of tomato soup. One blood sample kept in the refrigerator over night, a creamy supernatant layer separated.



Eruptive xanthoma

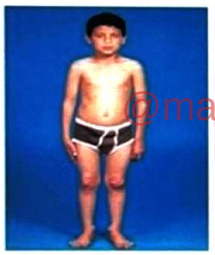


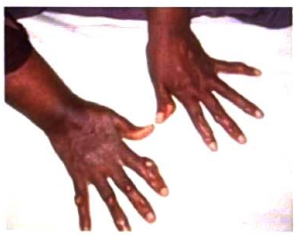
Lipemia retinalis

Milky white plasma

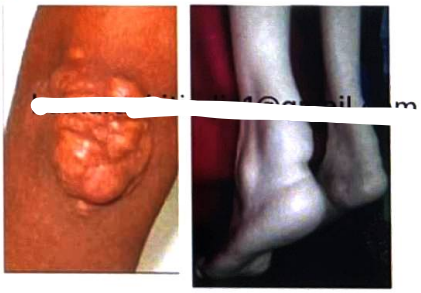
Answer : This is a case of Type I Hyperlipoproteinemia.

Question : A boy presented with the following features. What is the diagnosis ?



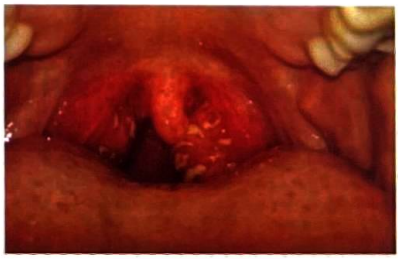


Answer : This is a case of Type II Hyperlipoproteinemia.



Question : Can you correlate ?

- Low level of HDL
- Mononeuritis multiplex
- Hepatosplenomegaly.



Answer : Tangier's Disease

Active space

LIPASES

Break covalent bond : Ester bond.

Triacyl Glycerol (TAG) $\longrightarrow$ Glycerol + 3 Fatty acids.

$\uparrow$

$3\ H_2O$

Class of hydrolase.

Hormone Sensitive Lipase (HSL)

00:03:19

Location : Adipose tissue.

Function : Hydrolyse TAG stored in adipocytes.

During fasting state :

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

Fasting state

$\downarrow$

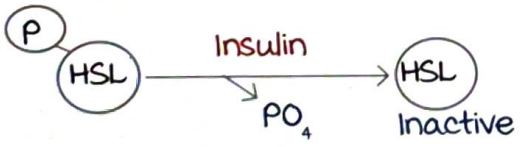
Glucagon

$\downarrow \oplus$

HSL

$\therefore$ Active in : 1) Fasting
2) Glucagon
3) Phosphorylated

Insulin $\rightarrow$ $\uparrow$ Phosphatase



In diabetes, HSL is active.

Hormone sensitive lipase

Active space

Lipoprotein lipase (LPL) 00:14:53

Anchored to endothelium of capillaries in heart, adipose tissue, spleen, renal medulla, aorta, diaphragm, lactating mammary gland.

Anchored to wall by a GAG → Heparan sulphate.

Injection heparin → LPL dislodged.

Activated by apo C II.

Action : Hydrolyse TAG in chylomicron and VLDL in fed state.

Hormone : Insulin increases 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	Fed
	↓	↓
	Glucagon	Insulin

In DM : HSL will be activated and LPL will be inhibited.

Hepatic lipase 00:23:25

Location : Sinusoidal surface of liver.

Metabolism of chylomicron remnants and conversion of HDL_3 to HDL_2.

Endothelial lipase 00:24:30

Action : HDL_3 → HDL_2

→ Pre. β HDL

Pre β HDL : Poorly lipidated, most active HDL.

Absorb maximum cholesterol from peripheral organ.

Active space

Intestinal lipase

00:25:40

Hydrolyses TAG (dietary) in intestine.

↓

Fatty acid + Glycerol

MCQs :

1. In a type I DM, which of the following is correct ?

A. Activation of LPL and activation of HSL.

B. Activation of LPL and inactivation of HSL.

C. Inactivation of LPL and inactivation of HSL.

D. Inactivation of LPL and activation of HSL.

CHEMISTRY OF AMINO ACIDS

Problem based questions

00:00:26

In HbS 6th position of beta globin chain, glutamate is replaced by valine. In deoxygenated state, what kind of mutation leads to polymerization of HbS, leading to sickle cell disease?

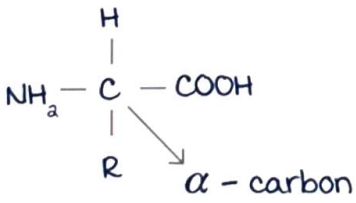
Answer : Nonconservative mutation.

Assertion (A) : Leucine is present in the interiors of albumin.
Reason (R) : Leucine is a non-polar amino acid.

i. A and R are true, R is the correct explanation for A.
ii. A and R are true but R is not the correct explanation for A.
iii. A and R are false.
iv. A is true but R is false.

Answer : A and R are true. R is the correct explanation for A.

Introduction :



Alpha amino acids :

Amino and carboxyl group are attached to alpha carbon atom.
most amino acids belong to this group.

Non-alpha amino acids :

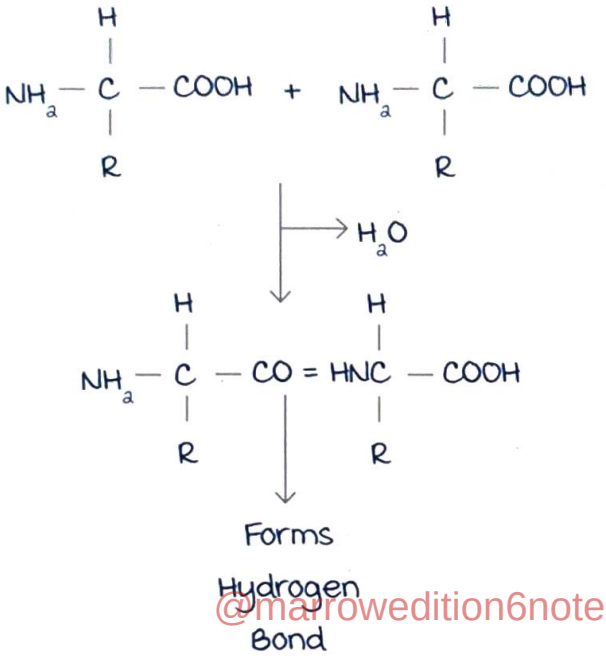
β alanine, β aminoisobutyrate γ isobutyrate.

Active space

Classification of amino acids :

- Based on side chain.
- Based on side chain characteristics.
- Based on metabolic fate.
- Based on nutritional requirements.

Classification of amino acids based on side chain :



Peptide can only form hydrogen bond.
In a long polypeptide chain, there is free ionizable group at the ends $-NH_2$ group at one end and $-COOH$ group at the other end.
Characteristic of polypeptide chain is determined by the variable side chain.

Aliphatic amino acids :

- Simple amino acid. Example : Glycine(Gly/G) and alanine(Ala/A).
- Branched chain amino acid. Example : Leucine (Leu/L), isoleucine (Iso/I), valine (Val/V). [Mnemonic – LIV].

Hydroxy group containing amino acids :
Serine (Ser/S), threonine (Thr/T), tyrosine (Tyr/Y).

Sulphur containing amino acids :
Cysteine (Cys/C), methionine (met/m).

Active space

Acidic amino acids :

Contain COOH group in the variable side chain.

Example : Aspartic acid (Asp/D), glutamic acid (Glu/E).

Amides :

Contain $CONH_a$ group in the variable side chain.

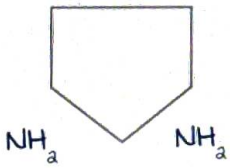
Example: Asparagine (Asn/N), glutamine (Gln/Q).

Basic amino acids :

Histidine (Hys/H) → Contains imidazole ring in the side chain.

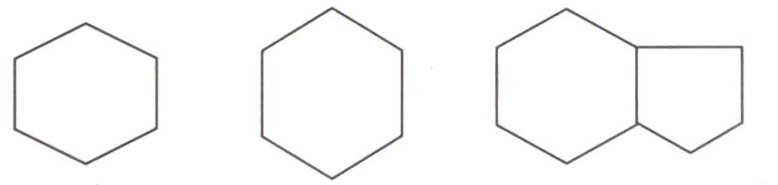
Arginine (Arg/R) : Guanidinium group.

Lysine (Lys/K) : Epsilon amino group.



Aromatic amino acids :

- Phenylalanine (Phe/F) : Benzene ring in the side chain.
- Tyrosine (Tyr/Y) : Phenol ring in the side chain.
- Tryptophan (Trp/W) : Indole ring in the side chain.



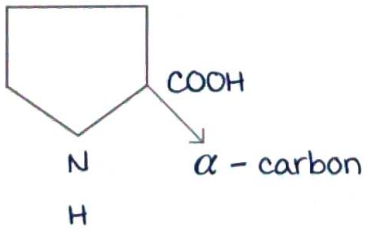
Benzene Ring

OH Phenol Ring

Indole (benzopyrole) Ring

Imino acid amino acids :

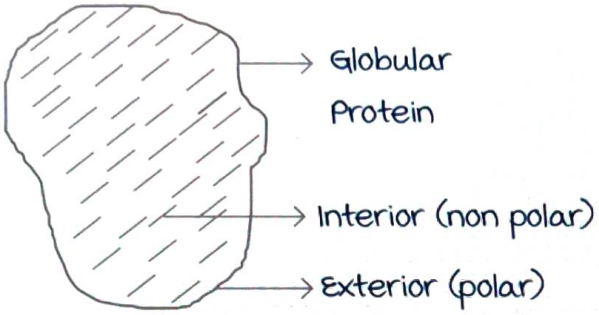
Proline (Pro/P) : Pyrrolidine ring ; Alpha carbon atom is a part of the ring.



Active space

Classification of amino acids based on side chain characteristics

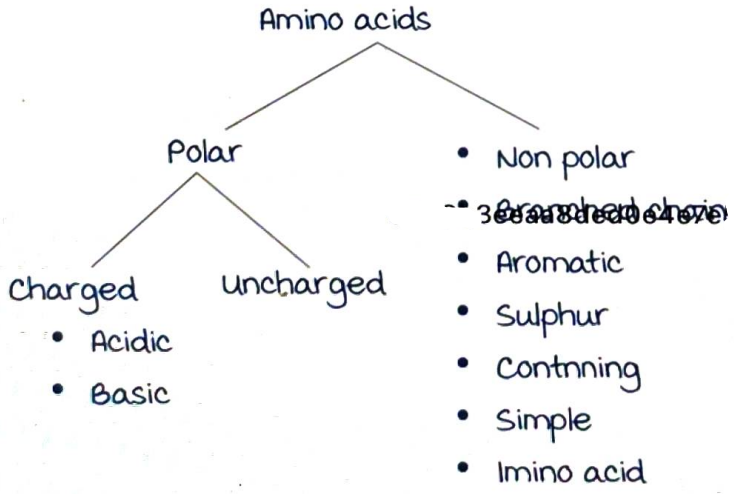
00:17:50



Interior of a globular protein contains non-polar amino acids (not soluble in water).
Exterior of a globular protein contains polar amino acids (soluble in water).

Conservative mutation : An amino acid is replaced by another amino acid of similar characteristics by a missense mutation.
Example : One polar amino acid is replaced by another polar amino acid.

Non-conservative mutation : An amino acid is replaced by another amino acid of different characteristics. Example : In HbS, glutamic acid, a polar amino acid is replaced by valine, a non-polar amino acid.



Charged amino acids :
Acidic and basic amino acids.

- Acidic : Aspartic acid, glutamic acid.
- Basic : Histidine, Arginine, Lysine (Mnemonic : HAL).

Active space

Uncharged amino acids :

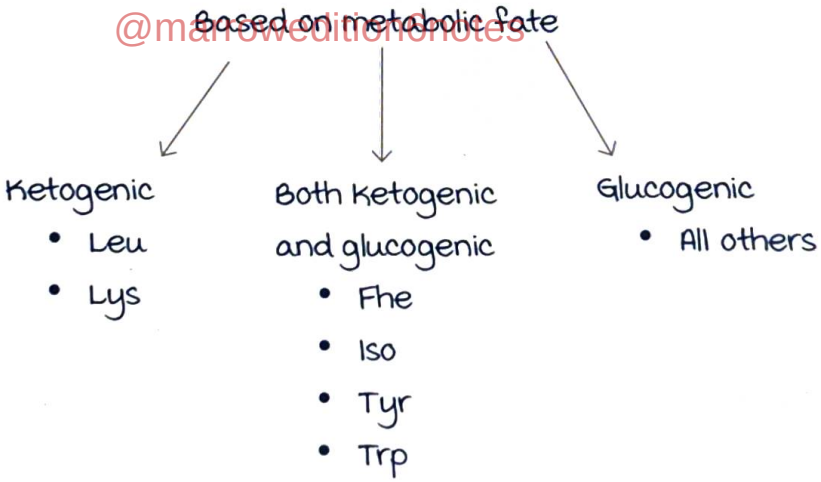
- Contain hydroxyl group : Serine, threonine.
- Amides : Asparagine and glutamine.
- Sulphur containing amino acid : Cysteine.
- Simple amino acid : Glycine.

Most polar amino acid is arginine.
Least polar amino acid is glycine.

Non polar amino acids :

- Branched chain amino acids (LIV).
- Aromatic amino acids except histidine.
- Sulphur containing amino acid : Methionine.
- Simple amino acid : Alanine.
- Imino acid : Proline.

Classification of amino acids based on metabolic 00:25:54



Ketogenic, glucogenic and both Ketogenic-Glucogenic amino acids.
Glucogenic amino acids provide glucose in the fasting stage.
Ketogenic amino acids provide Ketone bodies in prolonged starvation.

Ketogenic amino acids : Leucine, lysine.
Both Ketogenic and glucogenic amino acids : Phenylalanine, isoleucine, tyrosine, tryptophan.
Glucogenic amino acids : All others.

Principle glucogenic amino acid is alanine.

Classification of amino acids based on nutritional requirements :
Nutritionally essential and nutritionally non-essential amino acids.

Nutritionally essential amino acids :

Cannot be synthesized in our body (takes >7 steps to synthesize).
Cause negative nitrogen balance if not taken through diet.

Example : Methionine, Threonine, Tryptophan, Valine, Isoleucine, Leucine, Phenylalanine, Lysine, Histidine, arginine (semi essential).
(Mnemonic : METT VIL PhLy + histidine, arginine).

Nutritionally non – essential amino acids : Can be synthesized in our body.

Every protein has an amino acid score based on amount of essential amino acids.
Complete protein : Contains all the essential amino acids.

Derived amino acids 00:33:58

Derived from a standard amino acid.

	Standard amino acid	Derived amino acid
Codon	++	--
modification	Translational/co-translational	Post translational
Example	Lysine	Hydroxylysine

Classification of derived amino acids :
Seen in protein :
Hydroxylysine and hydroxyproline : Formed by hydroxylation by hydroxylase enzyme. They are present in collagen.
Vitamin C and alpha Ketoglutarate are co factors for

Active space

hydroxylase enzyme.

Gamma carboxy glutamate : Require gamma carboxylation. Vitamin K is required.

Example : Clotting factors -2/7/9/10, protein C, protein S, osteocalcin, nephrocalcin and matrix glutamic acid proteins.

Desmosine :

Derived from lysine. It is present in elastin.

Methyl lysine :

Present in skeletal muscle protein (myosin).

Not seen in protein :

- Ornithine, argininosuccinate, citrulline (Part of urea cycle).
- Homoserine, homocysteine (Sulphur containing amino acids).

Selenocysteine :

- Standard amino acid.
- Codon is UGA (stop codon).
- Formed by recoding → Stop codon is converted to coding codon.
- Co-translational modification : Serine (precursor amino acid) → Cysteine → Selenocysteine.
- Proteins/enzymes containing selenocysteine : Thioredoxin reductase, glutathione peroxidase, deiodinase, selenoprotein P, glycine reductase.
- Selenocysteine is also called as 21st protein forming amino acid.

Pyrolysine :

- 22nd protein forming amino acid.
- Codon is UAG.
- Formed by recoding.
- Precursor amino acid is lysine.

Properties of amino acids

00:43:38

Amino acids can :

- Absorb UV light.
- Exhibit isomerism.
- Exist in different charged states.
- Exhibit buffering capacity.

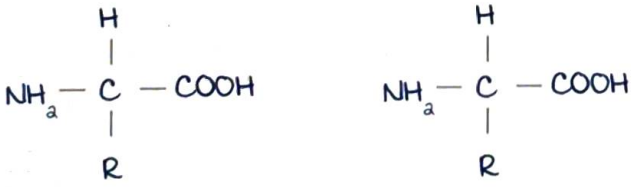
Absorb UV light :

- Amino acids are colorless as they do not absorb visible light.
- Proteins can be estimated by UV absorption spectrophotometer as amino acids can absorb UV light.
- Amino acids absorb 250-290 nm of UV light. Maximum absorption is for 280 nm.
- Aromatic amino acids absorb UV light as they have conjugate ring structure. Example : Tryptophan (maximum), phenylalanine, tyrosine.

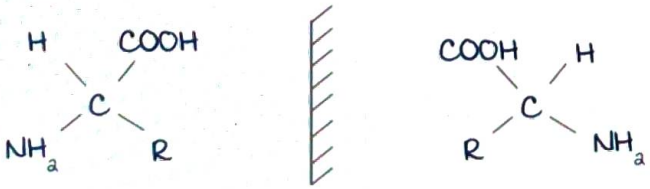
Exhibit isomerism :

Amino acids exhibit isomerism due to asymmetric carbon atom. Four valencies are occupied by four different groups except glycine. Glycine is optically inactive and do not exhibit isomerism.

Glycine :



D and L isomerism :



Mirror images

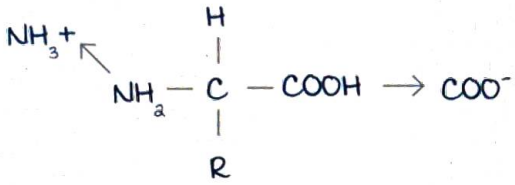
Due to presence of asymmetric carbon atom, mirror images

Active space

in structure can be seen.
They are known as D and L isomerism.
Most amino acids exist in L form as enzymes can act only on L amino acids.
Racemase is an enzyme that converts D form to L form.
Hence, it can act both on D and L forms.
D-aspartate and D-serine are D amino acids present in brain.

Isoelectric point

00:49:26



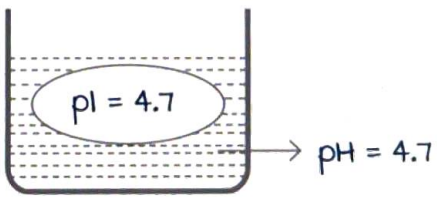
NH_2 group can accept a proton and can exist as NH_3^+
-COOH group can liberate a proton and can exist as COO^-
So, there are two ionizable groups.
Ionization constant (pK) is a particular point for an ionizable group.

Isoelectric point is average of the ionization constants of the ionizable groups present in the amino acids. Isoelectric point is

$$(pK_1+pK_2)/2 = pI$$

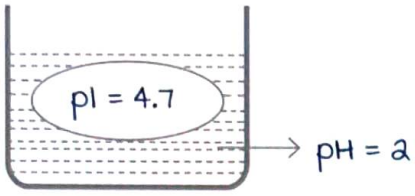
When the pH of the medium is equal to pI, then the protein is called as zwitter ion or ampholytes. In a zwitter ion, positive charges = negative charges → Neutral.

At pH = p^I :

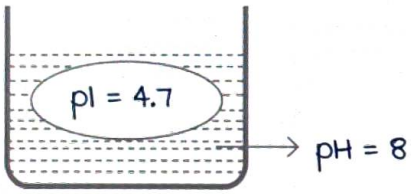


- There is no net charge.
- No mobility in electric field.
- Cannot attract net water → No shell of hydration around the protein → Maximum precitability and minimum solubility.
- Least buffering.

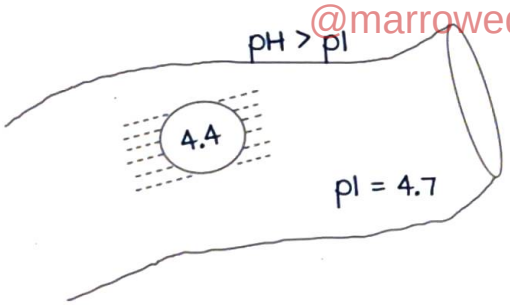
If pH < pI → Positive charge (protonated).



If pH > pI → Negative charge (deprotonated).



Albumin is negatively charged in blood as pH of blood is more than pI of albumin.
In the stomach pH is less than pI of albumin. Hence albumin carries positive charge in stomach.



Buffers are solutions that resist changes in pH.
Henderson Hasselbach equation : pH = pKa + log (base/acid)
Maximum buffering capacity is seen if pH = PKa. Example : Imidazole group of histidine (pKa = 6.5 to 7.4 = pH of blood).

Active space

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 amino acid is glycine.

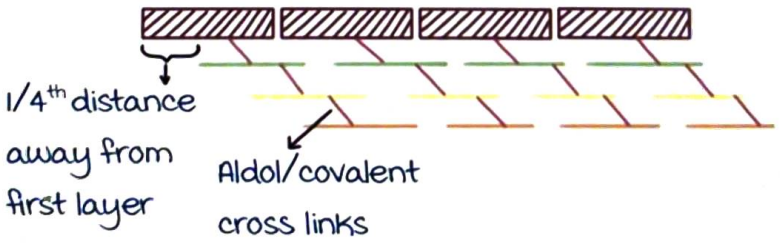
33% of α chain is Glycine.

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.

Intracellular events :

- Hydroxylation of proline and lysine residue
  ↓ Vitamin c , α Ketoglutarate
  By prolyl/ lysyl hydroxylase (monooxygenase)
- Glycosylation of hydroxy lysine.
- Intra chain and inter chain disulfide bond formation.
- Formation of triple helix → Golgi apparatus :
  Procollagen packed into secretory vesicle.
  Transported to extracellular matrix.

} RER

Extracellular events :

Cleavage of N and C terminal polypeptides.

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) and 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 COL1A2	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, COL1A1	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 (Requires Cu)	Menkes disease (ATP7B)

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 (ADAMTSa

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.

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 is the disulphide bond.

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.
Geleophysic 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.

MCQs :
Which if the following is not a fibrous protein ?
A. Collagen.

B. Elastin.
C. Keratin.
D. Myoglobin.

Desmosine cross link is found in
A. Elastin.
B. Keratin.
C. Collagen.
D. Silk fibroin.
E. Laminin.

Which of the following regarding Keratin is false ?
A. Present in hair, nail and outer layer of skin.
B. Rich in cysteine. 60c6b3eeaa8ded0e4e7e5ea7
C. Beta plated structure.
D. The more the disulphide bond in the Keratin harder the structure.
E. Alpha helix cross linked by disulphide bond.

The disorder associated with deletion of elastin gene is

A. marfan syndrome.

B. Congenital contractural arachnodactyly.

C. EDS.

D. William Beuren syndrome.

E. Epidermolysis bullosa.

Which of the following statement regarding strcuture of collagen is/are false ?

A. Forms unique triple helix.

B. A repetitive Glu-X-Y pattern.

C. Each polypeptide chain is twised into right handed polyproline helix.

D. Each polypeptide is approximately 1000 amino acids.

E. Type III O glycosidic linkage present.

Recurring amino acid present in collagen is

A. Glycine.

B. Proline.

C. Hydroxyproline.

D. Hydroxylysine.

Match the following :

Epidermolyis bullosa : Type VII collagen.

Alports syndrome : Type IV collagen.

Menkes disease : Lysyl oxidase.

Scurvy : Lysyl hydroxylase.

Schmid metaphyseal chondrodysplasia : Type X collagen.

OA : Type II collagen.

The gene defect in alport syndrome is

A. COL 2AI.
C. COL 4AI.
B. COL 3AI.
D. COL 7AI.

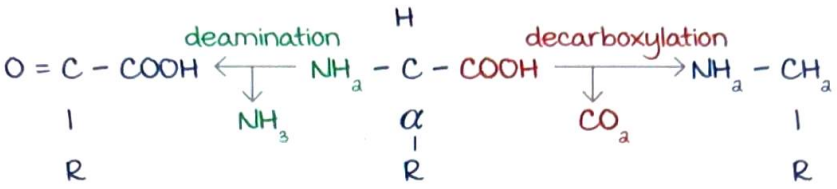
The type of collagen found in anchoring fibrils ?

A. Type I.
C. Type V.
B. Type III.
D. Type VII.

Active space

GENERAL AMINO ACID METABOLISM

Deamination & decarboxylation 00:01:04



α – Keto acids

Handling of amino group :

1. Transamination.
2. Transport of ammonia.
3. Oxidative deamination.

Transamination 00:03:55

Definition : Transfer of amino group from one amino acid to a ~~keto acid to form another~~ pair of amino acid and keto acid.

Takes place in all organs/cells.

Ammonia is not released freely.

Reversible.

Coenzyme : PLP.

Organelle : cytoplasm.

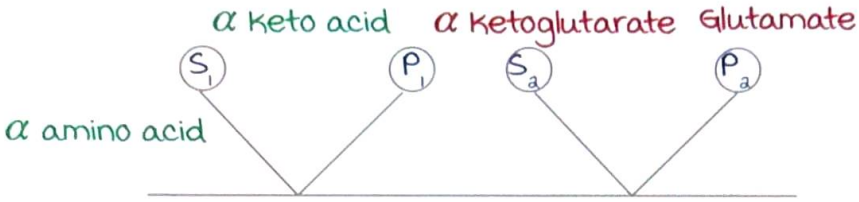
Examples :

1. SGPT/ALT (Alanine Aminotransferase)	2. SGOT/AST (Aspartate Aminotransferase)
Alanine → Pyruvate; α Ketoglutarate → Glutamate	Aspartate → Oxaloacetate (Specific); α Ketoglutarate → Glutamate (non specific)

Transamination is specific for one pair of the substrate but not specific for the other pair.

α amino group of any α amino acid is getting concentrated as glutamate (only amino acid to undergo oxidative deamination).

Ping-Pong mechanism : Two substrate two product reaction.
Bibi reaction.



Biosynthesis of non-essential amino acids :

α Ketoglutarate — Glutamate
Pyruvate — Alanine
Oxaloacetate — Aspartate

The non-α amino group that undergoes transamination :

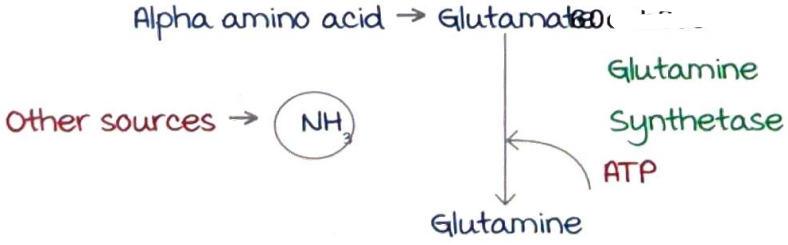
δ amino group of ornithine.
Enzyme : δ Ornithine aminotransferase.
Deficiency : Gyrate atrophy of retina & choroid.
Treatment : Restrict ornithine & arginine (source of ornithine).
Gives co-enzyme : PLP.

Sources of ammonia 00:20:05

α amino group amino acid (getting concentrated as glutamate).
Non-protein nitrogenous substances (NPN).
Amino sugars.
Gut bacteria.

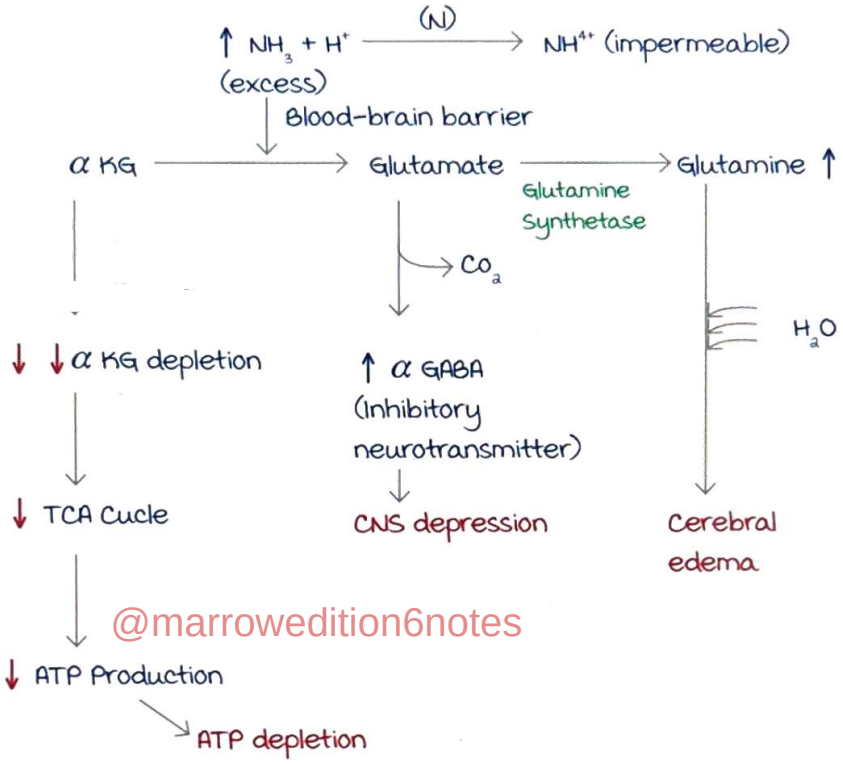
Transport of ammonia from most organs (including brain) 00:20:33

α amino group : Concentrated as glutamate.
Other sources : Release NH_3 (toxic).



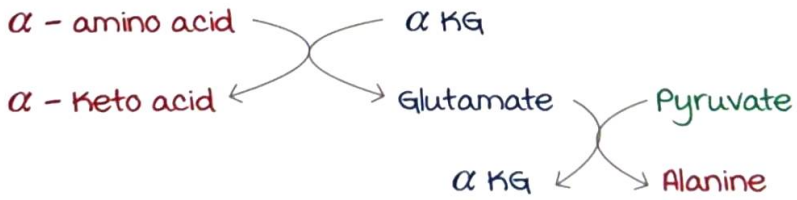
Glutamine is a transport form of ammonia from most organs (including brain).
The reaction takes place in mitochondria.
First line trapping of ammonia.
Ligase reaction.

Toxicity of ammonia to brain :

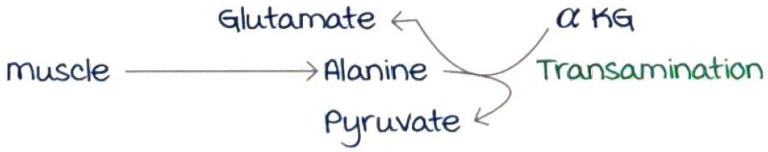


Transport of ammonia from muscle

00:30:01



In the muscle, glutamate is converted back into α-ketoglutarate and pyruvate takes up amino group and becomes alanine.
Alanine is the transport form of ammonia in the muscle.
Final destination : Liver (urea synthesis can only take place in the liver).



Active space

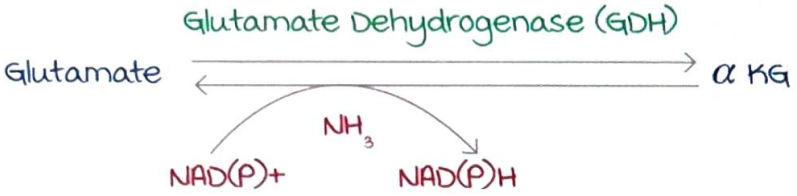
Glutamate metabolism

00:33:17

Glutamine (most organs) & alanine (muscle) are concentrated as glutamate.

Glutamate undergoes oxidative deamination in the liver.

Oxidative deamination :



NH_3 (released freely) : Enters urea cycle.

GDH can use either NAD+ and NADP+ as coenzyme → forms NADH and NADPH respectively.

GDH oxidative deamination takes place in the liver & kidney.

Reversible reaction.

Organelle : Mitochondria.

Allosteric activator : ADP.

Allosteric inhibitor : ATP/GTP/NADH.

Trans-deamination : Transamination (occurs in all cells) is coupled with oxidative deamination (occurs in the liver).

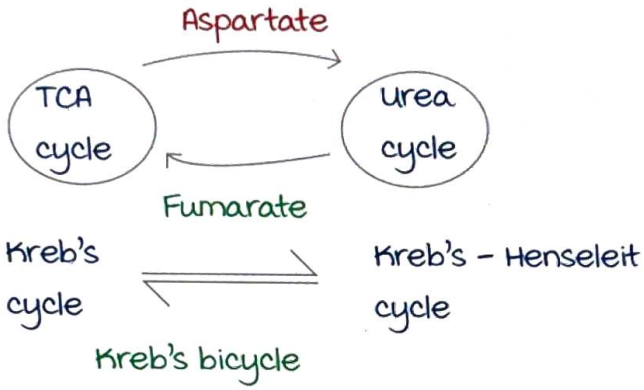
Active space

UREA CYCLE AND DISORDERS

Introduction

00:02:13

Also known as : Kreb's Henseleit cycle (scientist's name).
Ornithine cycle (ornithine is regenerated).
Urea Bi-cycle (linked to another cycle).



Sources of atoms of urea : NH_2 – CO – NH_2

↓ ↓ ↓

NH_3 CO_2 Aspartate

Compounds consumed in the Urea cycle : NH_3 (ammonia), CO_2 and, Aspartate.
Site : Liver (exclusively).
Organelle : Cytoplasm and mitochondria.
Other pathways taking place in both cytoplasm & mitochondria : HUG Pathway

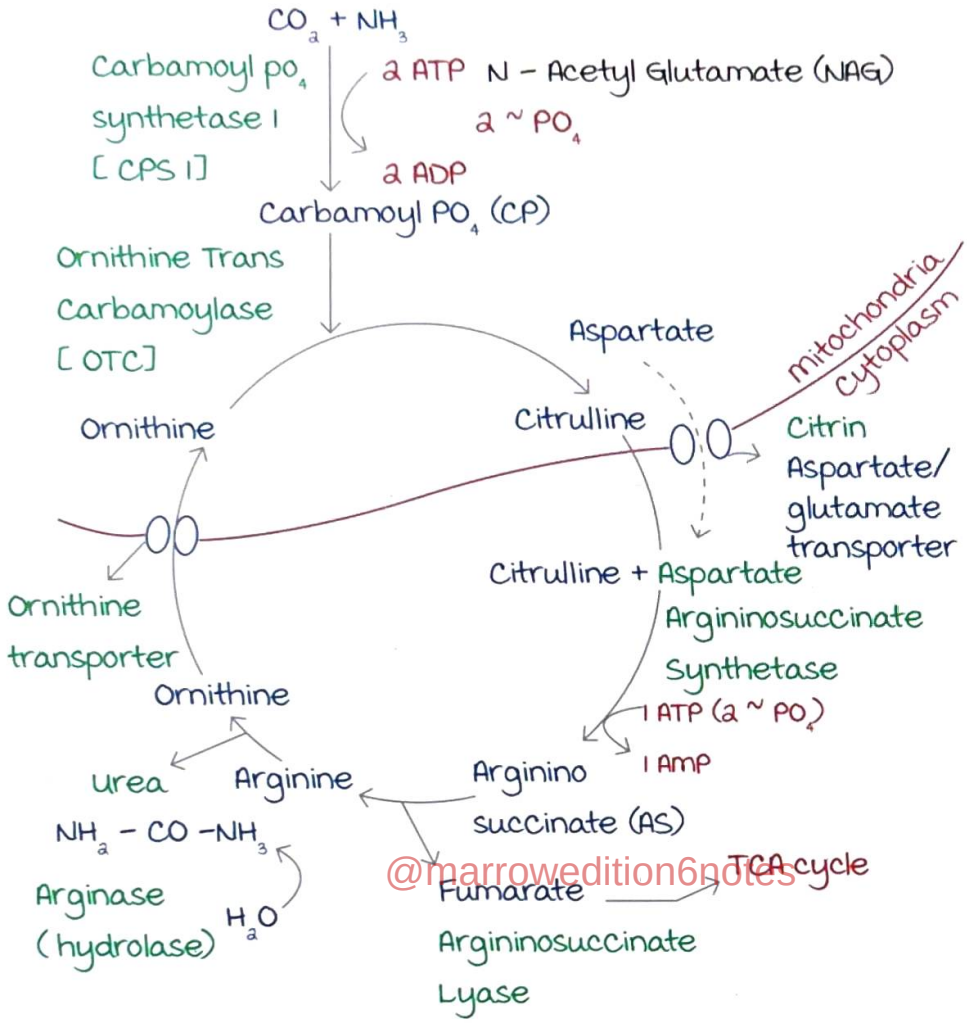
- Heme synthesis
- Urea cycle
- Gluconeogenesis
- Pyrimidine synthesis

Rate limiting step of urea cycle : CPS I (Carbomyl phosphate synthetase I)

Active space

Reactions of urea cycle

00:07:35



CPS I : Rate limiting step.

Uses 2 high energy PO_4.

N acetyl glutamate (NAG) is the obligate allosteric activator (positive) of the first step.

Energetics :

CPS-I : 2~PO_4 (2 ATP → 2 ADP)

Argininosuccinate synthetase : 2~PO_4 (1 ATP → 1 AMP)

- Total : 4~PO_4.
- 3 ATPs used directly
- 4 ATP equivalent used

Regulation :

Dietary : Increased protein intake induces urea cycle enzymes.

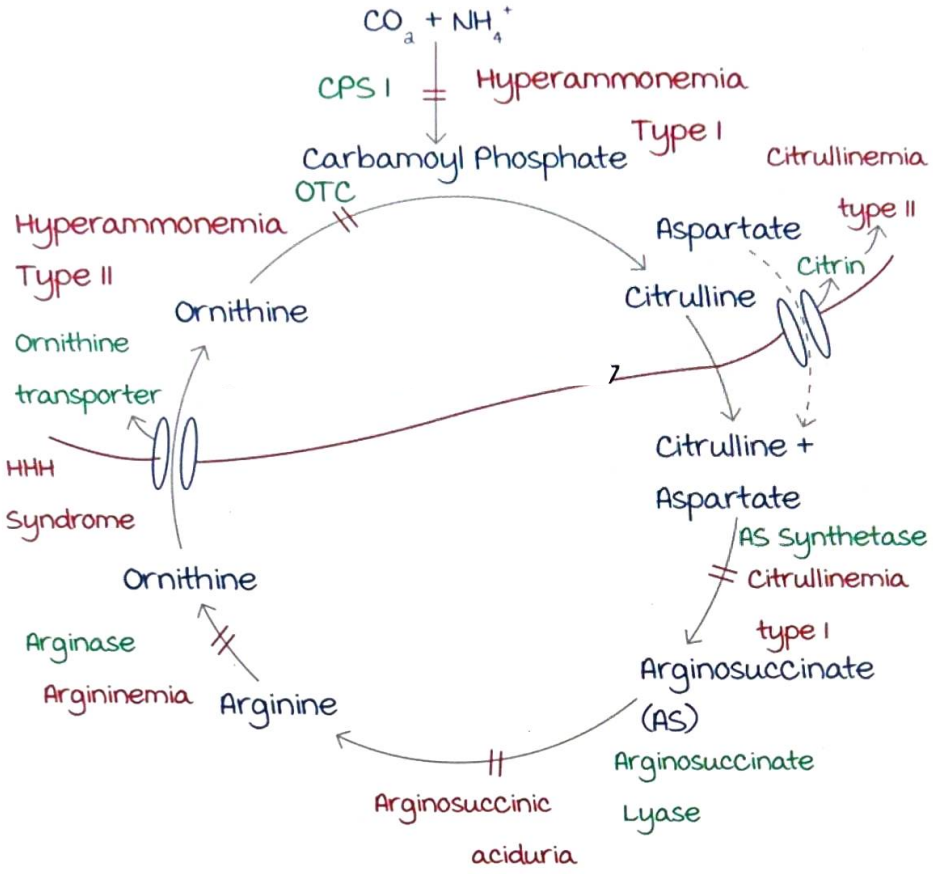
NAG is a positive allosteric regulator of CPS-I.

Compartmentation: partially in mitochondria & partly in the cytoplasm.

Active space

Urea Cycle Disorders

00:20:40



Hyperammonaemia & plasma glutamine

00:24:22

Relationship between hyperammonaemia and plasma glutamine :

↑ ↑ NH_3 → Glutamate

↓

↑ ↑ Glutamine

Types :

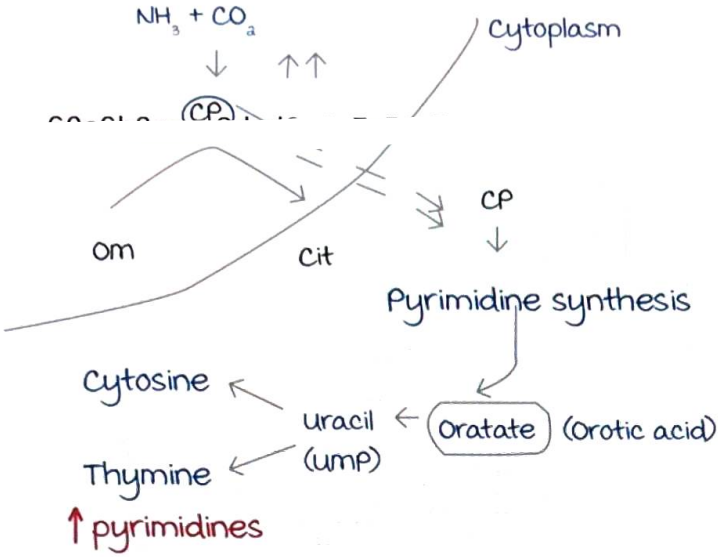
Type-1		Type-2
NH_3	>	NH_3
Plasma Glutamine	>	Plasma Glutamine

Type-2 Hyperammonaemia :

Defective enzyme : OTC.

Uracil is increased.

Active space



Characteristic features :

- X-linked partially dominant (only males affected).
- Increase orotic acid secretion.
- Most common urea cycle disorder (40%).

Trichorhexis nodosa :

Tufted hair.

Seen in argininosuccinic aciduria.

HHH syndrome :

Hyperammonaemia hyperornithinaemia homocitrullinuria syndrome.

Due to defective ornithine transporter :

- Ornithine accumulates → Hyperornithinaemia
- No ornithine : CP had nothing to combine with → hyperammonaemia
- CP combines with lysine : homocitrulline → homocitrullinuria

Arginemia :

Least hyperammonaemia : Arginase is the last enzyme.

Arginase has 2 isoforms.

Progressive spastic diplegia & scissoring of gait.

Active space

Clinical features & investigations of urea cycle disorder

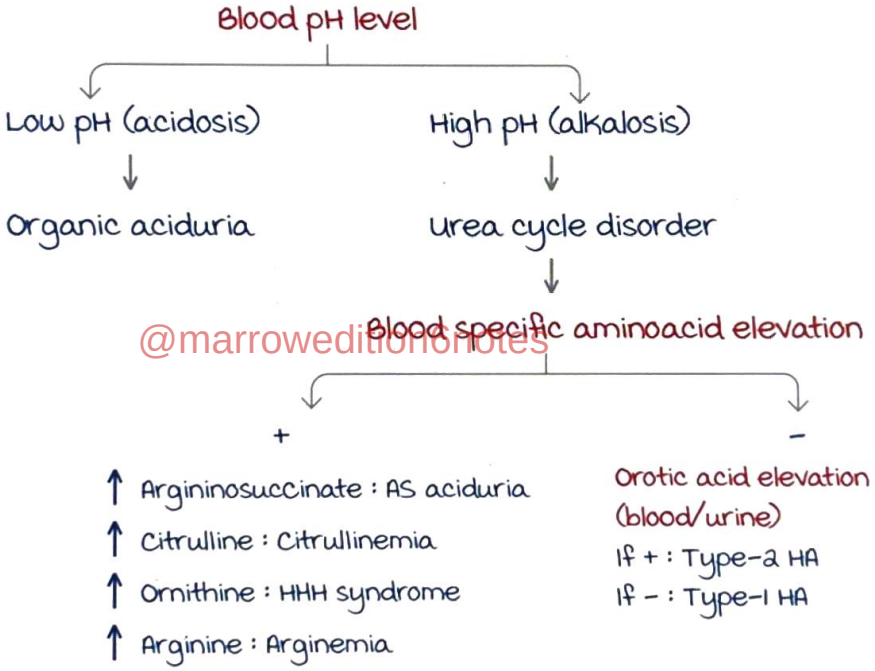
0:33:40

Clinical features :

1. Encephalopathy
2. Respiratory alkalosis : ↑ NH_3 → Hyperventilation → Tachypnoea → CO_2 washout.
3. Hyperammonaemia
4. Neonates present with feeding difficulty, lethargy, vomiting, failure to thrive, convulsion.

Tachypnoea.

Investigation :



Treatment

00:39:27

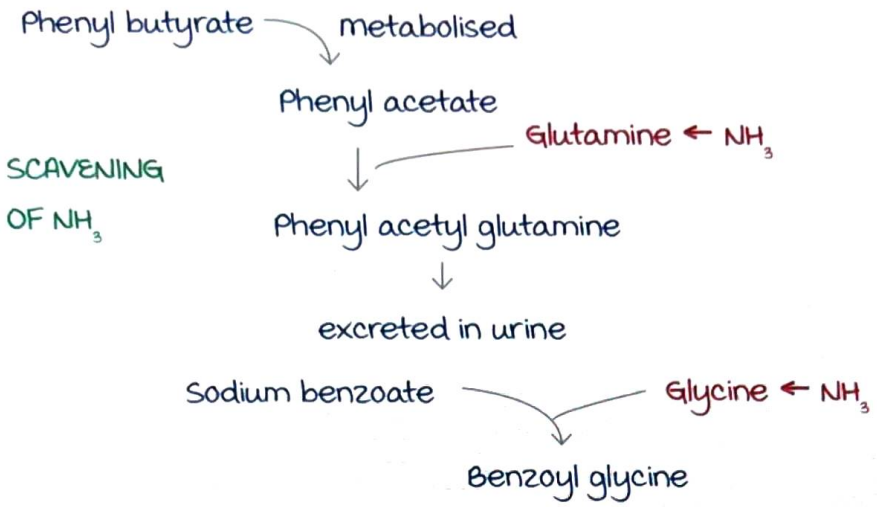
First-line treatment is arginine :

- Essential amino acid
- Provide ornithine
- Positive regulator of NAG synthase → increase NAG → + CPS-1

Contraindication : Do not give in arginase defect.

Second line : Acylation therapy

- Sodium benzoate and phenylbutyrate used.



Question : The given table is the lab [illegible] suspecting urea cycle disorder. Identify the enzyme involved in the correct sequence.

Infant	Urine orotate	Blood citrulline	Blood arginine	Blood NH_3
1	Low	Low	Low	High
2	-	High (>1000 μm)	Low	High
3	-	-	High	Moderately high
4	High	Low	Low	High

Options :

A. CPS-I
B. OTC
C. Argininosuccinate synthetase
D. Arginase

Answer : 1-A, 2-C, 3-D, 4-B.

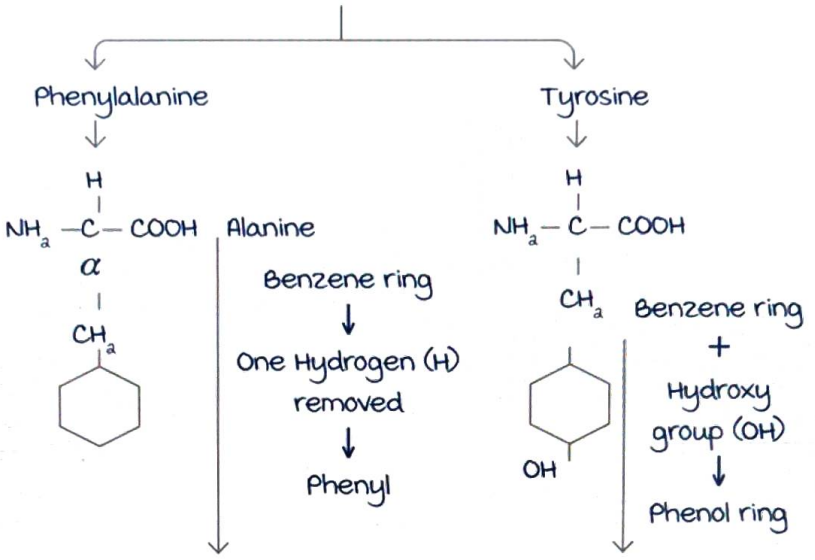
Question : A 6 month old boy admitted with failure to thrive with high Glutamine & Uracil in urine, hypoglycemia, high blood ammonia. Treatment given for 2 months. At 8 months again admitted for failure to thrive to gain weight. Gastric tube feeding was not tolerated. Child became comatose. Parental dextrose given. Child recovered from coma within 24 hrs. What is the enzyme defect ?

A. CPSI.
B. Ornithine transcarbamoylase.
C. Arginase.
D. Arginosuccinate synthetase.

Active space

AROMATIC AMINO ACIDS

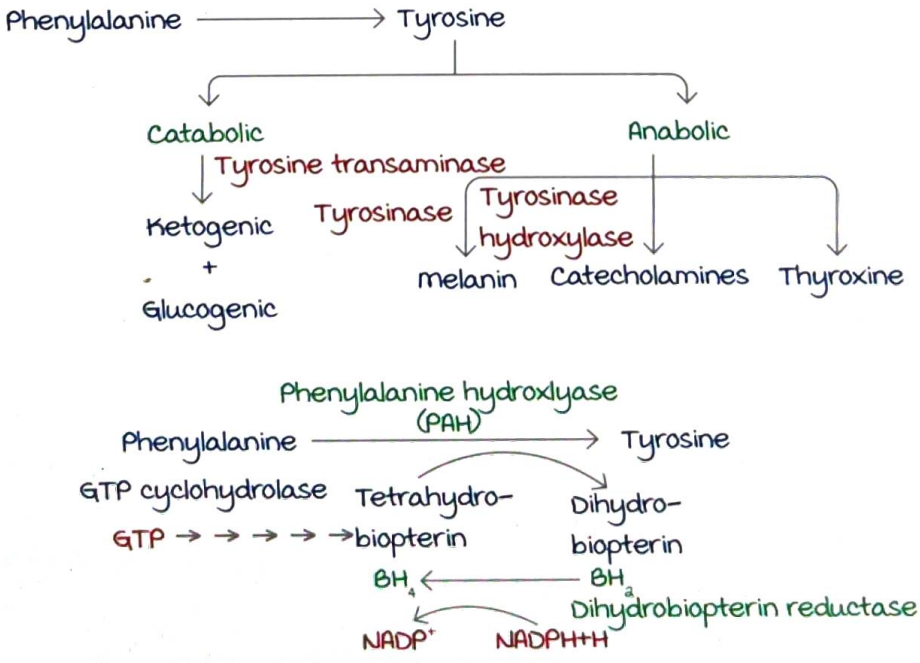
Phenylalanine and tyrosine: Structure 00:00:37



Essential amino acid (AA)
Both Ketogenic & glucogenic
Non-polar amino acid

Non essential amino acids
Both Ketogenic & glucogenic
Least non polar among non-polar aromatic amino acid

Phenylalanine & tyrosine: Metabolism 00:06:25



Active space

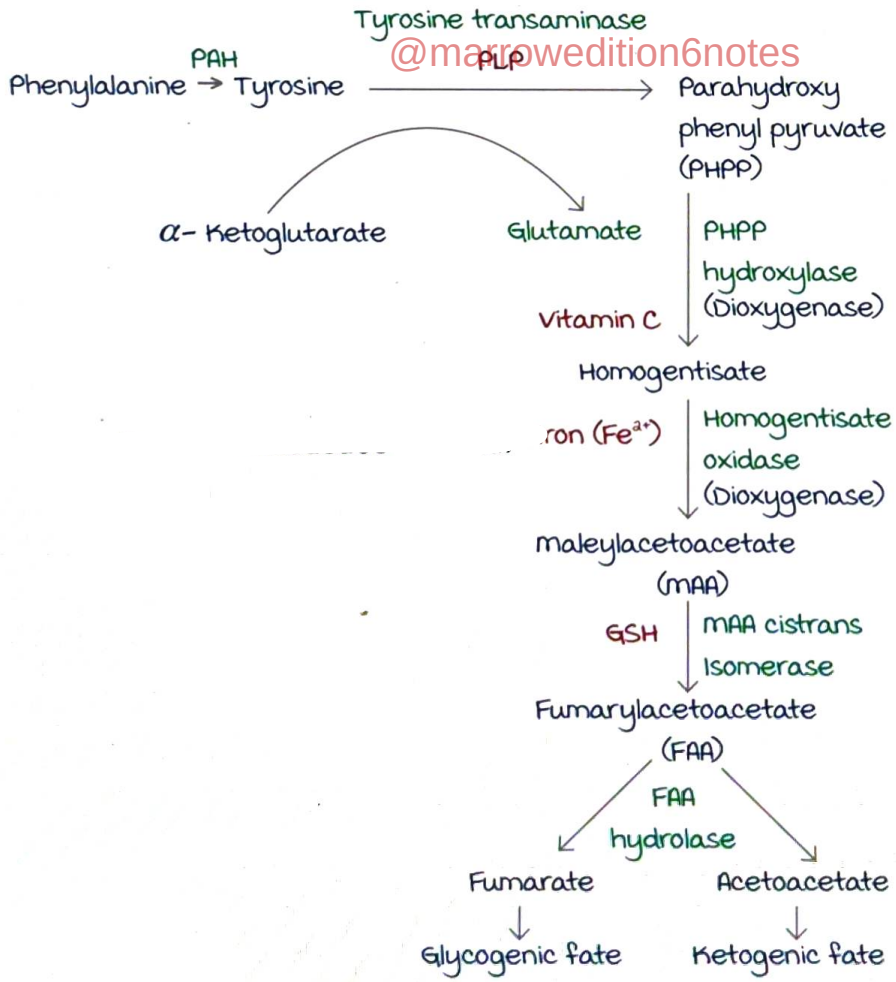
Properties of this reaction :

- Irreversible reaction.
- Phenylalanine hydroxylase is monooxygenase.

Concepts of Phenylalanine hydroxylase (PAH) :

1. Phenylalanine → Tyrosine
   ↑ ✕ ↓
   Phyenylalanine is an essential amino acid.
   Tyrosine is a non essential amino acid.
2. Phenylalanine doesn't have any function in the body per se.
   It converts into tyrosine and enters into a metabolic pathway.

Catabolic fate of phenylalanine and tyrosine 00:14:06

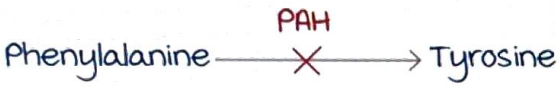


Active space

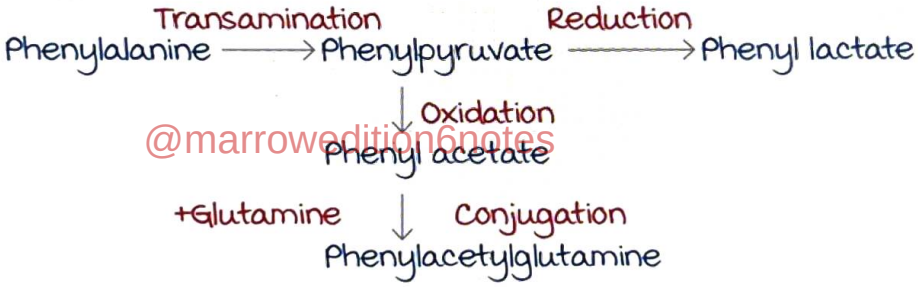
- Classic phenylketonuria : Defect in phenylalanine hydroxylase (PAH).
- Non classic phenylketonuria type II & III : Defect in dihydrobiopterin reductase.
- Non classic phenylketonuria type IV & V : Defect in formation of tetrahydrobiopterin.
- Alkaptonuria : Defect in homogentisate oxidase.
- Type I tyrosinemia : Defect in enzyme FAA hydrolase.
- Type II tyrosinemia : Defect in tyrosine transaminase.
- Type III tyrosinemia : Defect in PHPP hydroxylase.

Phenylketonuria

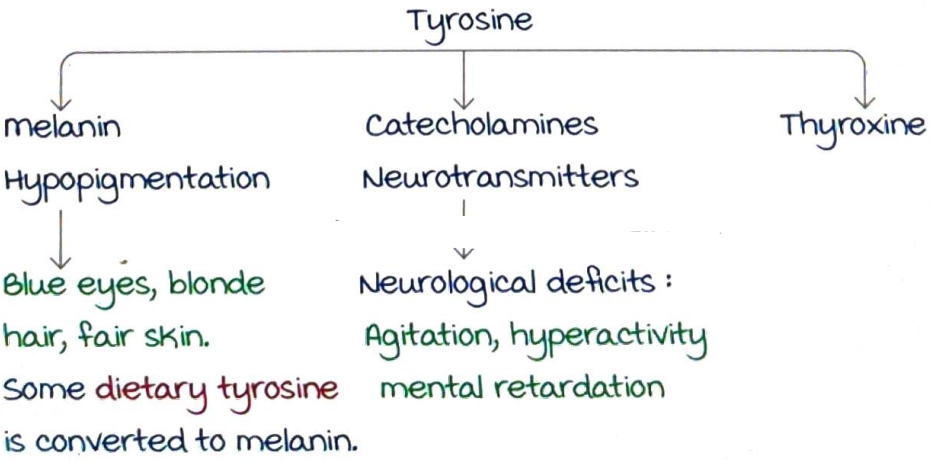
00:22:54



Phenylalanine enters into alternate metabolic pathways.



Phenyl acetate causes mousy body odour.



Causes of neurological manifestations :
In the brain : Phenylalanine, tyrosine, tryptophan passes through the blood brain barrier through a transporter.

In phenylketonuria, phenylalanine saturates all transporters.

↑ Phenylalanine, ↓ tyrosine, ↓ tryptophan.

- Phenylalanine cannot do any function as it is unable to convert to tyrosine.
- Phenylalanine also prevents dietary tyrosine to enter through the transporter.

↓ Tyrosine : ↓ Neurotransmitters, ↓ catecholamines
↓ Tryptophan : ↓ Serotonin

~~Clinical feature in infants~~: Intractable vomiting.
misdiagnosed as congenital hypertrophic Pyloric stenosis.

Laboratory diagnosis and treatment of phenylketonuria

00:33:34

1. Guthrie's test (Bacterial inhibition test) :
   Bacillus subtilis requires phenylalanine for growth.
   If phenylalanine present in blood : Bacterial cultures seen.
   In neonates, heel prick sample taken.

2. Ferric chloride urine test :
   1 ml of ferric chloride added to urine sample : Blue green colour indicates a positive test.
   Transient reaction.

3. Blood phenylalanine :
   Normal : 2 – 6 mg/dl.
   >20 mg/dl : bad prognosis.
4. Enzyme studies
5. Genetic mutation studies.
6. Tandem mass spectrometry : Gold standard screening method for all metabolic disorders.

Treatment :

- Restrict phenylalanine (cassava based diet).

- Concentrate of large neutral amino acid (LNAA).
- Sapropterin dihydrochloride (Kuvan) : Synthetic BH_4
- Enzyme replacement therapy under trial : Phenylalanine ammonia lyase.

Alkaptonuria

00:40:11

Homogentisate → Benzoquinone

Homogentisate Oxidase ↓ : Malyl acetic acid (MAA)

Polymerise ↓ : Alkaptone bodies

Alkaptone bodies accumulate in nose, pinna & sclera : Black spots.

Homogentisate excreted in urine, gets oxidised and results in blackish discoloration.
In infants, black/ reddish discoloration of diaper.
Increased discoloration on washing with soap : 'Alkalinization' increases darkening of urine.

No mental retardation.
Clinical features : Back pain, black spots/ pigmentation in middle age.
Ochronosis : Alkaptone bodies accumulate in intervertebral discs.

Garrod's Tetrad : First inborn error of metabolism studied by Archibald Garrod.
C – Cystinuria
A – Alkaptonuria
A – Albinism
P – Pentosuria

Laboratory diagnosis :
Alkalinisation of urine : ↑ darkening of urine.
Ferric chloride test : Positive.
$AgNO_3$ test : Positive
x – ray spine : Parrot beak appearance.

Active space

Treatment :

Nitisinone/NTBC : inhibits PHPP hydroxylase and reduces homogentisate.

Segawa syndrome :

Autosomal dominant.

Clinical features : Dystonia with diurnal variation.

Females > males.

GTP cyclohydrolase enzyme is defective.

↓ BH_4 but blood phenylalanine levels normal.

Tyrosinemia 00:51:26

Type I tyrosinemia/ Hepatorenal/ Hereditary tyrosinemia.

A/K/A tyrosinosis.

Most common type.

Hepatic and renal failure.

Enzyme defect : Fumarylacetoacetate hydrolase.

Accumulation of succinyl acetone.

Liver failure → MAT enzyme affected →

accumulation of methionine (boiled cabbage odour)

Resemble porphyria.

Treatment : Nitisinone.

Type II tyrosinemia :

A/K/A Oculocutaneous tyrosinemia/Richner hanhart syndrome.

Enzyme deficiency : Tyrosine transaminase.

Clinical features : Corneal ulcer,

Cutaneous non pruritic hyper keratotic plaque.

Type III tyrosinemia/ Neonatal tyrosinemia : Least common

Hawkinsinuria :

Mutation of PHPP hydroxylase (partially active).

Gives swimming pool odour.

Anabolic fate of tyrosine

00:56:57

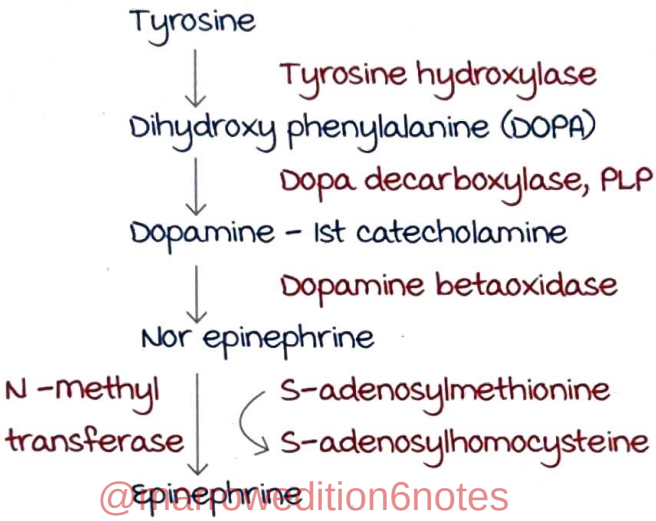
1. Catecholamines :

Synthesized in the chromaffin cells.

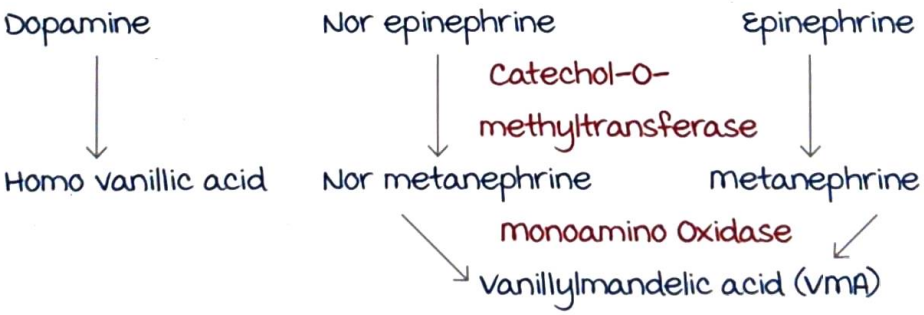
Adrenal - 80% adrenaline

Extra adrenal : Nor-epinephrine.

Nerve endings of sympathetic ganglia.

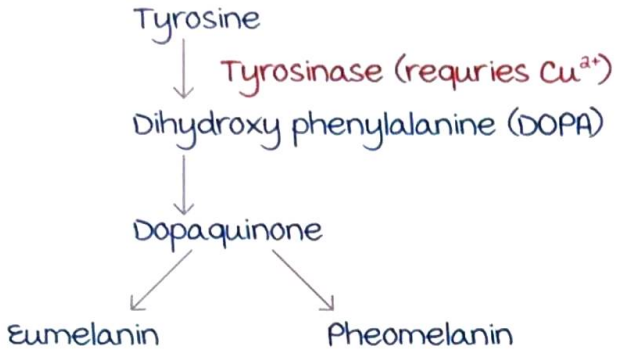


S-adenosylmethionine derived from methionine.



2. Melanin :

Synthesized in melanosomes in melanocytes (deeper layers of epidermis).



3. Thyroxine :
   Synthesized in thyroglobulin in thyroid follicles.
   Has 115 tyrosine residues.
   If iodinated : MIT – Monoiodo thyronine
   DIT – Diiodo thyronine

Pheochromocytoma 01:05:01

Tumour of adrenal medulla
Excess catecholamines.
Symptoms :
Profuse sweating, headache, palpitations, hypertension.

Diagnosis :
24 hr urinary examination for
- Vanillylmandelic acid
- Fractionated metanephrines (highly sensitive)

In plasma, catecholamines and free metanephrines.

Question : A patient has a "pill rolling" tremor, "cogwheel" rigidity, bradykinesis, speech difficulties, and a shuffling gait. The chemical that is lacking in this syndrome is a derivative of which of the following amino acids ?

A. Alanine.
B. Serine.
C. Tyrosine.
D. Tryptophan.

Active space

Question : A 4 month old infant normal at birth, developed sudden twitching movement. She noticed a mousy body odour in her wet diaper. She was taken to a pediatrician, sample taken from heel prick was send for Guthrie's bacterial inhibition test.

Q1) What is the probable diagnosis ?

Phenylketonuria (PKU).

Q2) What is the reason for mousy body odour ?

Phenyl acetate.

Q3) Which is the amino acid elevated here ?

Phenyl alanine.

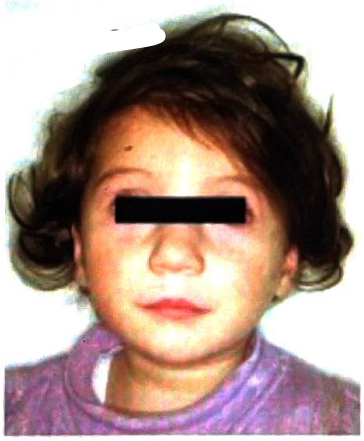
Q4) What is the bacteria employed for this test ?

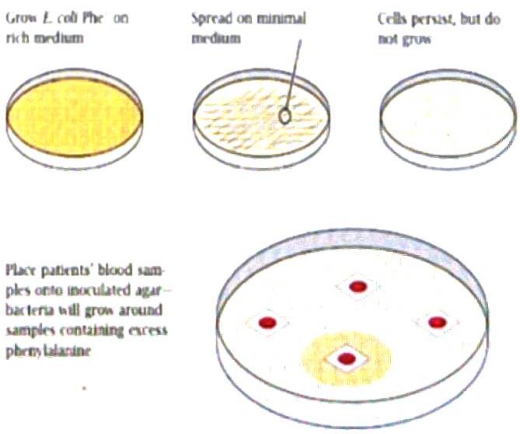
Bacillus subtilis.

Question : What is the rationale of using this Rx in PKU ?

Ans : In phenylketonuria, phenylalanine saturates all transporters, thereby not allowing tyrosine & tryptophan to enter into the brain. LNAA has high amount of tyrosine, tryptophan. Thus they are strong enough to compete with phenylalanine.

Question : A 5 yr old boy presented with blue eyes, blond hair, fair skin, eczematous rash, unusual mousy odour forced to eat fruits and other special diet.

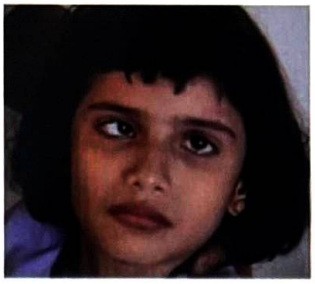




Answer : This is a case of Phenyl Ketonuria.

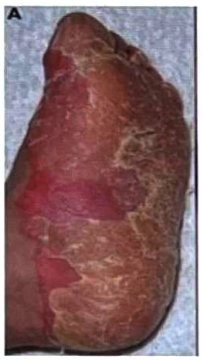
Active space

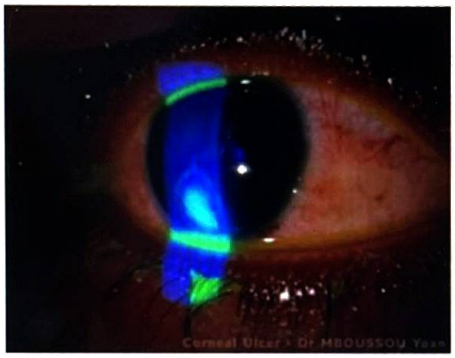
Question : A 4 year old girl presented to Pediatric OPD with complains of sudden weakness of both lower limbs especially in the evening, but improves in the morning. On investigation, Tetrahydrobiopterin level is reduced, but Phenyl Alanine level is normal. What is your diagnosis ?



Answer : Segawa syndrome.

Question : Identify this disorder associated with catabolic pathway of phenyl alanine ?



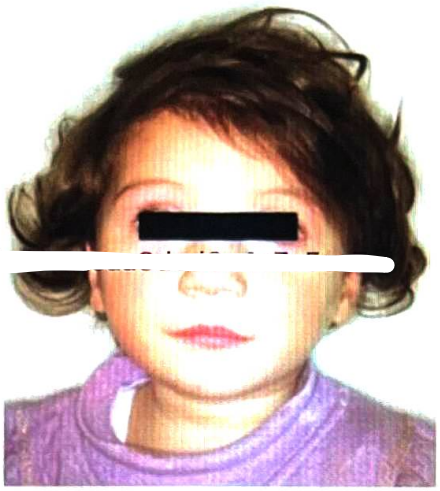


Answer : Type II Tyrosinemia (Hyperkeratotic plaques, Corneal ulcer). Aka Oculocutaneous tyrosinemia/Richner hanhart syndrome.

Question : A 6 month year old boy presented with severe metabolic acidosis, ketosis, failure to thrive and unusual odour of swimming pool as the weaning started. Urine Tandem Mass Spectrometry - Hawkinsin.

Answer : Hawkinsinuria.

Active space



Blonde hair, fair skin, blue eyes.
This is a case of PKU.

Melanine synthesis is affected.

Tyrosine is less → Less melanine.

But dietary tyrosine can be converted to melanin.

Milky white hair, more depigmented/milky white skin, red eyes. This is a case of Albinism.

Melanine synthesis is affected.

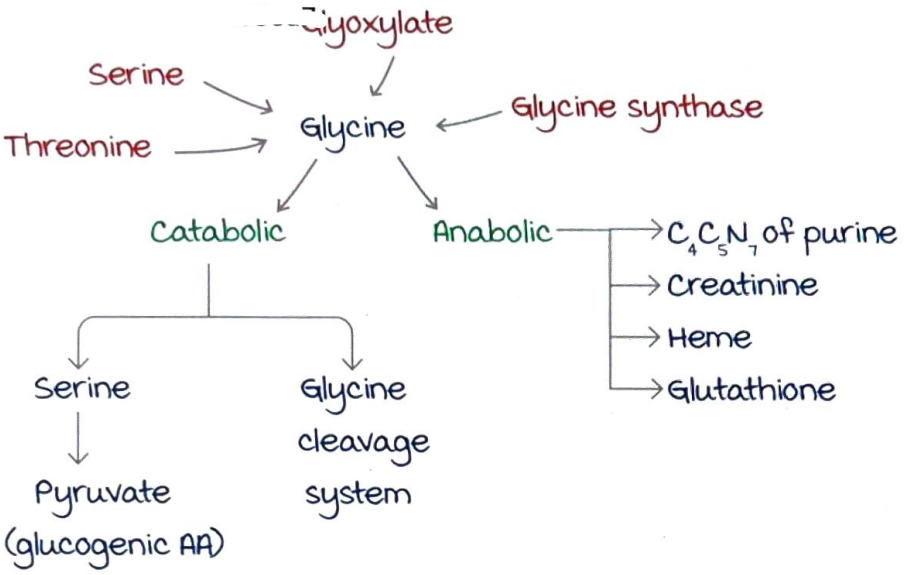
Tyrosinase enzyme itself is deficient.

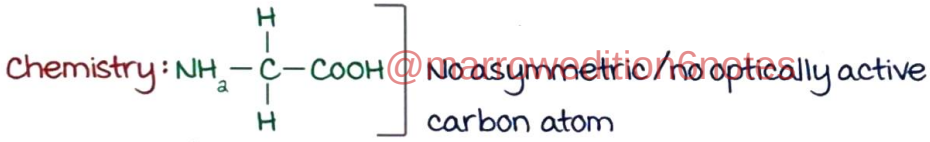
Even if dietary tyrosine is present, it can not be converted to melanin.

GLYCINE AND SERINE

Glycine

00:02:34

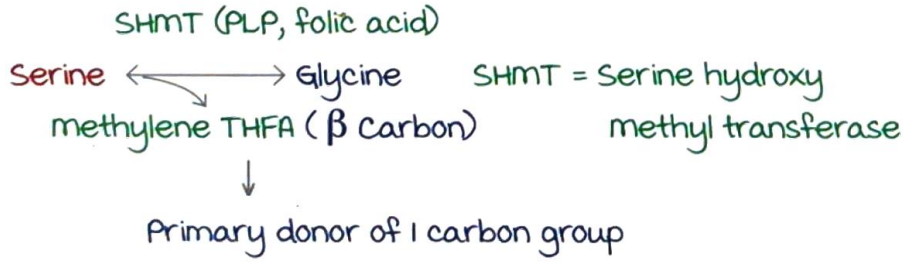




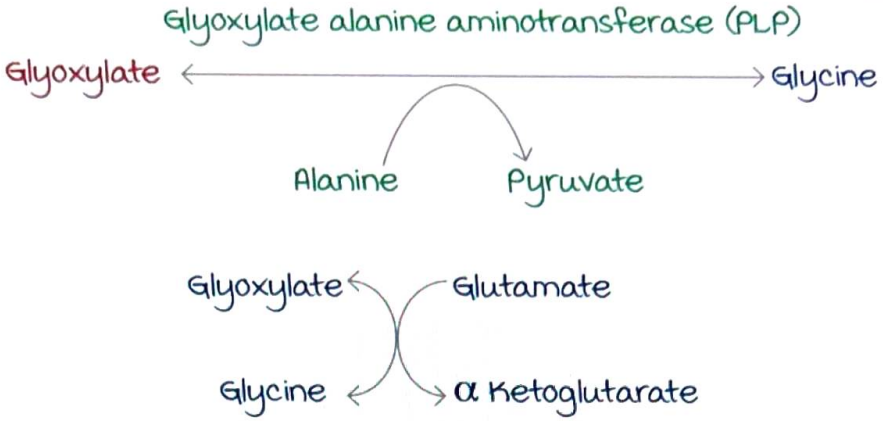
Non essential amino acid.
Purely glucogenic amino acid.
Polar amino acid.
Simple amino acid.

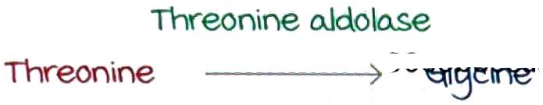
Glycine : Pathway

00:07:38



Active space





Glycine Cleavage System (GCS) :

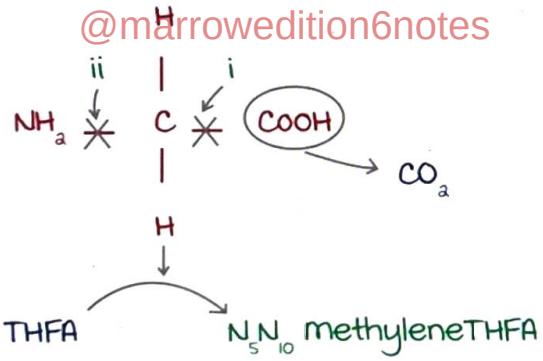
Multienzyme complex.

H - Protein (covalently linked to 3 enzymes).

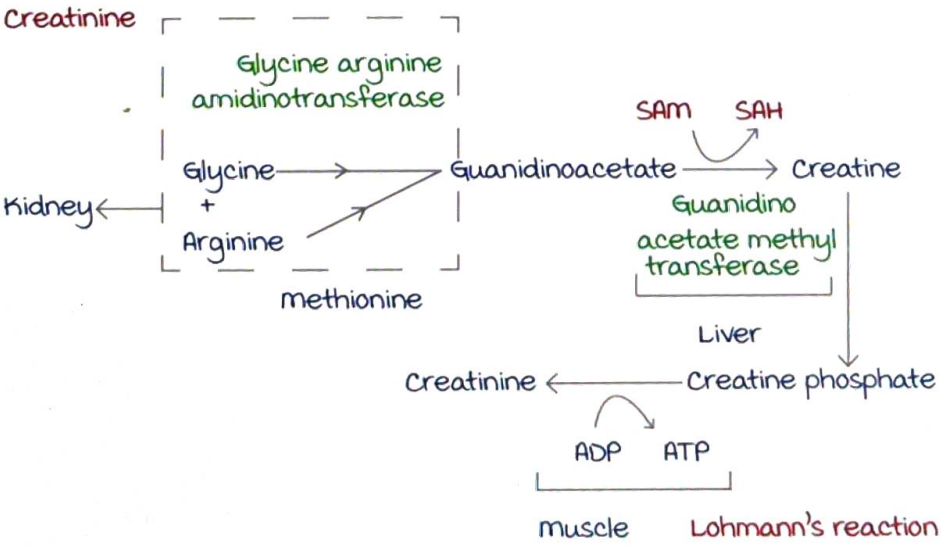
i) Glycine dehydrogenase : Cuts the COOH.

ii) Aminomethyl transferase : Separate methyl group.

iii) Dihydrolipoamide dehydrogenase.



Glycine : Anabolic fate & function 00:16:19



Active space

SAM : S-Adenosyl methionine.

SAH : S-Adenosyl homocysteine.

Creatine PO_4 : Immediate replenisher of ATP in muscle (first 3 - 4 seconds of exercise).
High energy compound.
a/k/a Phosphagen.

Heme :

Succinyl Co A + Glycine → → → → Heme

Glutathione :

C_4, C_5, N_7 of purine.
Neurotransmitter.
Conjugating agents : Bile acids.
Benzoyl Co A : Hippuric acid.
most abundant/recurring amino acid in collagen
Induces bends in 2° structure of proteins.

Glycine : Clinical correlation 00:23:09

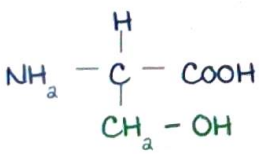
- Hyperoxaluria
  - 1°
    - Type 1 → defect in Glyoxylate Alanine Aminotransferase
    - Type 2 → defect in Glycerate dehydrogenase/Glyoxylate reductase
  - 2°
- Vit B_6 deficiency
- Vit C toxicity
- methoxyflurane
- Ethylene Glycol poisoning
- Enteric hyperoxaluria

→ Glyoxylate accumulation → Oxaluria

If defect occurs in GCS : Non-Ketotic hyperglycinemia.

Serine 00:27:14

most common site of phosphorylation.



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

Active space

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 Co A).

Selenocysteine precursor.

Serine —PLP→ Ethanolamine
↘ CO_2

Ethanolamine → Choline (Trimethyl ethanolamine) → Betaine (Trimethylglycine)

Glycoproteins → O-linked glycoproteins

Most common site for phosphorylation (covalent modification of enzymes).

1. Which is the amino acid involved in heme synthesis ?

Glycine.

2. State true/false ?

Amino acid can conjugate compound in xenobiotic reaction : T.

3. Why oxalate stones in urine in vitamin B6 deficiency ?

Because of deficiency of alanine glyoxylate aminotransferase.

4. Which is the amino acid completely incorporated to purine ring ?

Glycine.

5. Compound derived from glycine are all except ?

A. Creatinine.

B. Glutathione.

C. Purine.

D. Pyrimidine.

6. State true/false :

The following statements are regarding hyperoxaluria.

A. Vitamin B6 deficiency can cause it : True.

B. Primary type is due to defect in alanine glyoxylate amino transferase defect : True.

C. Vitamin C deficiency is a cause : False.

D. Glycerate dehydrogenase defect can cause it : True.

E. It is peroxisomal targeting defect : True.

7. Match the following :

Glycine : Glyoxylate.

Non Ketotic hyperglycemia : Glycine cleavage system.

Hyperoxaluria : Ethylene glycol.

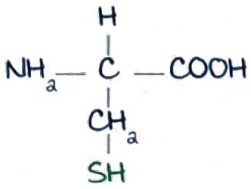
Serine : 1 C metabolism.

Active space

SULPHUR CONTAINING AMINO ACIDS

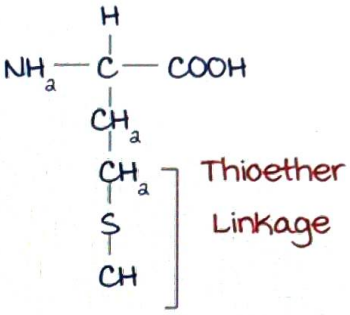
Introduction 00:03:26

- Cysteine



Sulfhydryl (-SH)/thioalcohol/ thiol group.
Non-essential amino acid (AA)
Purely glucogenic.
Polar amino acid.

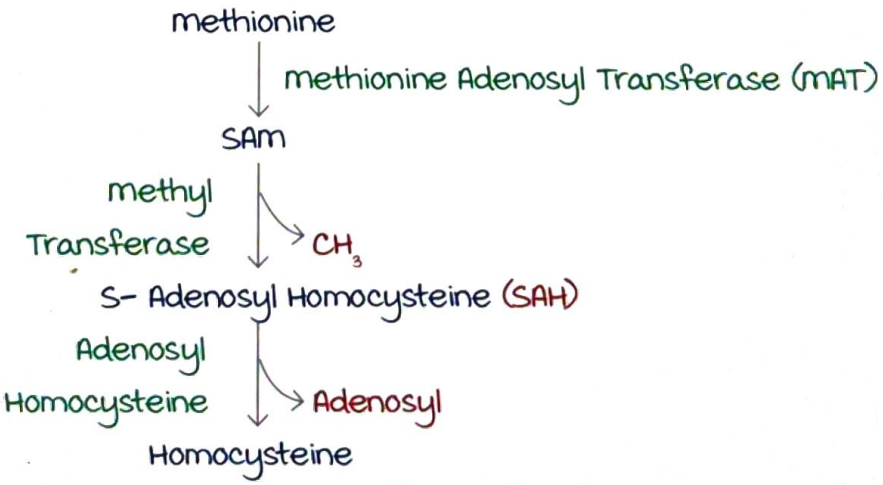
- methionine



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.

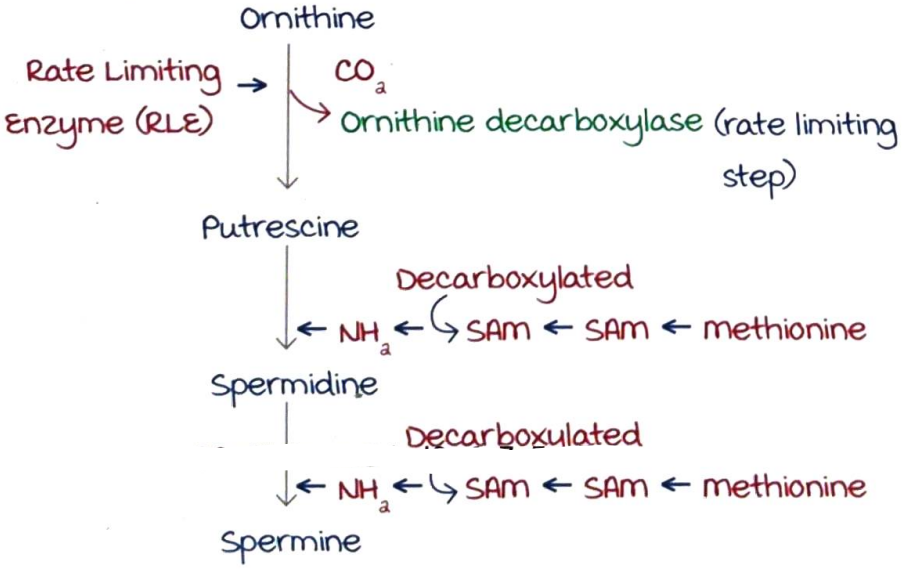
Transmethylation reaction & polyamine synthesis

00:13:56

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



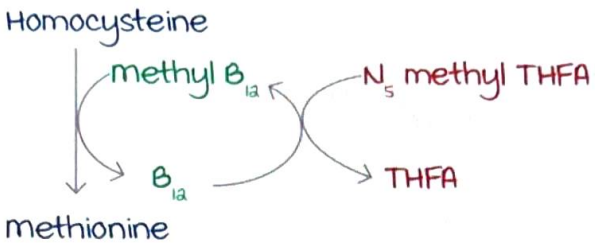
- Lysine on decarboxylation : Cadaverine
- Ornithine on decarboxylation : Putrescine
- Other polyamines : Spermine & spermidine (Precursors : Ornithine & methionine).

Active space

Fate of homocysteine

00:24:34

1. Regeneration of methionine :
   - THFA : Tetra Hydro Folic Acid



   - If there is 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

   - If there is deficiency of B_{12} /folic Acid,

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

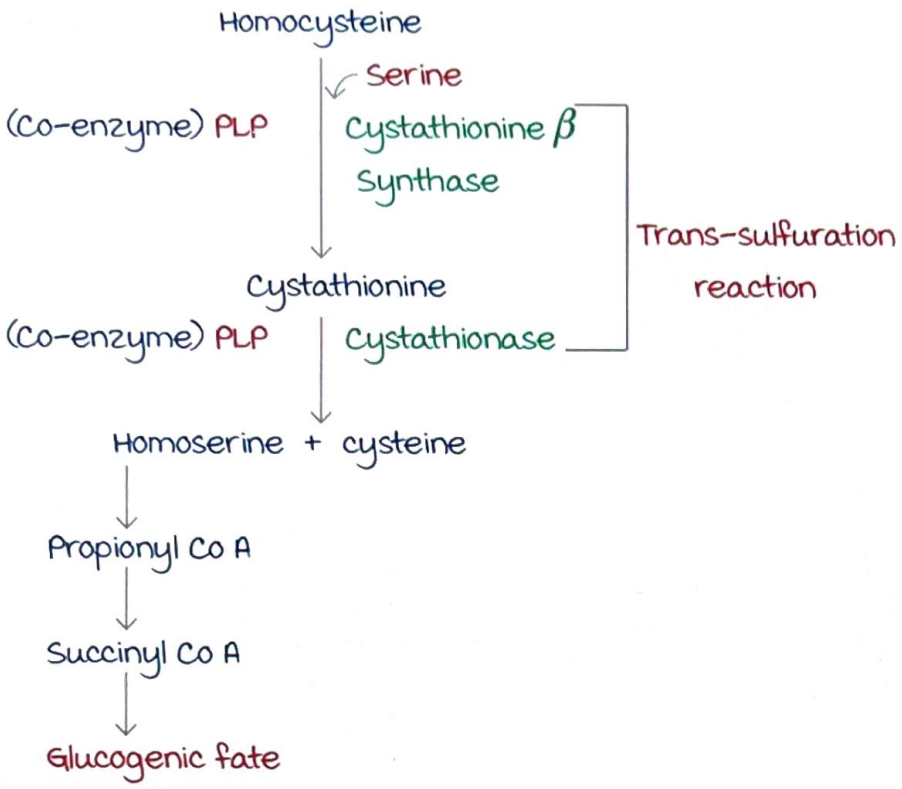
Deficiency of B_{12} / FA ⟶ ↑ ↑ Homocysteine
↓
Risk factor for thrombosis
↓
(CAD & CVA)
Coronary Artery Disease
Cerebrovascular Accidents

   - Deficiency of B_{12} and Folic Acid (FA) :
     ↑ Homocysteine in Blood;
     Homocystine (2 homocysteine joined by a disulphide bond) excreted in Urine.

2. Glucogenic fate :

methionine
↓

Active space



- Deficiency of B_6 :
  - ↑ Homocysteine in blood : CAD / CVA
  - ↑ Homocystine in urine

Biochemical disorders of Sulphur containing aminoacids

00:38:20

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

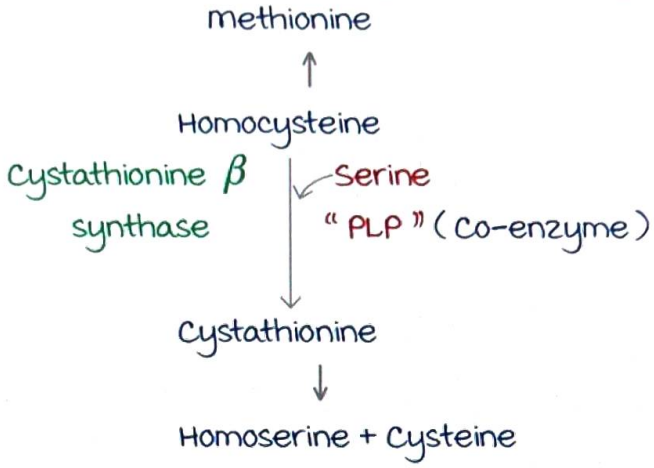
4. Cystathioninuria :
   - Defect : Cystathionase.

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

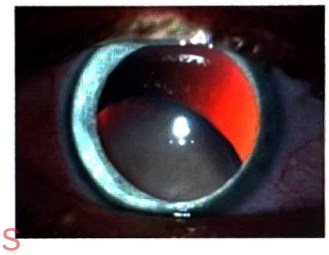
Active space

Classic homocystinuria : Defect and features 00:42:26

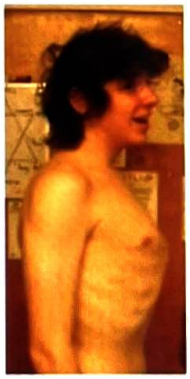
- Autosomal Recessive (AR).
- Biochemical defect : Defective cystathionine β synthase.

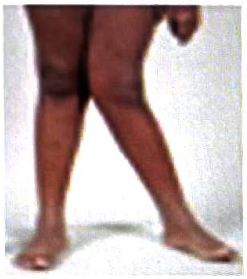


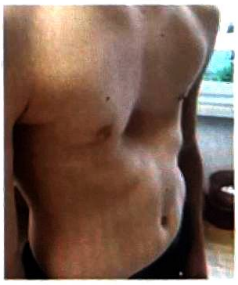
↑ Homocysteine in Blood.
↑ Homocystine in urine.
↓ ↓ Cysteine synthesis.
Methionine level : Normal.



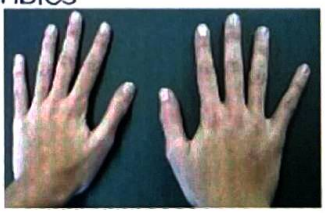
- Clinical features :
  Initially : Asymptomatic.
  Later, Developmental Delay.
  At 3 yrs of age : ↓ Vision, Progressive myopia, Quivering iris (iridodonesis). On Examination : Ectopia lentis.
  (Lens : Dislocated medially and downwards)
- Skeletal deformities (Arachnodactyly, Pectus carinatum Pectus excavatum).
- Severe mental retardation.
- Thromboembolism.
- Genu valgum/varum
- Coxa vara
- Pes cavus
- High arched palate







Homocystinuria resembles marfan's syndrome.

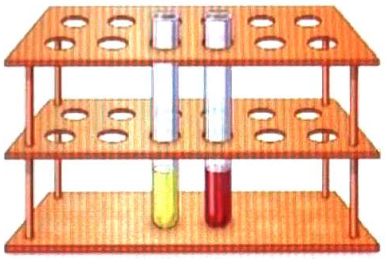


Classic Homocystinuria - management 00:52:12

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.



Cyanide nitroprusside test

2. Treatment :
   - High dose of vitamin B_6.
     Reason : Vitamin B_6 (PLP) → Coenzyme of cystathionine β synthase.
   - Restriction of methionine with cysteine supplementation.
     Reason : methionine is synthesized but cysteine is not.
   - Betaine supplementation.
     Reason : Trimethyl glycine (Betaine) → Remethylation of homocysteine

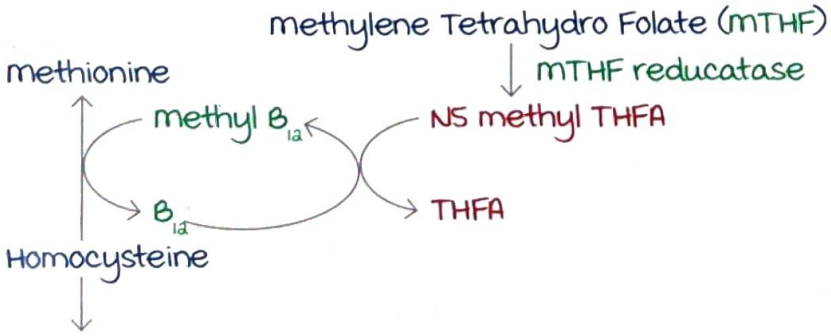
Homocysteine $\xrightarrow[\text{Betaine}]{CH3}$ methionine.

   - 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



Active space

Cystathionine

↓

Cysteine + homoserine

- Level of methionine ↓ ↓

  Cysteine : Normal

Homocystinuria - Comparison

00:58:48

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

01:00:09

1. Cystathioninuria :

   Defect : Cystathionase

   Cyanide nitroprusside test : Negative

2. Cystinuria :

   Defect : Dibasic AA transporter in intestine & renal tubules

 Hallmark of Garrod's tetrad : C – Cystinuria

A – Alkaptonuria

A – Albinism

P – Pentosuria

Excretion in urine : C – Cystine (not cysteine)

O – Ornithine

L – Lysine

A – Arginine

Cyanide nitroprusside test : Positive.

3. Cystinosis : Lysosomal storage disorders.

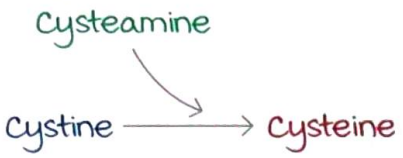
   Defect : Cystine transporter ⟶ Cystonosin (product of CTNS gene)

   (Lysosomal H^+ driven)

Active space

Affects : Liver → Hepatic failure.
Renal → Renal failure.
Cornea → Corneal opacity.
Bone marrow.

Treatment : Cysteamine.



Specialised products from cysteine 01:05:59

1. Cysteine on decarboxylation gives Betamercapto ethanolamine.
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) 01:06:56

- 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 :

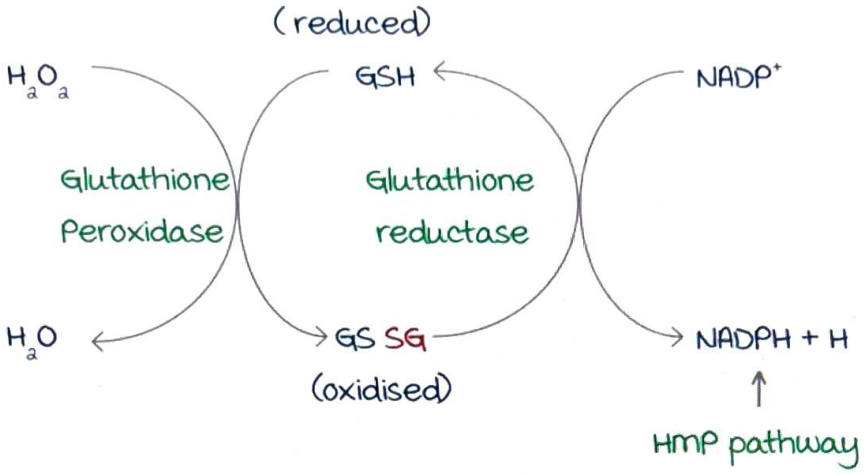
- Amino acid transport : meister's cycle/gammaglutamyl cycle.
- Free radical scavenging.
- Maintains RBC membrane integrity.
- Keeps Iron in ferrous state in hemoglobin.
- Antioxidant.
- Conjugation : In phase-II xenobiotic reaction.
- Acts as co-enzyme for various enzymes.

Active space

Glutathione (Co-enxyme)

Maleyl acetoacetate $\xrightarrow{\text{Cistrans isomerase}}$ Fumaryl acetoacetate

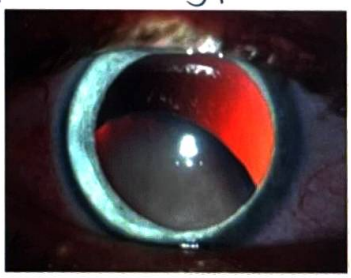
Free Radical Scavenging :



- Glutathione peroxidase :
  A Selenocysteine containing enzyme.
- Glutathione reductase :
  Flavin containing enzyme.
  Helps to assess B_2 level in Blood.

MCQs :

Question : In which IEM this clinical manifestation is seen ?
A Child is normal at birth, slight developmental delay present. After 3 yrs, he developed severe myopia, shivering of iris is seen on examination.



Ans : Classic homocystinuria.

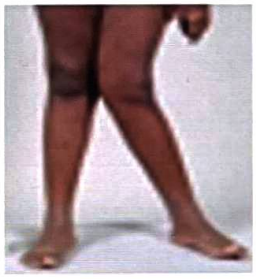
Question : A 14 year old boy developed sudden onset of weakness of left side of body with facial deviation to right side. He is admitted in neurology ICU with a suspected cerebrovascular accident .His left sided neurological weakness settled within a week.
The treating doctor noticed a waddling gait in the same boy. The mother gave a past history of defective vision due to dislocation of lens for which surgery was done. He has poor

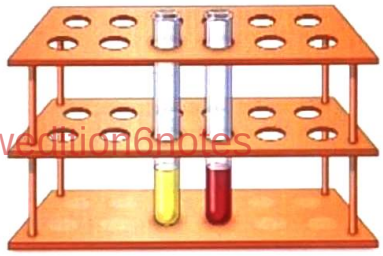
Active space

scholastic performance in school for which he is going to special school. Further studies confirmed that there is decreased mineralization of bone, increased length of long bones and slight curvature of spine to one side. What is the IEM suspected here ?

A. Cystinuria.
B. Homocystinuria.
C. Cystinosis.
D. Alkaptonuria.

Question : A 4 year old girl came to paediatric OPD with complaints of decreased vision and poor scholastic performance. O/E ectopia lentis and multiple skeletal deformities present. A test conducted is given in the picture. What is the diagnosis ?





Answer : Classic Homocystinuria.

Question : Identify the biochemical defect from the description given below.

- Homocysteinemia.
- Hypermethioninemia.
- Hypocysteinemia.

A. Methylene THFA Reductase defect.
B. Methionine Synthase defect.
C. Cystathionine beta synthase defect.
D. Methyl Cobalamine deficiency.

Question : Lysosomal storage disorder with CTNS gene defect is

Active space

A. Cystinosis.
B. Cystinuria.
C. Cysteinemia.
D. Homocystinuria.

Question : The amino acid with thioalcohol group
A. Methionine
B. Serine.
C. Cysteine.
D. Threonine.

Question : Vitamins needed in the metabolism of S containing amino acids are all except
A. PLP.
B. Folic acid.
C. Riboflavin.
D. Cobalamin.

Question : SAM is needed for formation of all except

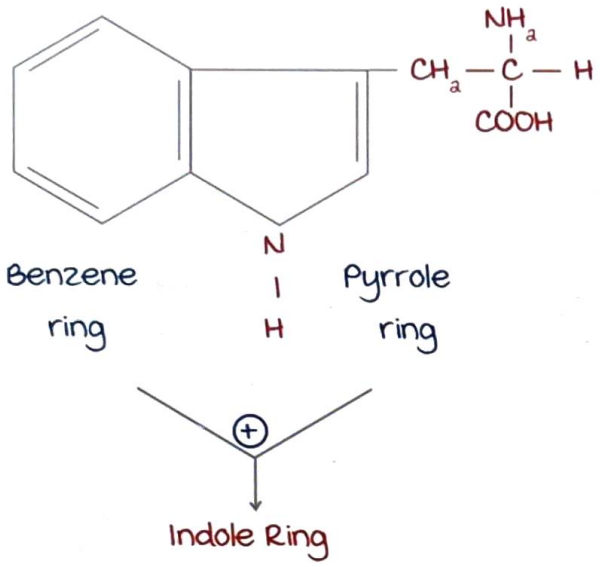
A. Melatonin.
B. Serotonin.
C. Epinephrine.
Creatine.

Question : All the following are functions of Glutathione except
A. Maintain integrity of RBC membrane.
B. Formation of methhemoglobin.
C. Transport of amino acids.
D. Free radical scavenging.

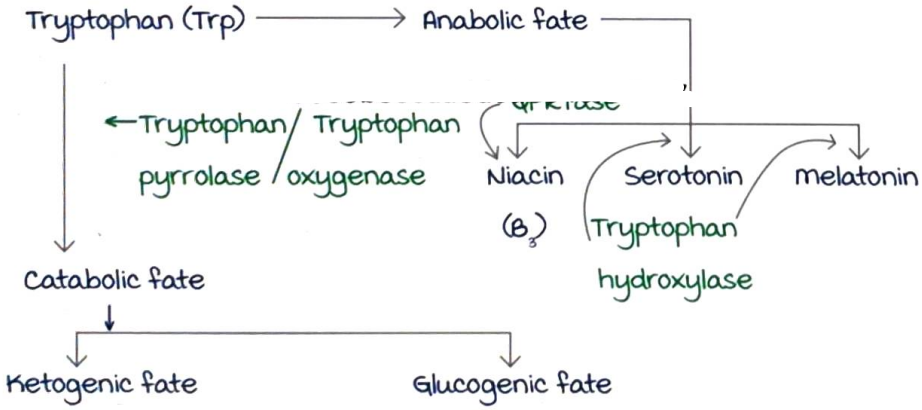
Active space

TRYPTOPHAN

Tryptophan : Chemistry 00:02:16

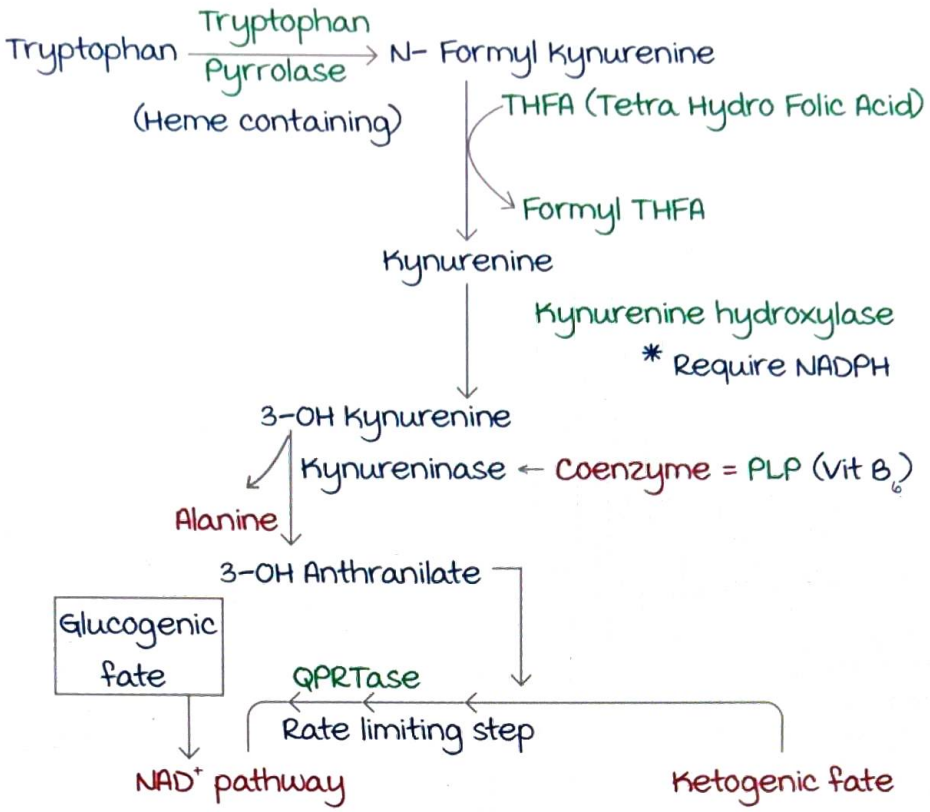


- Aromatic amino acid
- Essential amino acid
- Both Ketogenic & glucogenic



Tryptophan : Metabolism - catabolic fate 00:06:27

Catabolic fate : A/K/A Kynurenine anthranilate pathway



QPRTase - Quinolinate Phospho Ribosyl Transferase

- In B_6 deficiency → 3-OH Kynurenine

Xanthurenic acid

↓

Excreted in urine

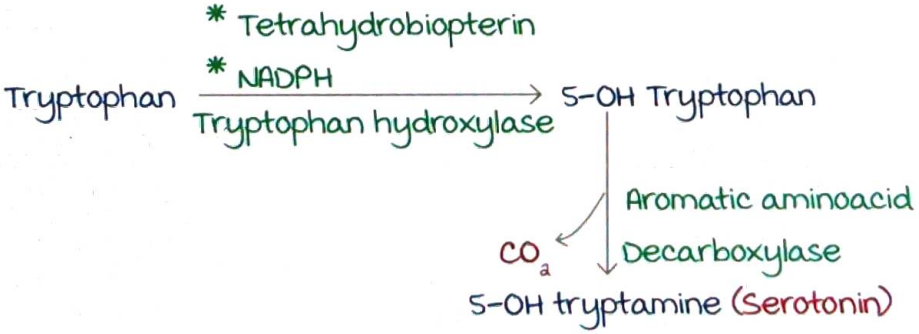
- ↓ Niacin → Pellagra like symptoms.

Conversion factor :

- 60 mg of tryptophan $\xrightarrow{\text{Converted}}$ 1 mg of niacin.

Tryptophan : Metabolism - anabolic fate 00:14:36

- Serotonin :



Active space

Degrades to 5-OH Indole acetic acid.

Functions of serotonin : • Neuro transmitter.

↓

Vasoconstriction, mood elevator, temperature regulation gastro intestinal tract motility.

Site of synthesis : Argentaffin cells.

↓

Intestine, mast cells, platelets and brain.

- Melatonin :

Synthesized in pineal gland.

Serotonin → N-acetyl serotonin

↓ SAM ← methionine

↓ 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.

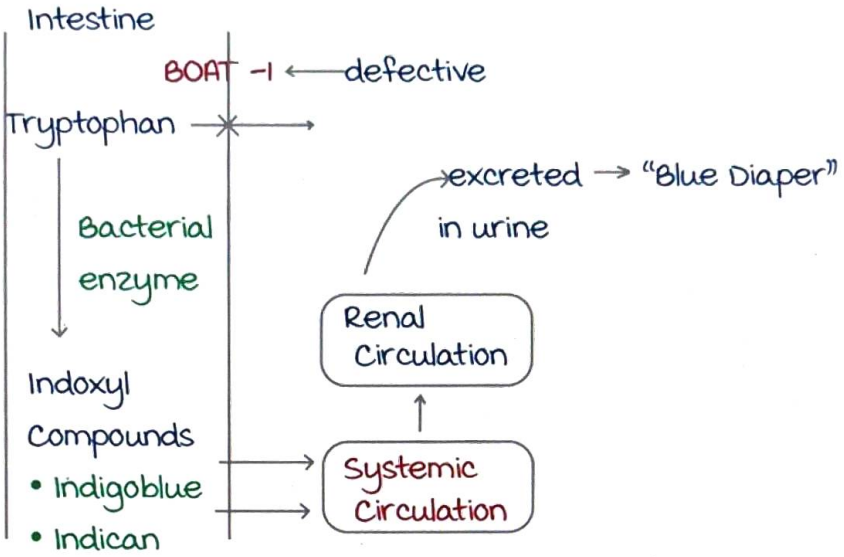
Diagnosis : ↑ Serum Serotonin.

↑ 5HIAA in 24 hr urine (N = < 5mg / day).

Hartnup's disease

00:25:45

Defect in absorption of tryptophan & other neutral amino acids from intestine & renal tubules.



• ↓ Tryptophan in cells → ↓ Serotonin and ↓ Niacin.

mc symptom : Cutaneous photosensitivity

(Photosensitive dermatitis due to niacin ↓).

Neurological manifestation (↓ serotonin) : Wide based gait, intermittent ataxia.

Diagnosis by 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 in intestine
  Blue diaper syndrome

BRANCHED CHAIN AMINO ACIDS

Chemistry of branched chain amino acids 00:01:59

Branched chain amino acids are :

Leucine : Ketogenic.

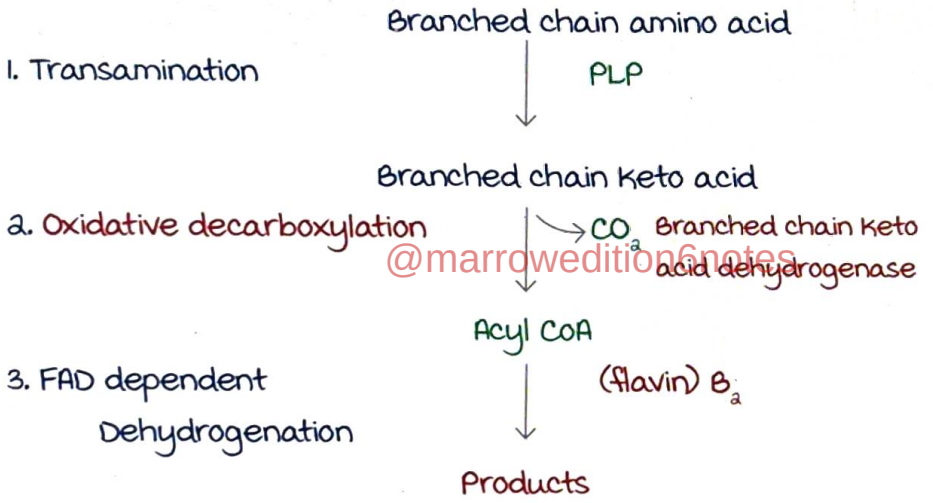
Isoleucine : Ketogenic and glucogenic.

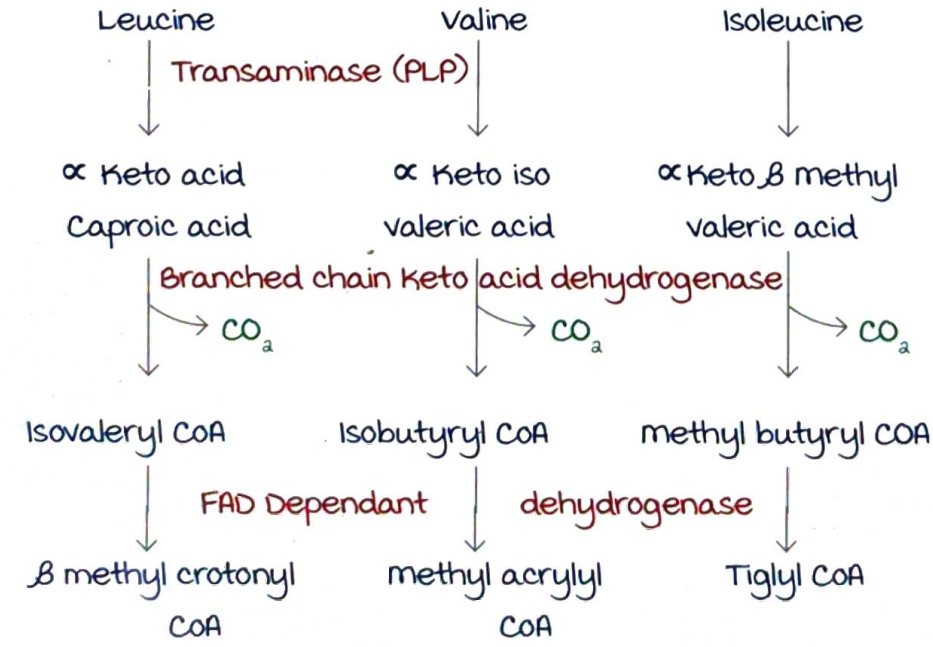
Valine : Glucogenic.

All are non-polar.

All are essential.

Metabolism of branched chain amino acids 00:04:03





Active space

β methyl crotonyl CoA → Ketogenic

Methyl acrylyl CoA → Propionyl CoA → Glucogenic

Tiglyl CoA → Acetyl CoA → Ketogenic

Branched chain keto acid dehydrogenase 00:09:10

- 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^+.

MSUD (Maple Syrup Urine Disease) 00:11:40

Defect in :

$E_1\alpha$: Type I A (m.c)
$E_1\beta$: Type I B
} Associated with thiamine pyrophosphate

E_2 : Type II
E_3 : Type III

Clinical correlation of MSUD :

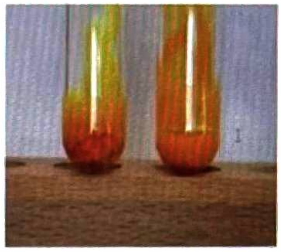
- Neonates.
- Biochemical defect :
  Branched chain Keto acid dehydrogenase.
  Decarboxylase enzyme (E_1 enzyme component).
  Defect in oxidative decarboxylation.
  Branched chain Keto acid ↑↑ (accumulates)
  ↓ × BCKD (defective)

Active space

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
↑ Branched chain Keto acid ┘ urine

Dinitro phenyl hydrazine test : Yellow colour precipitate.
Rothera's test : Purple ring.

Treatment :

Restrict branched chain amino acid.
Supplement thiamine.

Isovaleric aciduria 00:18:10

Defect in _____

Enzyme defect : Isovaleric acid dehydrogenase.

Smell of sweaty feet.

MCQs :

A 3 week old neonate presents with pure hypotonia, poor suckling reflex. An intern who examined the baby collected some urine and keep it in refrigerator for one night. The next day a sweety smell was appreciated in the urine sample. She did a DNPH test and got a positive result.

Ans : Maple Syrup Urine Disease.

2. An 11 day old neonate presented with vomiting, difficulty to feed, lethargy. The characteristic posture is seen. The

Active space

caramel odour is present. What is the diagnosis ?

A. Neonatal septicemia.

B. MSUD.

C. Isovaleric aciduria.

D. Organic aciduria.

E. Hyperammonemia.

3. Assertion : Thiamin is used in the treatment of MSUD.

Reason : Thiamin is coenzyme for the first step of catabolism of BCAA.

A. A & R are true, R is the correct explanation for A.

B. A & R are true but R is not the correct explanation for A.

C. A is true but R is false.

D. A is false & R is true.

Active space

ACIDIC AND BASIC AMINO ACIDS

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	most polar most basic	Polar
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

(∝ 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 00:11:40

Arginine

- metabolic functions : Synthesis of :

1. Agmatine → Antihypertensive.
2. Creatine → Glycine + Arginine + methionine.
3. Urea → Arginine $\xrightarrow{\text{Arginase}}$ Ornithine + Urea.
4. Ornithine.
5. Nitric oxide.

- Nitric oxide
  Endothelium Derived Relaxing Factor (EDRF)
  Free radical.
  Gaseous molecule.
  Short half life : 0.1 sec.
  Arginine $\xrightarrow[\text{Synthase}]{\text{Nitric oxide}}$ Nitric oxide + citrulline
  +
  NADPH
  Functions : Vasodilator.
  Penile erection.
  Neurotransmitter.

Treatment of :

1. Pulmonary hypertension.
2. Impotence (Sildenafil)

Active space

Sildenafil
↓
(Inhibits cGMP phosphodiesterase)
↓
↑ cGMP

3. Angina pectoris :
Glyceryl nitrite → Nitric oxide

- Nitric oxide synthase :
  Mono oxygenase
  5 cofactors : 1. Heme
  2. BH_4 (Tetrahydrobiopterin)
  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 & amides

00:21:28

Aspartic acid (4c) — Extra – COOH in side chain — Glutamic acid (5c)
Extra – $CONH_2$
Asparagine — Glutamine

Active space

- Chemical properties :

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

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 NH_3 → Renal regulation of Blood PH.)

   Enzyme required : Glutaminase enzyme

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

1 Aspartic Acid (Transmination)

Oxalo acetale ⟶ Aspartate

Glutamate → α Keto Glutarate

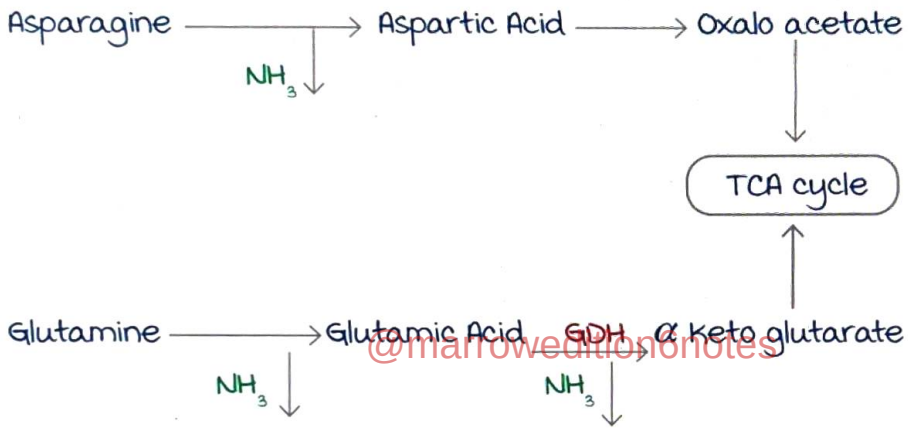
2. Glutamic Acid (Reductive amidation)

α Keto glutarate —GDH→ Glutamate

3. Aspartate —(Asparagine Synthetase)→ Asparagine

Glutamate —(Glutamine synthetase; NH_3 + ATP)→ Glutamine

Catabolism :



Canavan disease

00:35:10

A 21 month old boy, born without complications after an uncomplicated pregnancy, failed to achieve expected developmental miletsones. As an infant, he developed nystagmus and poor muscular head control.
Physical examination findings were notable for generalized hypotonia & progressive macrcephaly.

- Enzyme deficient : Aspartoacylase
  N Acetyl Aspartic Acid —X→ Aspartic Acid.
- Clinical features :
  Progressive macrocephaly.
  Persistent head lag .
  Developmental delay.
- On examination :
  Distorted mitochondria.

Active space

Severe leukodystrophy.
↑ N-acetyl aspartic acid in blood, CSF, urine.

Questions :

1. Fatty acids cannot be transported to mitochondria, if lysine is deficient. Why ?
Ans : Because carnitine, which is the transporter of fatty acids, is derived from lysine.

2. Histones are positively charged. Why?
Ans : As they are rich in basic amino acids like arginine & glycine.

3. Sildenafil is used in the treatment of impotence?
Ans : Inhibits cyclic GMP phosphodiesterase and thereby increases cyclic GMP (2nd messenger of nitric oxide (derived from arginine)).

4. FIGLU is used to assess folic acid vitamin deficiency. Why?

Ans : Folic acid is an acceptor of formamine group. SO when there is folic acid deficiency, FIGLE gets excreted in urine.

MCQs.

1. The amino acids that form oxaloacetate are
A. Asparagine & Glutamate.
B. Asparagine & Aspartate.
C. Glutamate & Glutamine.
D. Glutamine & Aspartate.

2. An infant normal at birth later develops progressive macrocephaly, severe hypotonia, and persistent head lag. MRI scanning of head revealed striking vacuolization and astrocytic swelling in white matter. N acetyl aspartic acid present in the blood & CSF. What is the diagnosis ?
A. Alexander's disease.
B. Reye's syndrome.
C. Canavan disease.
D. Tay sach's disease.

Active space

E. Sandhoff's disease

3. The nitrogen donor for asparagine synthetase is

A. Ammonium ion.

B. Glutamine.

C. Aspartate.

D. Glutamate.

4. The donor of formimino group in one carbon metabolism is

A. Serine.

B. Tryptophan.

C. Histidine.

D. Arginine.

5. Urocanate is an intermediate in which amino acid metabolism?

A. Proline.

B. Histidine.

C. Threonine.

D. Arginine.

6. Ornithine is derived from which amino acid?

A. Proline.

B. Histidine.

C. Threonine.

D. Arginine.

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 also 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.
- β mercaptoethanolamine → Cysteine on decarboxylation.
- GABA → Glutamate on decarboxylation.

- Carnosine → β alanyl Histidine.
- Anserine → Carnosine on methylation.
- Homocarnosine → GABA + Histidine.

Urine odour in various inborn erros of metabolism

00:13:50

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

00:18:14

- 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).

Q. The amino acids that form Oxaloacetate are :

A. Asparagine and Glutamate.

B. Asparagine and Aspartate.

C. Glutamate and Glutamine.

D. Glutamine and Aspartate.

Active space

KREB'S CYCLE

Concept of TCA Cycle

00:01:22

It is A/K/A TCA cycle (Tricarboxylic acid)/citric acid cycle/ Kreb's cycle..

Carbohydrate, proteins, lipids

↓

Acetyl CoA [2C] : Completely oxidized

↓ Enters

TCA cycle releases : 2 C atom as CO_2

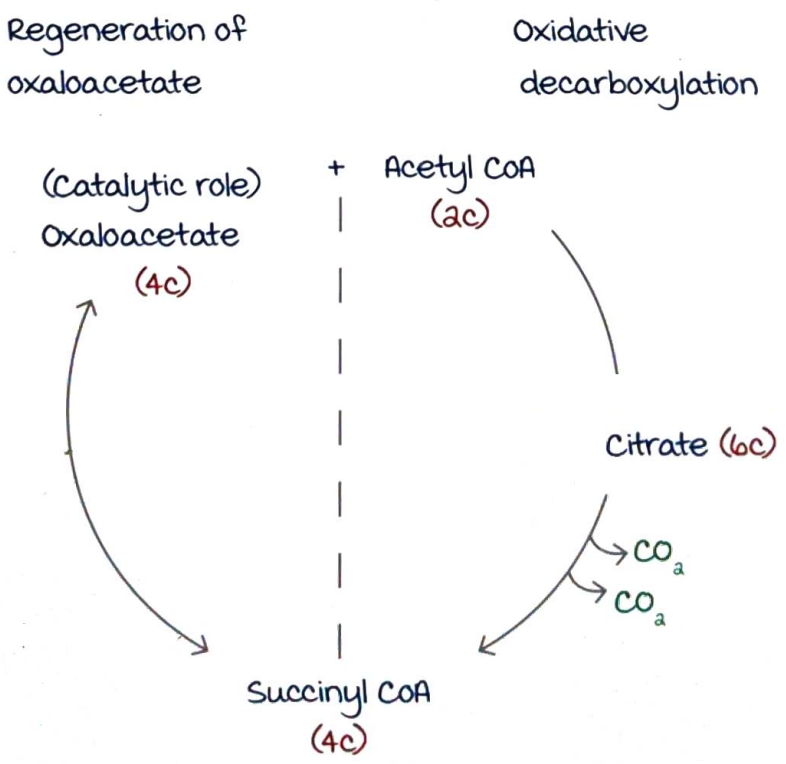
$NADH, FADH_2$ → enters ETC to produce ATP.

Site : All organs with mitochondria.

All enzymes of the TCA cycle are present in the mitochondrial matrix except succinate dehydrogenase.

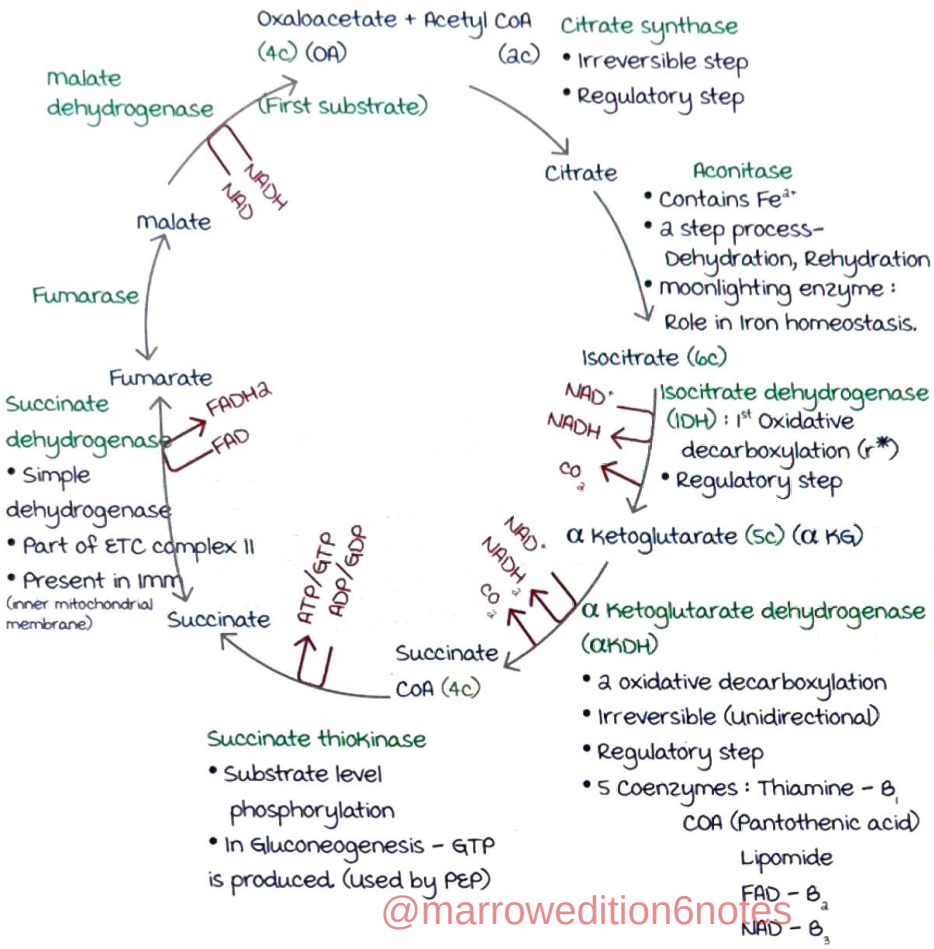
Overview of The TCA Cycle

00:06:24



Active space

Reactions of TCA cycle 00:08:07



Inhibitors and energetics of TCA cycle 00:22:51

Inhibitors of TCA cycle :

Non competitive [-]

Fluoroacetate + OA = Fluorocitrate ⟶ aconitase.

Arsenite : Non-competitive inhibition of αKDH.

Malonate : Competitive inhibition of SDH.

Energetics of TCA cycle :

3NADH = 3 × 2.5 ATP = 7.5
1 FADH2 = 1 × 1.5 ATP = 1.5
} Oxidative phosphorylation
Total = 9 ATP

1 ATP (substrate level phosphorylation).

Total = 9 + 1 = 10.

Significance of TCA cycle 00:26:12

Complete oxidation of acetyl CoA : Final common oxidative

Active space

pathway.

It is an amphibolic pathway [both catabolic and anabolic].

Catabolic : Acetyl CoA oxidized

Anabolic : Citrate enters fatty acid synthesis

α KG → glutamate → GABA

Succinyl CoA → Heme

Oxaloacetate → Glucose

Anapleurotic reactions :

It is a filling-up reaction.

Reactions that replenish depleted intermediates of the TCA cycle.

Most important reaction : Pyruvate —(Pyruvate carboxylase)→ oxaloacetate.

Amino acids entering TCA cycle :

Glutamine, Histidine, Arginine, Proline ↓ Glutamate ↓ αKG	Methionine, Threonine, Isoleucine, Valine ↓ Succinyl CoA	Phenylalanine, Tyrosine ↓ Fumarate

TCA cycle & cancer – Oncometabolism 00:33:35

Normal : Isocitrate —(IDH)→ αKG → succinyl CoA

If IDH mutant : αKG → 2 hydroxyglutarate (oncometabolite)

↓

Inhibits TET 2 gene (epigenetic modification)

↓

Increased DNA methylation & histone modification

↓

Changes the epigenome

Cancers related to mutant 2 hydroxyglutarate :

- Cholangiocarcinoma, glioma.
- Acute myeloid leukemia, various sarcomas.

Drugs inhibiting mutant IDH : Sidenibs
[Evasidenibs, Ivosidenibs].

Mutation in SDH complex (B & D) causes :
Familial glioblastoma.
Familial pheochromocytoma.

Vitamins in TCA cycle :

- CoA : Pantothenic acid
- FAD : B_2
- NAD+ : B_3
- αKGDH : B_1

Regulation of TCA cycle :
Regulatory steps :

1. Citrate synthase.
2. Isocitrate Dehydrogenase.
3. aKG Dehydrogenase.
4. Pyruvate Dehydrogenase. (Especially in brain).

↑ATP/ADP & ↑ NADH/NAD ratio : Inhibits TCA cycle regulatory enzymes.

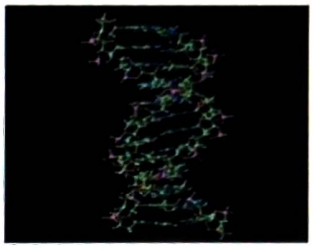
Active space

CHEMISTRY OF NUCLEOTIDES

Chemistry of nucleic acid

00:01:25

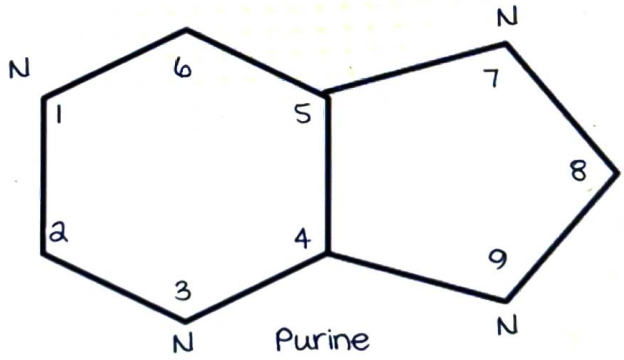
Two types of nucleic acid : DNA and RNA.
The nucleic acids are made up of nucleotides.
The nucleotide is made up of :
nitrogenous base (purines & pyrimidines)
Pentose sugar [ribose, deoxyribose]
Phosphate group.
nitrogenous base + pentose sugar=
Neucleoside.



Purine bases

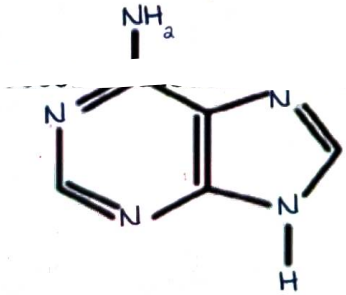
00:03:30

2 heterocyclic ring structures with nitrogen positions at 1,3,7,9.
Purines : Adenine and guanine.
Minor purines : xanthine, hypoxanthine, and uric acid.



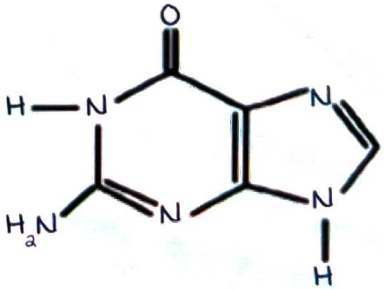
Purine

6 amino purine



Adenine (A)

2 amino 6 oxopurine



Guanine (G)

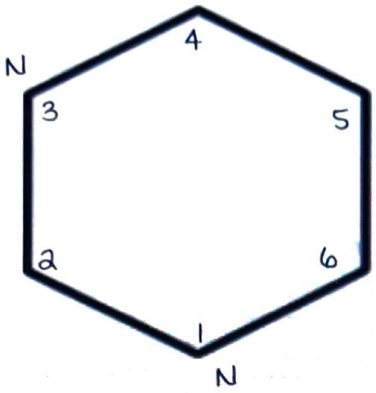
Active space

Pyrimidines :

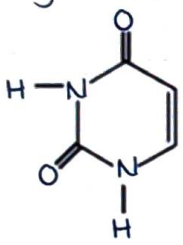
Single heterocyclic ring structure with nitrogen positions at 1,3.

Pyrimidines :

Uracil [RNA only], thymine [DNA only], Cytosine.

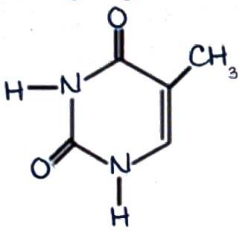


2,4 dioxo Pyrimidine



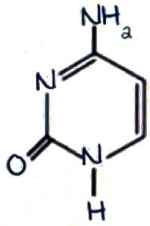
Uracil (U)
RNA only

2, 4 dioxo 5 methyl pyrimidine



Thymine (T)
DNA only

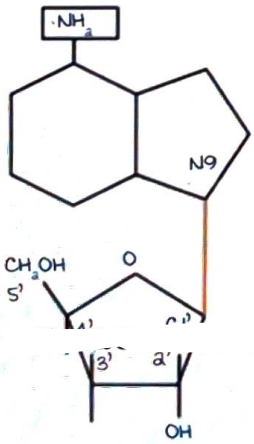
2 oxo 4 amino pyrimidine



Cytosine (C)
both DNA ana RNA

Nucleoside formation

00:10:29



Purine:

β N glycosidic bond between C 1' of pentose sugar & N 9 of purine.

C 1 of pentose sugar & N 1 of pyrimidine.

Pentose sugar.

If OH group is present at 2', 3' position- ribose.

If OH group is present only in 3' position- deoxy ribose.

Active space

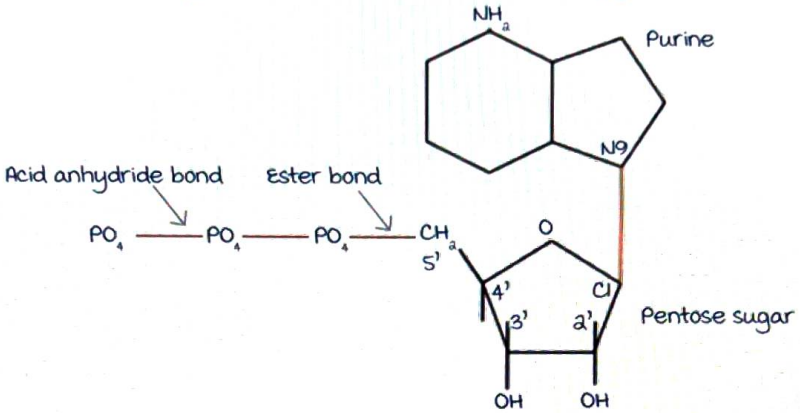
Nucleotide formation

00:14:12

Addition of PO4 group by ester bond to the 5' C atom : Nucleoside monophosphate.

Further PO4 groups are attached by acid anhydride bond.

If an acid anhydride bond is broken, energy is released.



Dinucleotide formation

00:16:37

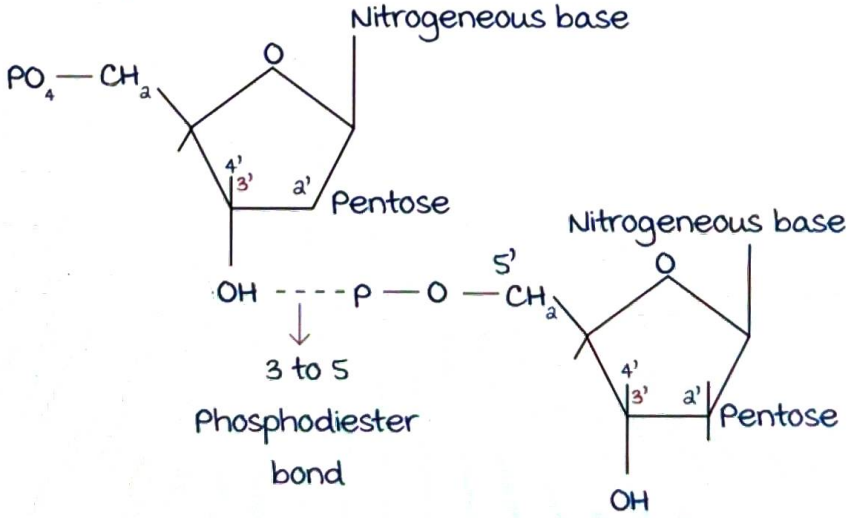
The two nucleotides are joined by a 3' to 5' phosphodiester bond : Between the 3' OH group to the 5' PO4 group.

The nucleotide with a free functional group at 5' position : First nucleotide [head end].

The nucleotide with the free functional group at 3' position : Last nucleotide [tail end].

These functional groups are ionizable – exhibit Polarity.

By convention, ~~the base sequence~~ of nucleic acid is written from 5' to 3'.



Active space

Ribonucleotide and deoxy Ribonucleotide 00:26:49

Nitrogenous base	Nucleoside	Ribose nucleoside monophosphate	Deoxy nucleoside monophosphate
Adenine	Adenosine	Adenosine monophosphate	Deoxyadenosine monophosphate
Guanine	Guanosine	Guanosine monophosphate	Deoxy Guanosine monophosphate
Uracil	Uridine	Uridine monophosphate	Deoxy Uridine monophosphate
Hypoxanthine	Inosine	Inosine monophosphate	Deoxy Inosine monophosphate
Xanthine	Xantho-sine	Xanthosine monophosphate	Deoxy Xanthosine monophosphate
Cytosine	Cytidine	Cytidine monophosphate	DeoxyCytidine monophosphate

ELECTRON TRANSPORT CHAIN

Basics of ETC

00:01:28

Oxidation : Loss of electrons

Reduction : Gain of electrons

Redox couple : Compounds that can exist in oxidised as well as reduced state

Eg : NAD^+ /NADH, FAD/$FADH_2$, FMN/$FMNH_2$

Redox potential : Ability to transfer electron/gain electron.

Greater the redox potential greater is the ability to gain electrons.

Electron moving from lower to higher redox potential :

Exothermic reaction

Components of ETC

00:04:40

ETC is a series of redox couples arranged (inner mitochondrial membrane) in ascending order of redox potentials.

Components :

Complex I : NADH-CoQ oxidoreductase

- Oxidises NADH, reduces coenzyme Q.
- Components : FMN & FeS

Coenzyme Q/ubiquinone/Q_{10}

- Mobile electron carrier

Complex II : Succinate Q oxidoreductase

- Oxidises Succinate, reduces coenzyme Q.
- Components : FAD/$FADH_2$
  Fe-S complex.

Complex III : Q-Cyt c Oxidoreductase

- Components : Cyt b, Cyt c_1
  Rieske Fe-S complex.

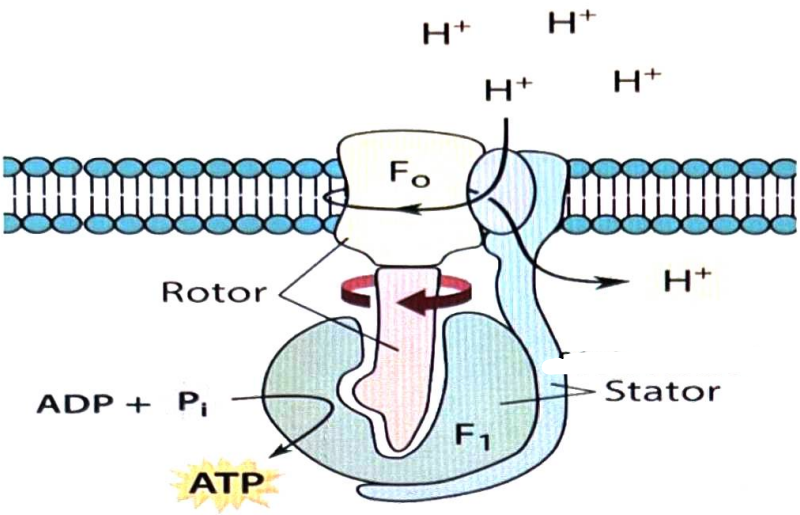
Complex IV : Cyt c Oxidase

- Irreversible complex
- Components : Heme a, a_3 / Cyt a, a_3
  Cu A-Cu B centre

Active space

Complex V

00:17:12



Also known as ATP synthase complex.

F_o subunit :

- Spans the inner mitochondrial membrane.
- Made of 10 C disc proteins.
- Hydrophobic
- Proton channel

F_1 subunit :

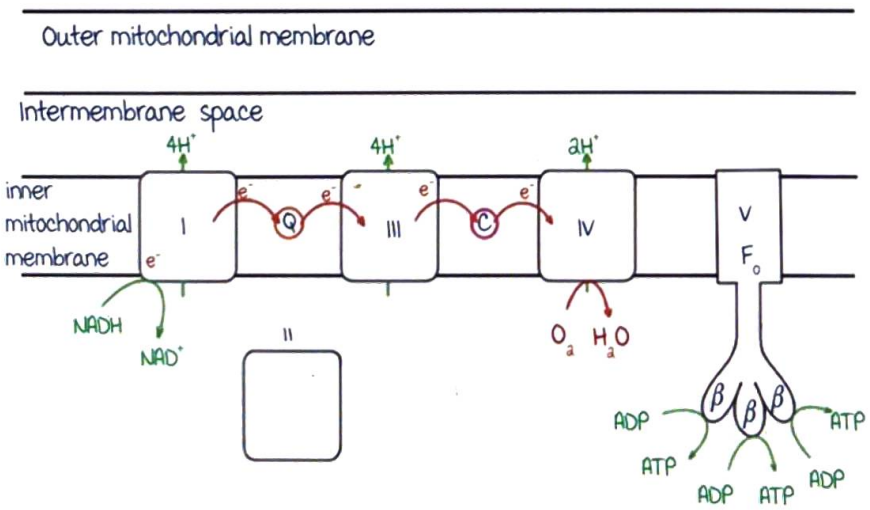
Made of 9 subunits : 3 α

3 β : ATP synthesising unit.

Υ (mobile), δ and ε

Oxidative phosphorylation

00:21:17



Active space

Oxidation :

- Movement of electrons, leads to release of free energy.
- Pump H^+ to intermembrane space.

Phosphorylation :

1. A proton motive force is created (difference in potential due to pumping of protons).
2. H^+ moves to mitochondrial matrix through F_o subunit.
3. Rotation of γ subunit.
4. Conformational change in β subunit (Binding change mechanism by Paul Boyer) : ADP → ATP.

Oxidation is coupled with Phosphorylation by a proton gradient.

NADH pumps 10 H^+

$FADH_a$ pumps 6 H^+

NADH generates 2.5 ATP (P:O ratio = 2.5)

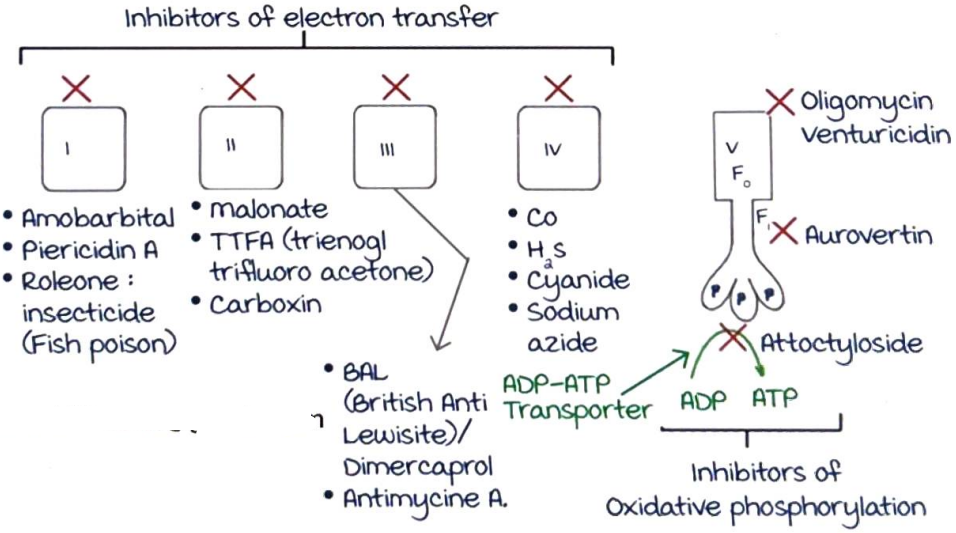
$FADH_a$ generates 1.5 ATP (P:O ratio = 1.5)

This is explained by the chemiosmotic theory by Peter Mitchell.

Inhibitors of respiratory chain

00:29:11

Inhibitors of electron transfer & oxidative phosphorylation :



Chemical uncouplers :

- Dinitrophenol (DNP)
- Dinitrocresol (DNC)
- Fluoro carbonyl cyanide phenylhydrazone (FCCP)
- Aspirin in high dose

Physiological uncouplers :

- Thermogenin/uncoupling protein-1 (UCP-1) : Non shivering thermogenesis :
  Found in brown adipose tissue (chubby cheeks in neonates). There is oxidation in the tissue but no phosphorylation (action of thermogenin). Movement of electrons leads to heat release. This is the principle behind prevention of hypothermia in newborns.
- Thyroxin
- Long chain fatty acid
- Unconjugated bilirubin

High energy phosphates 00:37:59

Produce free energy > 7 KCal.

Examples : Phosphoenol pyruvate.
Carbamoyl phosphate.
1,3 BPG
Creatine phosphate
ATP → ADP + Pi
ATP → AMP + PPi

METABOLISM OF NUCLEOTIDES

Purine metabolism

00:00:57

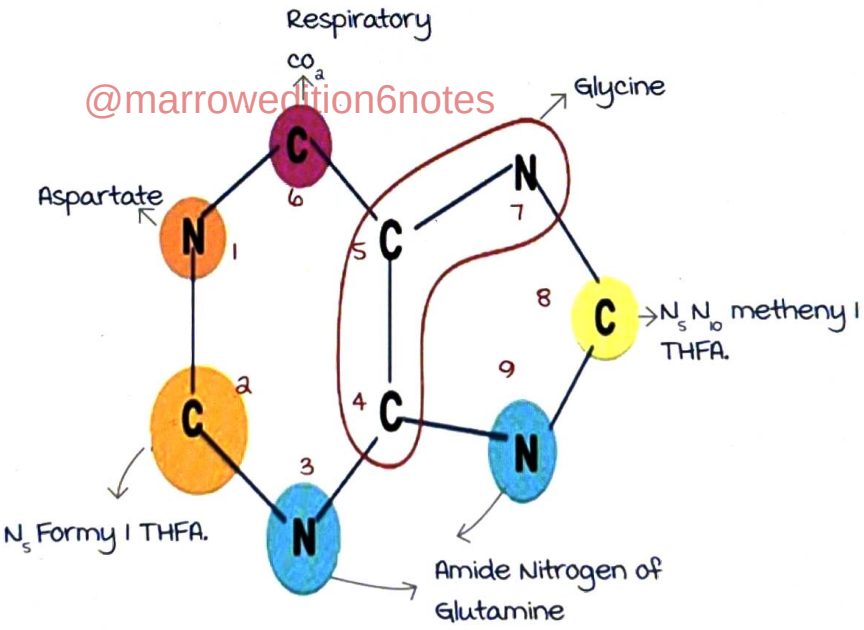
De Novo Purine synthesis :

Occurs in most of the tissues (cytoplasm) but mainly in the liver.

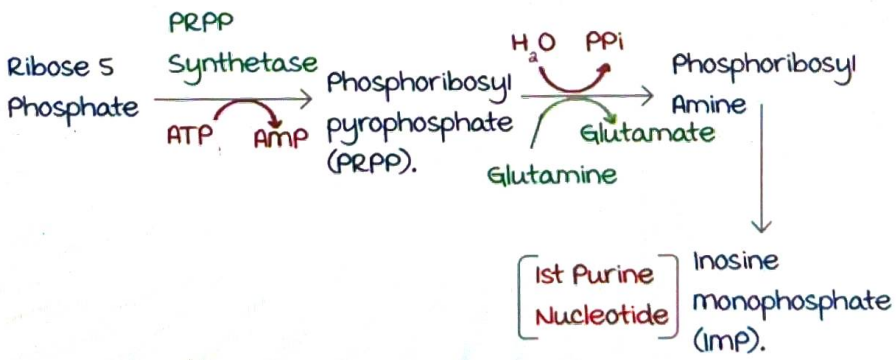
De Novo synthesis doesn't take place in : Brain.
Erythrocyte.
Leukocyte.
Bone marrow.

They purely depend on the Salvage pathway.

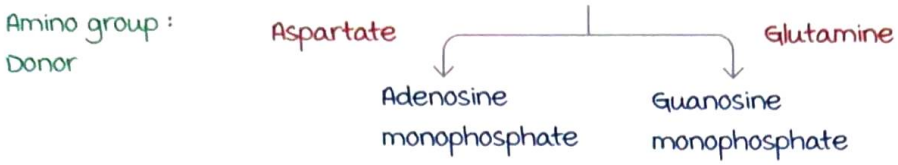
Contributors to Purine ring : The folic acid component in the purine ring plays an important role in cancer treatment and its deficiency can cause defective DNA synthesis.



Steps of De Novo Purine synthesis :



Active space

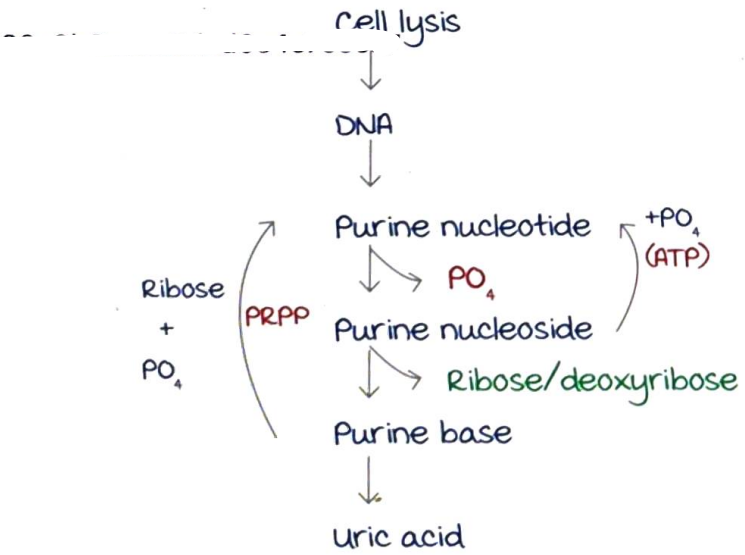


- PRPP Synthetase : Preliminary step
  Regulatory step
- PRPP can go into different pathways :
  Salvage pathway.
  NAD^+ synthesis.
  Pyrimidine synthesis.
- PRPP Glutamyl Aminotransferase : Committed step of purine synthesis
  Regulatory step
  Rate limiting step

Salvage pathway

00:11:13

Purine nucleoside and Purine bases are recycled to Purine nucleotides :



Features :

1. Saves energy.
2. Effective recycling.
3. Important in organs without De Novo Purine synthesis.

Donor for Ribose and PO_4 : PRPP. The reaction is called Phosphoribosylation.

Active space

Donor for PO_4 : ATP. The reaction is called Phosphorylation.

Reactions :

1. Phosphoribosylation $\xrightarrow{HGPRTase}$ Phosphoribosyl transferase

   Pu $\longrightarrow$ PuRP

   HGPRTase : Hypoxanthine Guanine PRTase.

   a. Hypoxanthine/ Guanine $\xrightarrow[HGPRTase]{PRPP \quad PPi}$ Inosine monophosphate/ Guanosine monophosphate.

   b. Adenine $\xrightarrow[PRPP \quad PPi]{APRTase}$ Adenosine monophosphate

   APRTase : Adenine Phosphoribosyl transferase.

2. Phosphorylation $\xrightarrow[ATP \quad ADP]{}$ Kinase

Adenosine $\xrightarrow{Adenosine\ Kinase}$ Adenosine monophosphate

Purine catabolism

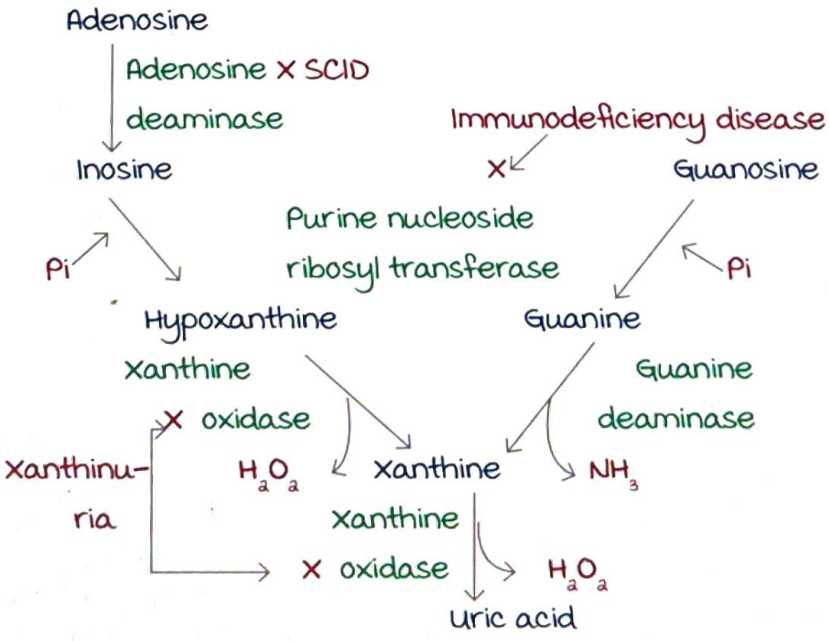
00:20:05

Site : Dietary Purine degraded in : Intestine.

Endogenous are degraded in : Liver.

End product : Uric acid. Organelle : Cytoplasm.

Steps of purine catabolism :



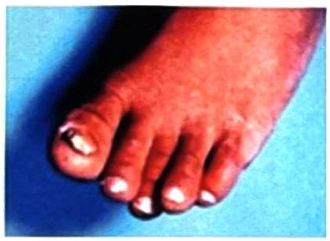
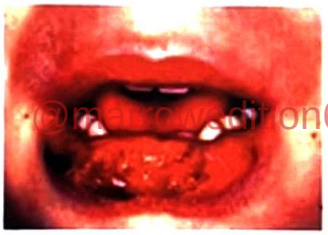
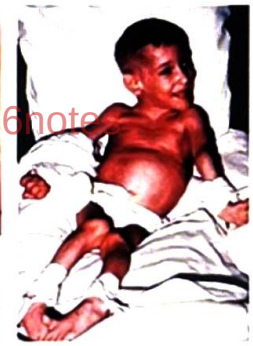
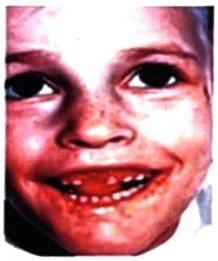
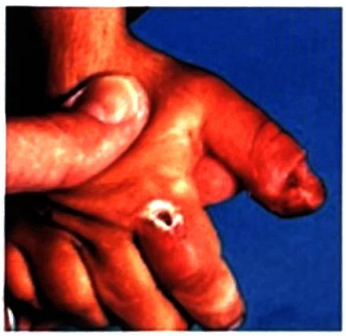
Active space

- SCID (Severe combined immunodeficiency defect) :
  Adenosine deaminase defect.
  Both B-cells & T-cells are affected.
- Immunodeficiency disease :
  Purine nucleoside ribosyl transferase defect.
  T-cells are affected & B-cells are normal.
- Xanthinuria :
  Xanthine oxidase defect.
  Xanthine crystals are seen with Hypouricemia.
  Xanthine oxidase requires Molybdenum.
  Allopurinol inhibits Xanthine oxidase.

Disorders associated with Purine metabolism 00:25:46

HGPRTase deficiency :

1. Lesch Nyhan syndrome :

X linked recessive disorder.

Biochemical defect : HGPRTase completely deficient.

Hypoxanthine/ Guanine $\xrightarrow{X}$ IMP/ GMP.

Purines accumulate ⟶ Increased uric acid (Hyperuricemia)

Clinical features :

- Compulsive self-mutilation.
- Gout.

Active space

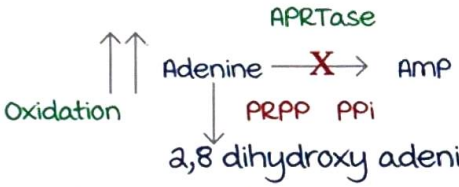
- Dystonic movements.
- Megaloblastic anemia.
- Neurological deficit.

Treatment :

- Allopurinol (Inhibits Xanthine oxidase).
- High fluid diet.
- Alkalinize urine.

2. Kelley-Seegmiller syndrome :
   HGPRTase is partially deficient.
   > 1.5-2% activity +

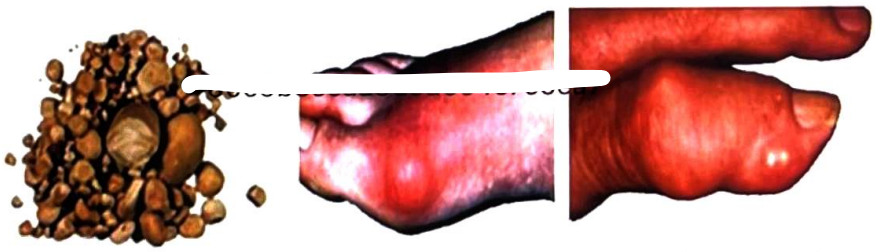
   APRTase deficiency :



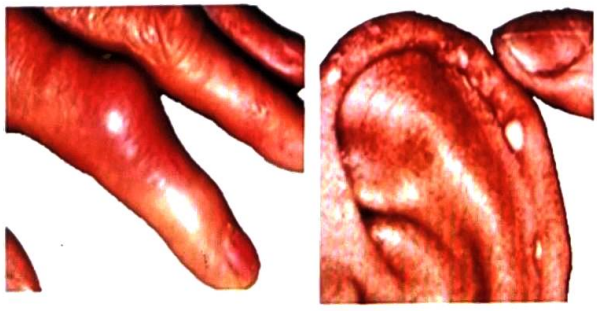
2,8 dihydroxy adenine causes : Severe crystalluria causing brown spots in the diaper.

Gout

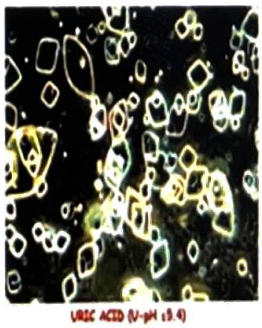
00:31:36



Uric acid crystals | 1st metatarsophalangeal joint | Chronic gout



Tophi : monosodium urate crystals in subcutaneous tissue



Negatively birefringent uric acid crystals (Seen in synovial fluid)

Active space

Biochemical defect :

- In primary gout

  Enzyme defect :

  ↑ Activity of PRPP synthesis → Purine → ↑ Uric acid

  ↑ Activity of PRPP Glutamyl amido transferase

Causes : Lesch Nyhan syndrome.

Glucose-6-phosphatase deficiency : Von Gierke's disease (Type I GSD)

- In secondary gout

↑ Cell turnover → Malignancy

Decreased excretion of Uric acid. Causes :

- Lactic acidosis
- Renal failure
- Diuretics (Thiazides)

Aggravating factors of gout :

- Alcoholism.
- Increased Purine intake (meat).
- Junk food : Fructose rich → Hyperuricemia

Symptoms of Gout :

Acute

- Acute inflammatory arthritis
- Uric acid nephrolithiasis

Chronic

- Tophi

Diagnosis of Gout : Aspiration & examination of synovial fluid. most commonly affects the first metatarsophalangeal joint because the acid crystalizes commonly at the cold peripheries.

Treatment :

- Allopurinol.
- High fluid diet.
- Alkalinization of urine.
- Anti-inflammatory drugs.
- Uricosuric drugs (Probenecid).
- Prevent aggravating factors.

SCID & Severe T-cell defect 00:40:25

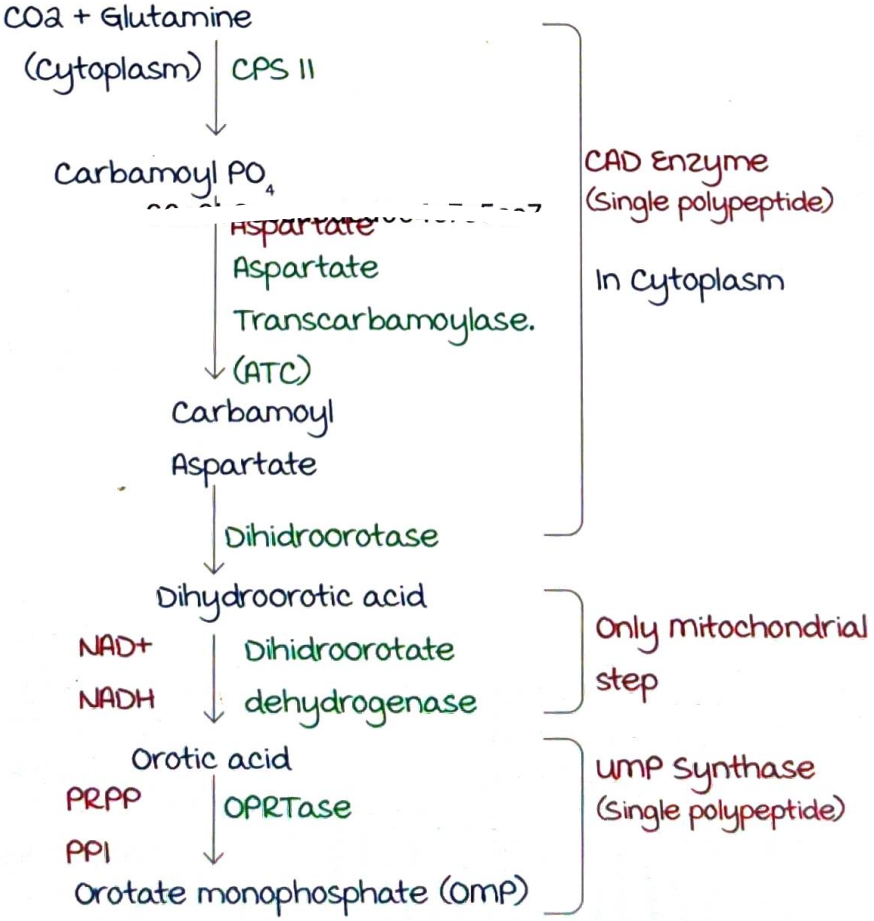
SCID : Severe combined immunodeficiency.
Defective enzyme : Adenosine deaminase.
Most common cause : Defect in gamma chain of Ig.
Non-homologous end joining (NHEJ) (defective DNA repair) is also a cause.
Both B-cells and T-cells are affected.
The first disorder to be treated by gene therapy (by Dr. French Anderson : Father of gene therapy).
Enzyme Replacement Therapy (ERT) : Polyethylene glycol modified ADA is also a treatment modality for SCID.

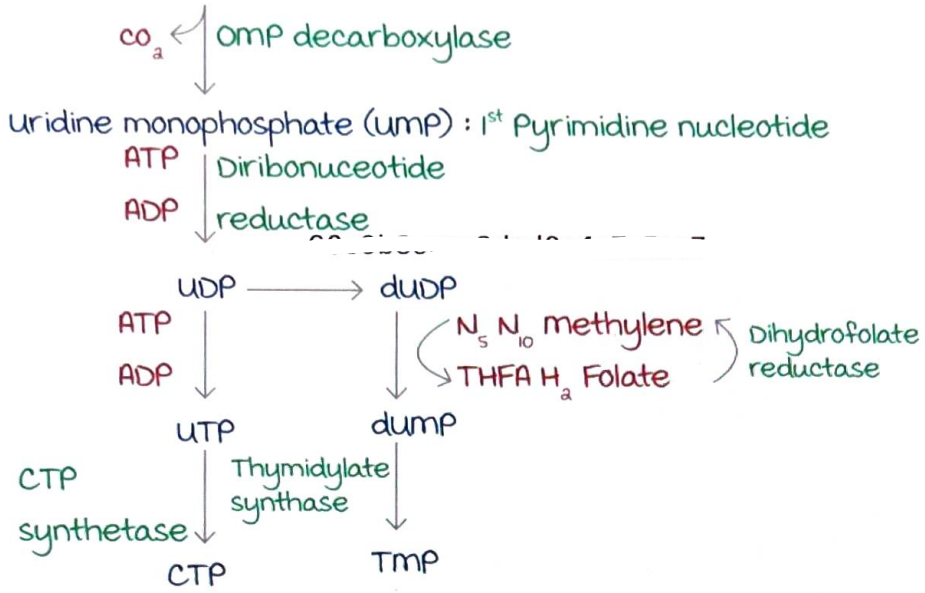
Purine nucleoside phosphorylase defect :
Severe T-cell defect and B-cells are apparently normal.

Pyrimidine metabolism 00:43:27

Site : Liver

Organelle : Cytoplasm and mitochondria.





5-Fluorouracil inhibits Thymidylate synthase.

Methotrexate inhibits Dihydrofolate reductase.

Orotic aciduria

00:54:04

Defect in the De Novo synthetic pathway of pyrimidines.

Autosomal recessive.

Type I Orotic aciduria : Defect in bifunctional enzyme UMP synthase.

Decreased UMP synthase leads to decrease in CTP & TNP.

Resulting in decreased pyramidines → decreased DNA synthesis → macrocytic anemia.

Type II Orotic aciduria : Defect in OMP decarboxylase only.

End products of Pyrimidine catabolism :

Cytosine Uracil Thymine

β – Alanine β – Amino isobutyrate] Water soluble

Therefore they do not crystallize and no symptoms seen.

Conditions causing Orotic aciduria :

- Type II hyperammonemia :

OTC → ↑ Carbamoyl PO_4 (mitochondria)

CP (Cytoplasm) → Pyrimidine synthesis

Orotic acid

Active space

- Reye's syndrome :
  Defect in mitochondrial enzymes (Urea cycle enzymes).
  Increased CP leads to orotic aciduria.

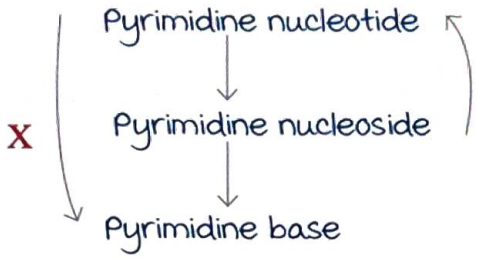
Pseudouridine :

It is found in the pseudouridine arm of tRNA.

Uridine = Uracil + Ribose sugar

Pseudouridine = C_5Pyr + C_1' Pentose sugar.

Pyrimidine Salvage pathway :



Case :

A 1 month old child presented with failure to thrive, macrocytic hypochromic anemia. Bone marrow examination showed megaloblast. No response to Vitamin B12 or Folic Acid or Iron. What is your diagnosis?

Ans. Orotic Aciduria.

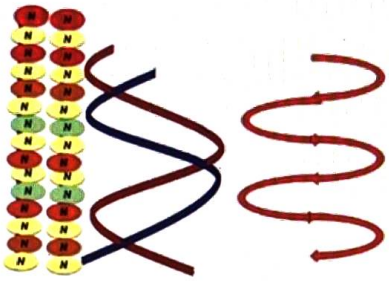
STRUCTURE AND ORGANISATION OF DNA

Introduction

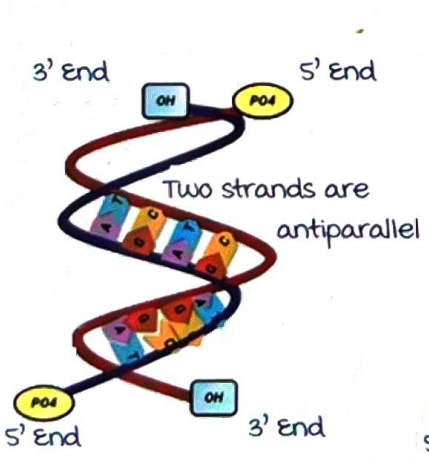
00:00:19

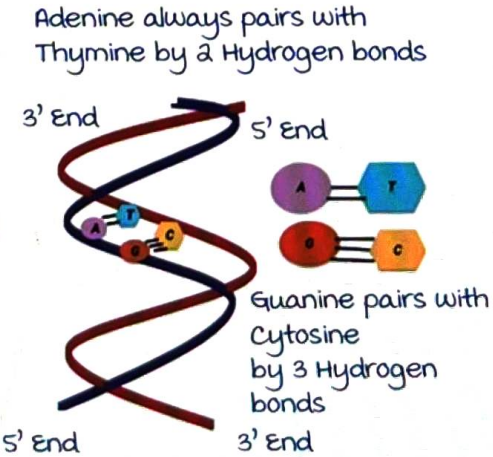
Dark lady of DNA : Rosalind Franklin.
Structure of DNA is called as 'Watson & Crick model of DNA', discovered in 1953, for which Watson & Crick was awarded Nobel prize in the year 1962.
Salient features of structure of DNA :

Two Polydeoxy ribonucleotide strand twisted around each other in right handed direction.



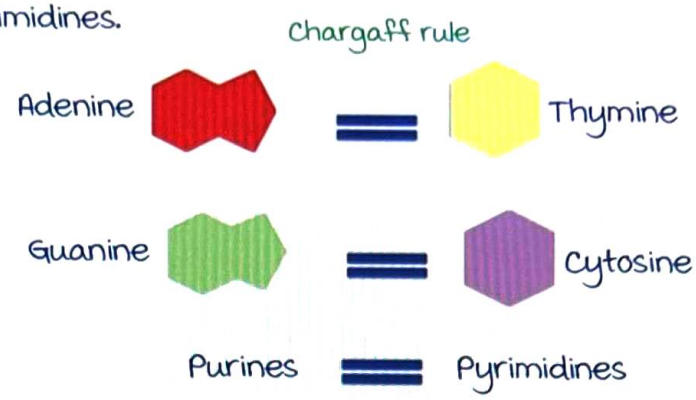
Hand rails : Formed by sugar + phosphate; Steps : Formed by bases.
Anti-parallel arrangement of 2 strands of DNA : One strand is always in 5' → 3' direction. While the other strand in 3' → 5' direction.
Diameter of DNA is 2nm.
Base pairing rule :
Adenine (A) always pairs with thymine (T) by two hydrogen bonds.
Guanine (G) pairs with cytosine (C) by three hydrogen bonds.





Active space

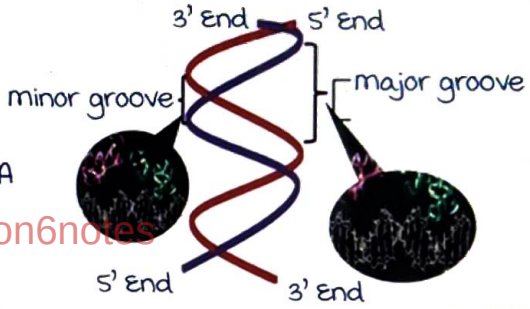
Chargaff's rule : It states that number of purines = number of pyrimidines.



Base stacking DNA :

- The bases are aromatic and nonpolar, they stack on each other by vanderwaal's forces called base stacking.
- The vanderwaal's thickness is 3.4A°; average of 10.5 bases per turn, thus the pitch of helix is 34 A°.

Diameter : 2 nm.



Minor & major grooves :
They are the sites for DNA protein interaction.

Different types of DNA

00:09:27

6 forms of DNA :

- A, B, C, D, E → Right-handed.
- Z → Left-handed.

A form	B form	Z form
Right-handed	Right-handed	Left-handed
11bp/turn	10.5bp/turn	12bp/turn
Broad & short	Longer & thinner	Elongated & thin

Important facts :

- Most stable DNA : B form.
- The form of DNA preferred in a solution devoid of water is A form.
- The back bone of DNA is zig zag in Z DNA.
- The DNA found in low salt concentration and high degree of hydration is B form.
- The DNA found in high salt concentration is A form.

- Z DNA is found predominantly in certain regions where pyrimidine alternates with purine (most commonly C & G).
- DNA complimentary to mRNA : cDNA.
- Chimeric DNA is formed when DNA of interest joins with vector DNA.
- Z form found at the ends of the chromosome.

Mitochondrial DNA :

- 1% of cellular DNA.
- Mitochondrial DNA is double stranded, circular and has about 16,569 bps.
- Human mitochondria has 2-10 copies of DNA.
- Mitochondrial DNA encodes 37 structural genes for :
  - 2 rRNAs (16SrRNA and 12 SrRNA).
  - 22 mitochondrial tRNAs.
  - 13 proteins of ETC (electron transport chain).

6×10^9 bp present in human diploid chromosome.

13 proteins of ETC 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 mitochondrial DNA that constitutes 19%.

Unique features of mitochondrial DNA :

- Mitochondria has a unique genetic code; Only 22 tRNAs are involved in translation in mitochondria.

Codons	Nuclear code	Mitochondrial code
AUA	Isoleucine	Methionine
UGA	Stop codon	Tryptophan
AGA, AGG	Arginine	Stop codon

- Mutation rate is very high because :
  1. No introns.
  2. No protective histones.
  3. No effective repair enzymes.
  4. Its exposed to oxygen free radicals, generated by oxidative phosphorylation.

Active space

- Mitochondrial DNA has non-Mendelian type of inheritance (cytoplasmic inheritance) known as matrilinear inheritance.

Denaturation of DNA 00:19:06

Also known as 'melting of DNA'.
The process by which two strands are separated in to component strands.

Features of denaturation :

- Breaking of hydrogen bonds.
- Base stacking also disrupted.
- Phosphodiester bond is not broken.
- No Covalent bonds are broken.
- Primary Structure is not altered.
- Secondary and tertiary structure altered.
- Viscosity is decreased.
- Increase in absorbance of UV light, called hyperchromicity. DNA absorbs 260nm UV light. Denaturation is identified based on the if the >40% of UV light passed through the DNA is absorbed or not.

Melting temperature (Tm) : midpoint of range of temperature over which the two strands of DNA separates.

Features of Tm :

- Base composition.
- Salt concentration.
- A 10-fold increase in monovalent metal ion concentration increases the Tm by 16.6 degree Celsius.
- Formamide : Destabilizes the hydrogen bond.

Supercoils & topoisomerases 00:24:56

Positive supercoils : DNA is wound in the same direction as of DNA helix i.e., clockwise or right hand.
Negative recoil : DNA is wound in the opposite direction as that of DNA helix i.e., anticlockwise.

Topoisomerases :

- Also known as nicking releasing enzymes.
- Relieves topological constraints of DNA.
- Types :

Type I	Type 2
makes single stranded nick in the DNA.	makes nicks in both the strands of the DNA.
ATP is not needed.	ATP is needed.

Organization of the DNA 00:27:30

Prokaryotic DNA is not organized.
Eukaryotic DNA is a highly organized structure.

Levels :

i. DNA double helix.
ii. 10nm chromatin fibril.
iii. 30nm chromatin fibril.
iv. Nuclear scaffold (Interphase chromosome).
v. Metaphase chromosome.

10nm chromatin fibril :

- made up of Nucleosomes (Nucleoprotein complex). Nucleosome consists of a histone octamer and DNA wound around it.
- 1.75 turns (~2 turns) in a histone octamer.
- Left-handed direction.
- Solenoid structure.

30nm chromatin fibril : Has total of 6 nucleosomes.

Histones :

- most abundant chromatin protein.
- Histones are positively charged (DNA is negatively charged). Ionic bound found between the DNA and the protein.
- Amino terminal of the histones is rich in basic proteins like arginine and lysine, hence the positive charge.

Active space

- Histones classification :

Core histones	Linker histones
Present in the histone octamer.	make nicks in both the strands of the DNA.
H_{2A}, H_{2B}, H_3, H_4 are the cores histones.	H_1 histone fou[illegible] region.

The linker DNAs connecting the nucleosomes has a 'beads on string appearance.'
Around 35bps are present in the linker DNA.

Euchromatin & heterochromatin 00:38:06

Euchromatin	Heterochromatin
Less densely packed.	Densely packed.
Transcriptionally active.	Transcriptionally inactive.
Chromatin stains less densely.	Chromatin stains densely.
Permissive chromatin.	Repressive chromatin.

Types of heterochromatin :

- Constitutive : Centromere, telomere.
- Facultative : One of the 'X-chromosomes' in females.

Q. If a sample of DNA has adenine 23% what will be the amount of guaninge present ?
a. 23%
b. 25%.
c. 46%.
d. 27%.
e. 54%.

Q. Which is more susceptible to alkali denaturation, RNA or DNA ?
RNA is more susceptible to alkali hydrolysis than DNA. The free hydroxyl group is responsible for rapid alkali hydrolysis.

Active space

DNA REPLICATION

Introduction

00:00:51

Definition :

DNA replication is the process by which copying of base sequence present in the parent strand to the daughter strand takes place. It helps in passing the genetic information to the progeny.

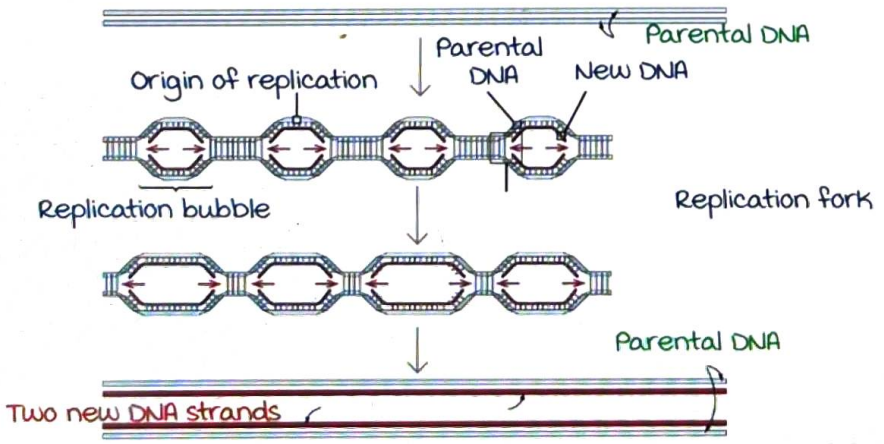
Salient features :

- It takes place during the 'S' phase of the cell cycle.
- Both the strands of DNA act as template.
- Half of parent strand is conserved (semiconservative model).
- Base pairing rule is always obeyed.
- New strand is always synthesized in 5'→3' direction.
- DNA replication is a bidirectional process.
- It's a semi-discontinuous process, comprising of a leading strand (continuous) & a lagging strand (discontinuous).
- Primer is required for DNA replication.

Steps of DNA replication :

1. Identification of origin of replication (Ori).
2. Unwinding of DNA.
3. Formation of replication fork.
4. DNA synthesis (leading & lagging strand).

Identification of Ori, unwinding & formation of replication fork :



Active space

Identification of origin :

Ori is identified by certain proteins :

Ori BP : Ori binding protein. This protein binds to the origin of replication.

Near the origin of replication, an AT rich sequence is present. The AT rich region in eukaryotes are known as DUE (DNA unwinding element).

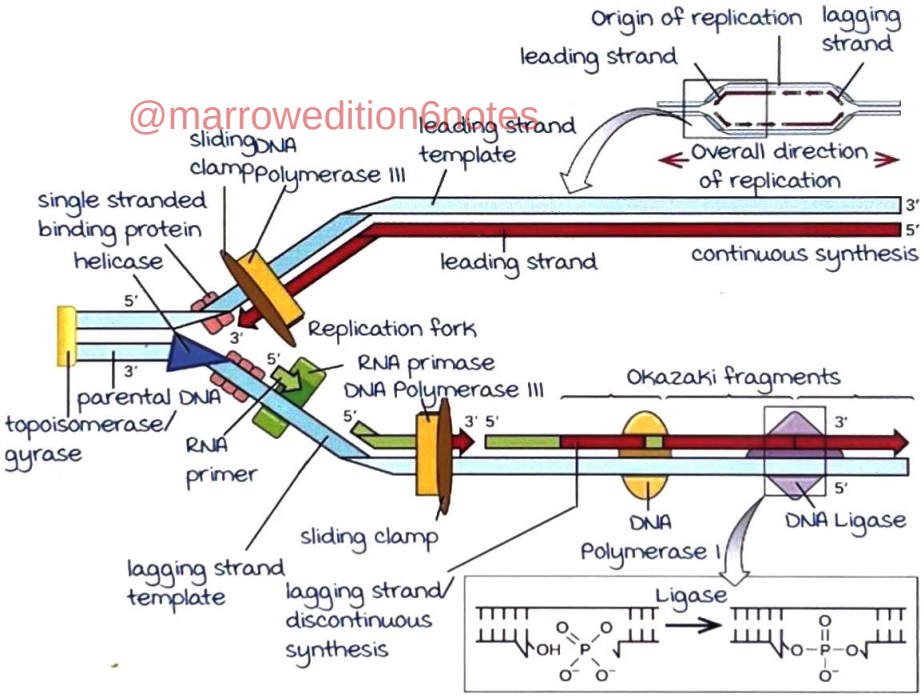
The OriBP binds to the Ori and the AT rich region unwinds.

The SSB (single strand binding proteins) prevent the relinking of reannealing of separated DNA. In humans the SSBs are known as RPA (repetition protein A).

Once the DNA is unwound, a bubble likes structure is formed with 2 replication forks at each ends.

DNA synthesis

00:12:41



Leading strand template :

The strand running in the 3' to 5' direction

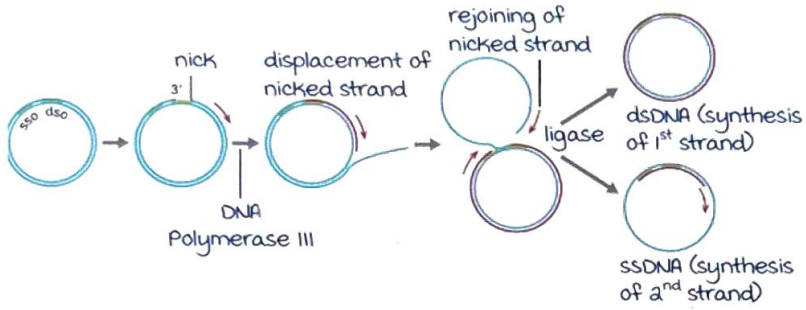
Leading strand synthesis :

- 1st step : RNA primer synthesized in 5'→3' direction; mediated by enzyme primase.
- 2nd step : Synthesis of new DNA (leading strand) in continuous manner; mediated by DNAP-III (in

prokaryotes).
New strand from 5' to 3' direction.

Lagging strand synthesis :

- 1st step : RNA primer synthesized (by enzyme primase).
- 2nd step : Okazaki fragment (short strand) synthesis; mediated by DNAP-III.
- 3rd step : Removal of primer & filling of gaps; mediated by DNAP-I.
- 4th step : Sealing of nicks by DNA ligase (requires ATP).



Enzymes of DNA replication & their function 00:22:40

- Topoisomerase : Prevents torsional strain.
- Helicase : Unwinding enzyme along the direction of movement of replication fork. ATP driven enzyme.
- DNA primase : RNA primer synthesis.
- DNA ligase : Seals the nicks.
- DNA polymerase : Acts only in 5'→3' direction & requires primer.

DNA polymerase : Acts only in 5'→3' direction. Requires primer.	
Prokaryotic : DNA polymerase I (DNAP-I). DNA polymerase II (DNAP-II). DNA polymerase III (DNAP-III).	Eukaryotic : DNAPα. DNAPβ. DNAPγ. DNAPδ. DNAPε.

DNAP-I :

- Also known as Kornberg's enzyme.
- Discovered by Arthur Kornberg in E.coli bacteria.

Active space

- Klenow polymerase/fragment : DNAP-I from which 5'→3' exonuclease activity is removed.
- Actions : Removal of primers, DNA proof reading, DNA repair function (major).

DNAP-II → DNA proof reading & DNA repair (minor).

DNAP-III → Leading strand synthesis, Okazaki fragment synthesis, DNA proof reding.
Most processive DNA polymerase.

DNAPβ → It's the major DNA repair enzyme.

DNAPγ → Required for mitochondrial synthesis.

DNAPδ → Required for lagging strand synthesis.

DNAPα → Responsible for primase activity.

DNAPε → Required for leading strand synthesis.

DNAPε, DNAPγ, DNAPδ → Have proof reading mechanism.

DNA – damaging agents, defects produced, repair mechanisms & associated disorders 00:33:11

Agent	Defects produced	Repair mechanism	Associated disorder
Ionizing radiation & anti-cancer drugs.	ds breaks. ss breaks. Cross links (inter/intra strand).	Non-homogenous end joining (NHEJ). Homologous recombination (HR).	NHEJ is associated with severe combined immune deficiency (SCID). HR is associated with : ATLD (ataxia-telangiectasia like disorder). Nijimen Break syndrome. Bloom's syndrome. Werner syndrome. Rothmund – Thomson syndrome. BRCA 1 & 2.
UV light-& chemicals.	Bulky adducts. Pyrimidine dimer.	Nucleotide excision repair.	Xeroderma pigmentosa. Cuckayne syndrome. Trichothiodystrophy.
Oxygen radicals & alkylating agents.	Abasic sites.	Base excision repair.	Mutyh polyposis.
Replication error	Base mismatch (insertion/deletion).	Mismatch repair.	Hereditary non-polyposis colon cancer (also known as Lynch syndrome).

Active space

NHEJ :

- There is loss of genetic material.
- It's the major mechanism in humans.

HR :

- There is exchange of genetic materials, finally leading to correction of the defect.
- It's the major mechanism in yeast.

Xeroderma pigmentosa : Helicase enzyme is the defective enzyme.

Telomere & telomerase 00:43:07

Telomere :

- It is the ends of the chromosome'.
- Has 'TTAGG repeats'.

End replication error :

- Once the primers are removed, it leaves a gap at the 5' end of the daughter strand.
- 3' end of a parent strand is not replicated.
- If not corrected, it may lead to shortening of DNA (generation after generation).

Telomerase enzyme :

- Also known as terminal telomere transferase.
- It's a ribonucleoprotein.
- It's a protective enzyme.
- It corrects the end replication error in certain cells.
- It has an intrinsic RNA primer, which is complementary to the sequence that is present at the ends of chromosomes (TTAGG repeat). This helps it to easily catch on to the ends of the chromosome and corrects the end replication error.
- It has reverse transcriptase activity.
- Absent in somatic cells.
- Present in germ like cell, stem cell, cancer cell.
- Increased telomerase activity causes cancer.
- Decreased telomerase activity causes premature aging

Active space

(progeria).

- It acts as target for drug development for anti-cancer drugs.
- Hayflick limit → After >50 cells division replication potential becomes nil.

  This is because of the absence of telomerase activity.

Difference between prokaryotic & eukaryotic DNA replication

00:54:21

	Prokaryotes	Eukaryotes
Ori	Single	multiple
SSB	SSB	RPA
Primase	DnaG	DNAPα
DNA synthesis	DNAP-III	DNAPε (leading strand); DNAPδ (lagging strand)
Removal of primers	DNAP-I	RNase H / flap endonuclease

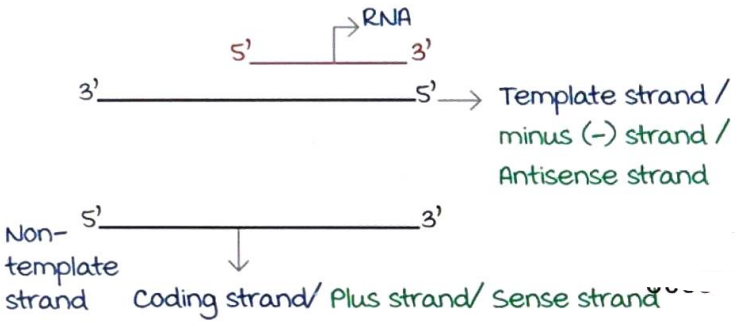
TRANSCRIPTION

The process by which RNA is synthesized from DNA.

Salient Features

00:01:39

1. Only 1 strand acts as a template.

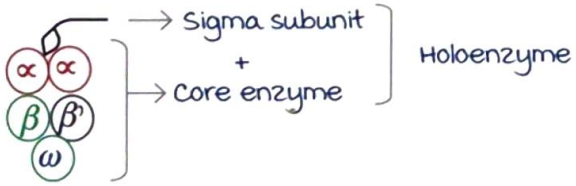


2. RNA is synthesized in the 5' → 3' direction. Because RNA polymerase can act in the same direction only.
3. No primer is required.
4. The newly synthesized RNA sequence is the same as the coding strand (exception : T is replaced by U) & complementary to the template strand.

RNA Polymerase (RNAP)

00:05:51

- Prokaryotic RNAP :
  Only 1 type RNAP.
  Multi-subunit enzyme.



  Sigma subunit : Helps in the binding of RNAP to Promoter site.
  β - subunit : Catalytic subunit.
- Eukaryotic RNAP :
  3 types RNAPs. Classified based on its sensitivity towards a poison in mushroom (amanitin).

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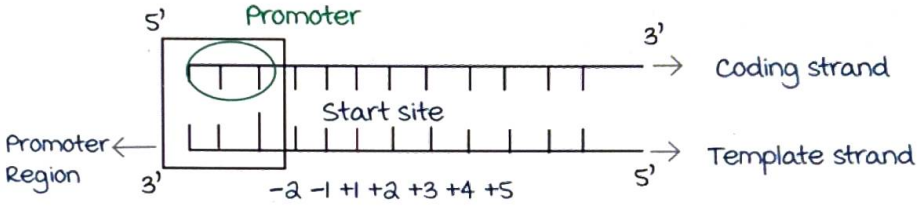
RNAP	α-amanitin sensitivity	Products
RNAP I	Least	rRNA (most abundant)
RNAP II	Highest	mRNA, miRNA, snRNA, lncRNA
RNAP III	Intermediate	tRNA, 5SrRNA, certain snRNA

Promoters of Transcription

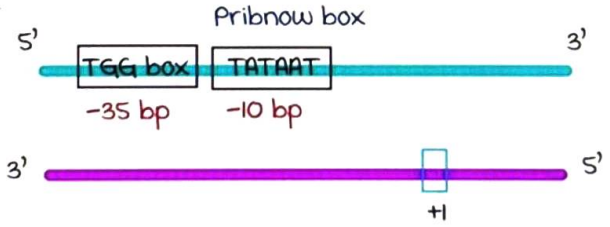
00:12:38

Promoter : Conserved sequence in the coding strand that specifies the start site of transcription (boxes or elements).



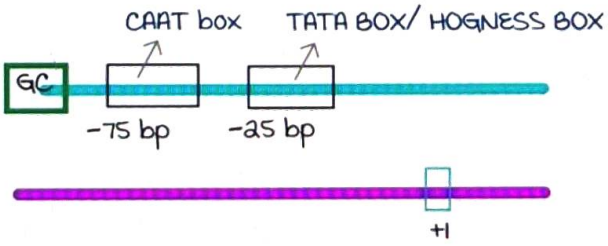
Bacterial promoters :

- Pribnow box : - 10bp
- TGG box : - 35bp



Eukaryotic promoters :

- TATA box/Hogness Box : - 25bp
- CAAT box : -75bp
- GC box



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Promoter less sequence : No promoters.

Additional promoter elements :

- Inr (Initiator sequence)
- DPE (Downward promoter element)

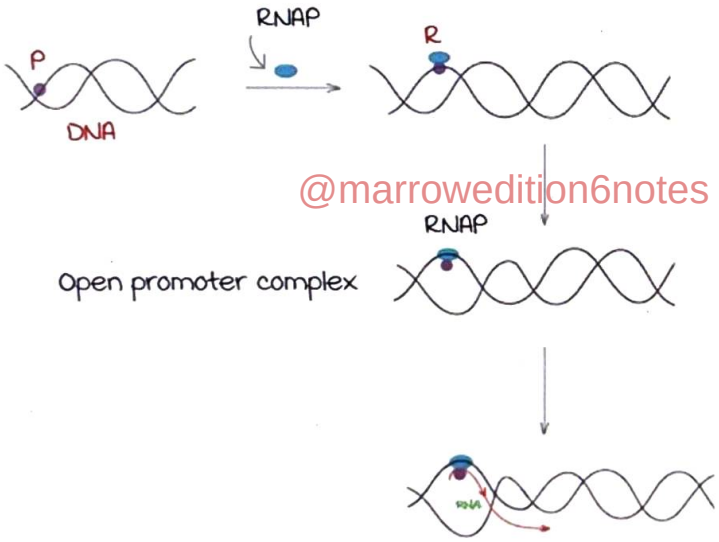
Promoters are upstream elements, and they are not transcribed.

Exception : Intragenic/Downstream promoter → Transcribed.

Example : RNAP II.

Transcription Cycle 00:20:46

1. Template binding & closed promoter complex.
2. Open promoter complex.
3. Chain initiation & chain elongation.
4. Chain termination.



Termination :

- ρ dependent
- ρ independent

ρ dependent termination :

- ρ factor binds to the RNA – DNA complex.
- ρ factor is an ATP-dependent RNA – DNA helicase → detaches the RNA from the DNA.

ρ independent termination : The template strand has termination signals (GC rich region and U rich region).

GC rich region forms intrastrand base pairing, that is, hairpin structure. This is followed by the U-rich region that forms bonds with the A region, which is comparatively weaker.

Hence, RNA detaches from the DNA.

Post – transcriptional modification (PTM) 00:27:13

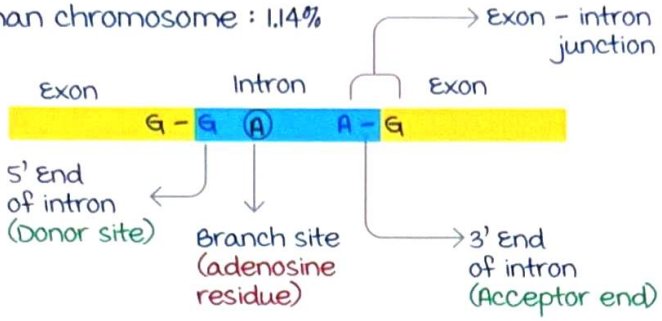
Prokaryotes : All RNA undergoes PTM except mRNA.
Eukaryotes : All RNA undergoes PTM.
PTM in mRNA :

1. 7 – methylguanosine capping :
   At the 5' end → 7 – methyl guanosine cap is added.
   Steps :
   - GTP is added to the 5' end by Guanylyl transferase, in the nucleus to the mRNA (a/k/a Primary transcript/ hnRNA (heteronuclear RNA)).
   - In the cytoplasm, methylation at N_7 of Guanosine.
     methyl donor: SAM (S-Adenosylmethionine).
     Enzyme : Methyltransferase.
     Functions :
   - Prevents the attack of 5' → 3' exonuclease.
   - Stabilizes the mRNA.
   - The cap binds with cap-binding complex → Helps in the attachment of mRNA to the 40S ribosome of the translating machinery → Initiation of translation.
2. Poly A tail at the end.
   40 – 200 Adenosine residues are added to the Primary transcript at the 3' end.
   Enzyme : Polyadenylate polymerase.
   Functions :
   - Prevents attack of 3' → 5' exonuclease.
   - Stabilizes the mRNA.
   - Poly A tail binds to PAB I (Poly A tail Binding protein) → Helps in the attachment of the mRNA to the 40S subunit → Initiation of translation.

PTM - Removal of introns & splicing of exons 00:35:54

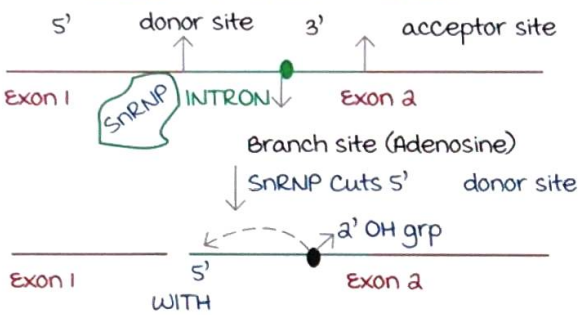
Introns : Unwanted regions/ non-coded to the proteins.
Exons : They are coded to the proteins.

Exons in human chromosome : 1.14%

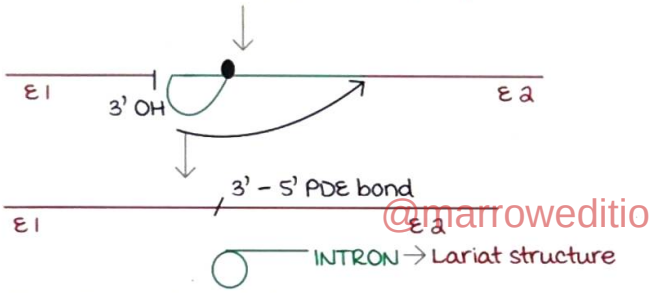


Steps :

- snRNA + Protein → snRNP



2' OH froms bond ∧ 5' po_4 ⇒ 2' – 5' phosphodiester bond



This intron is degraded

Spliceosome

00:45:29

3 components :

- snRNA : Transcribed by RNAP II, Uracil rich (U1, U2, U3)
  Example of the ribozyme.
- snRNA + Proteins → snRNP (small nuclear Ribonucleoproteins).
  A/K/A snurps.
  An autoimmune disorder associated with snurps is SLE.
- snRNP + exon – intron junction of primary transcript (hnRNA) → Spliceosome.

Self-Splicing introns :

- Discovered by Thomas Cech.
- Group I introns :
  No Lariat formation.
- Group II introns :

Active space

Lariat structure is formed.

No ATP is required for both groups.

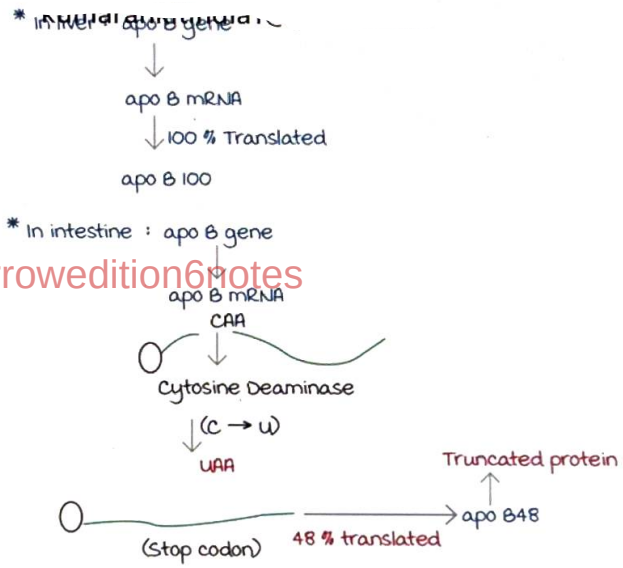
3. Alternate mRNA processing/ Differential RNA processing.
   The exons can be spliced in different alternative ways so that different protein products can be produced from the same gene.
   - Different Isoforms of the same protein can be generated.
   - membrane bound & secretory immunoglobulins.

RNA editing

00:50:55

- 0.01% mRNA undergoes this mechanism.

Example :



Questions :

1. mRNA without polyA tail : mRA of histones.

2. Poly A (AAAAA) codes for : Poly lysine.
   Poly G : Glycine
   Poly U : Phenylalanine.
   Poly C : Proline.

3. hnRNA without intron : hnRNA of histones.

4. Site of post transcriptional modification : Nucleus.

5. SnoRNA : Small nucleolar RNA that is involved in RNA processing.
Site of RNA processing : Nucleolus. So, called nucleolar RNA.

TRANSLATION

Introduction 00:01:04

Definition : Translation is the process in which protein is synthesized from RNA (mRNA).
Site of translation : RER (rough endoplasmic reticulum), free ribosome.

Genetic code & Representation of amino acids in DNA 00:01:57

Genetic code :
Definition : the relationship between a sequence of DNA and a sequence of amino acids in the corresponding polypeptide is called genetic code.
Cracking of genetic code was done by Marshall Nirenberg and H. Gobind Khurana.

Cistron : it is the smallest unit of genetic expression, which codes for a polypeptide chain.
Eukaryotic mRNA is monocistronic and prokaryotic mRNA can be polycistronic.
Representation of amino acids in DNA :
If 1 base represents 1 amino acid, then only 4 amino acids can be represented.
If the base sequence is of 2, then 16 amino acids can be represented.
If the base sequence is of 3, then 64 amino acids can be represented.
(Triplet nucleotide sequence is sufficient to represent the 20 amino acids).

Codon and salient features of genetic code 00:08:49

Codon :
Definition : the triplet sequences present in mRNA representing specific amino acids are called codons.

Stop codons : Among the 64 codons, 3 are stop codons (terminator codons), namely UAA–Ochre, UGA–Opal and, UAG–Amber.
(UGA can be recoded to selenocysteine & UAG can be recoded to pyrrolysine).
Initiator codon : AUG is the initiator codon; (met in eukaryotes & N-formyl methionine in prokaryotes).
Amino acids are represented by a single codon – AUG–met (methionine), UGG–Trp (tryptophan).
Amino acids are represented by the largest number of codons (6 codons) – Ser, Leu, Arg.
Salient features of genetic code :

- Genetic code is degenerate (redundant) i.e., amino acids are represented by more than 1 codon.

Degeneracy of the codon mostly lies on the 3rd base of the triplet sequence.

- The genetic code is unambiguous; a codon represents only a specific amino acid.
- Genetic code is non – overlapping.
- Genetic code is non – punctuated i.e., there is no comma or semicolon in between.
- Genetic code is universal i.e., a specific codon represents a specific amino acid, in all the species.
  An exception is mitochondrial DNA.

Cellular machinery for protein synthesis and wobbling

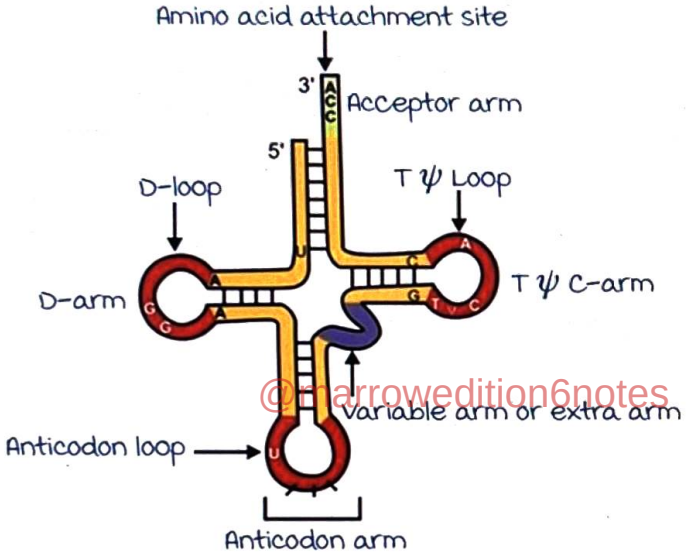
00:16:50

Cellular machinery :
Ribosome = rRNA + specific proteins.

- Eukaryotic ribosomes contain 4rRNAs and 80 different proteins.
- 4 types of rRNA in eukaryotes : 28S rRNA, 18S rRNA, 5.8S rRNA & 5S rRNA.
- Eukaryotic ribosome is also known as 80S ribosome, divided into 40S and 60S subunits.
- The 40S subunit has 18S rRNA and 30 proteins; the 60S subunit has 28S, 5.8S, and 5S rRNA and 50 proteins.

Transfer RNA (tRNA) :

- Also called soluble RNA (sRNA).
- The shape of the tRNA : the secondary structure is clover leaf-shaped, and the tertiary structure is L-shaped.
- Single tRNA contains 74–95 nucleotides.
- tRNA contains the largest number of unusual bases (dihydrouracil, pseudouridine, hypoxanthine).
- tRNA contains Thymine (pseudouridine arm contains ribothymidine).
- Arms of tRNA :



i. Acceptor arm : Has 3 unpaired nucleotides CCA at 3' end; binds the specific amino acid.
ii. Anticodon arm : Nucleotide sequence complementary to the codon; binds to the codon in the mRNA.
iii. DHU arm / D - arm – recognizes the specific aminoacyl tRNA synthetase enzyme (helps in addition of the amino acid to the acceptor's arm).
iv. Pseudouridine arm : Binds the charged tRNA to the ribosome.

Wobbling :

Codon : Anticodon interaction at the 3rd base is not very strict.

For example : The anticodon loop of tRNAPhe (AAA or AAG) can interact with codons UUU or UUC in mRNA.

The reduction of the number of tRNAs is possible due to wobbling.

Active space

The phenomenon of wobbling was put forward by Watson and Crick.

Steps in translation & Energetics 00:29:54

Steps in translation :

1. Charging of tRNA.
2. Initiation.
3. Elongation.
4. Termination.

Charging of tRNA :

Anticodon arm : UAC → DHU arm recognizes the specific tRNA synthetase → specific aminoacyl tRNA synthetase (ATP -> AMP) → methionine → amino acid is attached to the acceptor's arm.

Identification of the initiator codon:

- The first AUG sequence after the marker sequence is defined as the start codon.
- Marker sequence in prokaryotes : Shine Dalgarno sequence.
- Marker sequence in eukaryotes : Kozak Consensus sequence.

Initiation of protein synthesis :

Involves 4 steps :

1. Disassembly of ribosomal units (using eIF3, eIF1A).
   80S → 60S
   → 40S
2. Formation of 80S initiation complex :
   GTP + eIF2 + met tRNA (Ternary complex)
   ↓ ← 40S subunit
   43S preinitiation complex
   ↓ ← mRNA
   48S initiation complex
   GTP, eIF2 ←← ↓ ← 60S subunit
   80S initiation complex

Elongation :

Catalyzed by proteins called elongation factors (EF).

It's a multi-step process that includes :

1. Binding of aminoacyl tRNA to A-site (requires EF-1, GTP hydrolysis).
2. Peptide bond formation
   Requires peptidyl transferase - ribozyme, 28S rRNA → 60S subunit. No ATP is required.
3. Translocation of the ribosome on the mRNA (requires EF-2, GTP hydrolysis).

Termination :

- Complexed with RF-3 (releasing factor), GTP & peptidyl transferase.
- Requires RF & GTP hydrolysis.

Energetics :

4 phosphates are required for 1 peptide bond formation.

- Charging of tRNA → $tRNA^{aa}$ - 2 ~P (requires 2 inorganic phosphates).
- EF1 – binding of tRNAaa to A-site → 1GTP.
- EF2 – translocation → 1GTP.

No ATP is required for actual peptide bond formation.

Classification of RNA

00:53:48

RNA		
Protein coding RNA	Non-protein coding RNA	
mRNA	ncRNA	
	Large : rRNA → 28S, 18S • lncRNA. • circRNA.	Small : rRNA → 5.8S, 5S • tRNA. • snRNA. • miRNA. • siRNA.

Circular RNA (circRNA) : responsible for the regulation of gene expression.

Micro RNA (miRNA) :

Active space

- It's a small, non-coding, single-stranded RNA.
- Length : 21 to 22 nt (nucleotide).
- Discovered by Craig Mellow & Andrew Fire.
- It is endogenously generated from pre-miRNA.

Pri-miRNA

↓

Pre-miRNA (Contains Cap + Poly A tail)

↓ DROSHA DGCR8

Trimmed miRNA

↓ Exportin - 5

Trimmed miRNA

↓ TRBP dicer nuclease

dsRNA

↓ Argonaute proteins

Loaded into RISC complex (RNA induced silencing complex)

↓

miRNA

- Mechanism of action :

miRNA is involved in the post-transcriptional regulation of gene expression. miRNA binds with the seed sequence present in the 3' UTR, on the mRNA.

  - If it is a perfect base pairing : mRNA degraded.
  - If one or more mismatches are present : Translation arrest.
    This process is called RNA interference/RNAi → gene silencing.
- miRNA is involved in the molecular pathogenesis of neoplasia.
- Oncogenic miRNA : Oncomirs.

siRNA (silencing RNA / small interfering RNA) :

- It's a small, non-coding, single-stranded RNA.
- Length : 21 to 22 nt (nucleotide).
- Mechanism of action : same as miRNA.
- It is exogenous in origin.

Active space

Long non-coding RNA (lncRNA) :

- It's a product of RNAP II (RNA polymerase II).
- Function : Regulation of gene expression.
- Different methods of gene expression of lncRNA :
  1. Gene activation : Facilitates TF (transcription factor) binding.
  2. Prevents gene transcription : lncRNA + TF inhibits the binding of TFs to the promoter site.
     Example : decoy RNA.
  3. Histone and DNA modification : By directing methylases & acetylases to the histones and DNA. Thus, helps in epigenetic modification.

GENE EXPRESSION

Regulation of gene expression :

Factors affect the expression of the genes :

Genes express in different ways in different situations.

Eg. Insulin although present in the liver they express only in the pancreas.

Levels of expression of gene

00:01:29

DNA (genes)
↓ Transcription
mRNA
↓ Translation
Proteins.

At Gene level	At Transcription level	At mRNA level (post-transcription)
Gene amplification Gene rearrangement Epigenetic modification Transposons Gene switching	Induction & repression ↓ Operon Concept	RNA editing Alternate RNA processing RNA i (RNA interference)

Two types of Genes

Housekeeping/Constitutive gene

Expressed at a constant rate.

A basal level of expression.

Eg. Hexokinase.

Inducible gene

Not expressed at a constant rate.

Expressed after a stimulus.

Eg. Glucokinase.

Active space

Operon concept

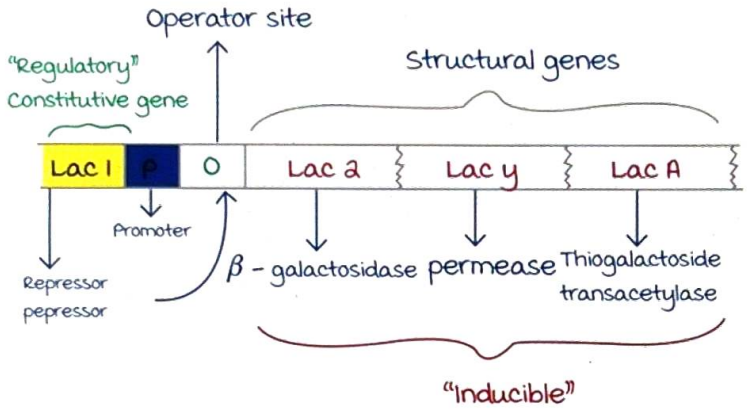
00:07:13

An array of genes having a specific purpose.

The concept was put forward by Francois Jacob & Jacques Monod.

Lac Operon :

An array of genes for metabolism of lactose in E. coli bacteria.



RNA polymerase enzyme binds to the Promoter site.

Regulatory/ Constitutive gene	Structural/Inducible gene		
Lac I : Secrete repressor/inhibitor ↓ Bind to operator site	Lac z ↓ β-galactosidase ↓ Lactose breakdown (Lactase) into glucose & galactose.	Lac Y ↓ Permease ↓ Permit lactose entry into the cell.	Lac A ↓ Thiogalactoside transacetylase ↓ Function unknown.

RNA polymerase cannot move forward if the repressor/ inhibitor (active state) is bound to the operator site.

Catabolite repression (CR) :

CAP/CRP : Catabolite activator protein/Catabolite repressor protein.

CAP/CRP can be in an active state (cAMP attached) or an inactive state (cAMP not attached).

Active space

CAP/CRP is a positive regulator of the Lac operon.

↓

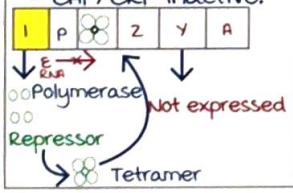
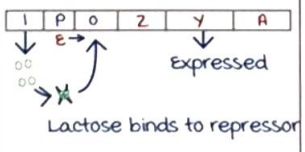
If CAP/CRP is active then the Lac operon is switched on.

Glucose is a better source of energy for E.coli (preferred fuel).

Two conditions :

- Glucose is absent : cAMP levels are high → CAP/CRP active.
- Glucose is present : cAMP levels are low → CAP/CRP inactive.

In the presence of glucose (catabolite), lac operon is switched off (repression).

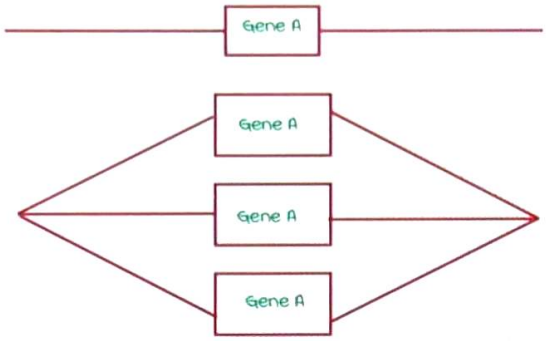
Scenario 1 : Glucose + & Lactose −	Scenario 2 : Glucose − & Lactose +	Scenario 3 : Glucose + & Lactose +
Lac Operon is switched off. Explained by Lac i & CR mechanism	Lac Operon is switched on. Explained by Lac i & CR mechanism.	Lac Operon is switched off. Explained by CR mechanism only.
Repressor/ inhibitor protein is active (in abscence of lactose) & is bound to operator site → No expression. cAMP level low (due to presence of glucose) → CAP/CRP inactive. 	Repressor/inhibitor protein is inactive (because lactose binds to it) & therefore is not bound to operator site → Genes expression → cAMP level high (due to absence of glucose) → CAP/CRP active. 	cAMP level low (due to presence of glucose) → CAP/CRP inactive

Isopropyl Thiogalactoside : Lactose analogue.

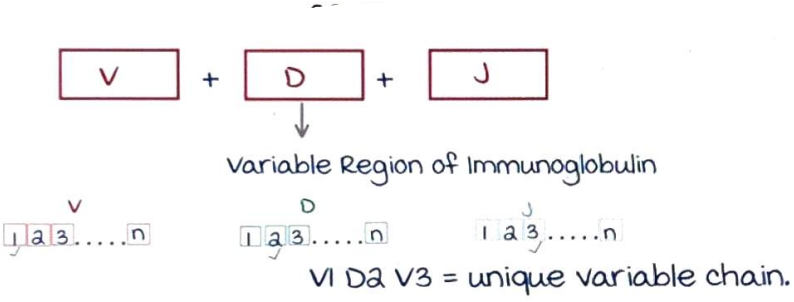
Gene amplification 00:24:03

The process by which the number of genes available for transcription is increased.

Eg. Patients on methotrexate for a long time → increase in DHF reductase by body → develop resistance.



Gene rearrangement 00:26:31



V1 D2 V3 = unique variable chain.

Different gene segments are brought together in different combinations. Acts at the DNA level.

Eg. Immunoglobulin variable chain is produced by joining 3 gene segments (V, D, and J segments).

V, D, and J have different numbers of segments which in variable combination produce a different product.

Transposons 00:29:02

Mobile DNA sequences that move from one location to another.

Discovered by Barbara McClintock.

Enzyme responsible for movement : Transposase.

Two Classes :

Class 1 (90%) : Retroposons
Mobile DNA elements moving with help of an expressed RNA product.

Class 2 (10%) : Transposons
DNA segment moves.
Mutation : Insertion & deletion.

Active space

Epigenetics 00:32:45

Definition : Reversible heritable chemical modification of DNA or chromatin without altering the nucleotide sequence.

Two Types

DNA modification — Histone modification

Epigenome : Constellation of covalent modification of DNA & histone → Regulating gene expression.

DNA methylation 00:36:00

Takes place at cytosine residue wherever CpG islands present (seen in promoter region).
Enzyme : DNA methyltransferase (DNMT).
methyl donor : SAM.
~~DNMT4 prefers~~ : Azacytidine.
5 Aza 2'-deoxycytidine.
Decitabine.

methylation of cytosine residues results in the Inhibition of gene expression → Favor heterochromatin formation.

Histone modification 00:40:05

Histone acetylation & deacetylation.
Post-translational modification.
Histone acetylation : Positively charged histone is added with negatively charged acetyl group (CH_3COO^-)

↓

Decreases the positive charge

↓

DNA becomes less condensed

↓

Euchromatin/Permissive chromatin.
Increased expression of gene.

Active space

Enzyme : Histone Acetylase (HAT).

Histone deacetylation : Increase in the positive charge by removing the negative charges.

↓

Increased condensation of the DNA.

↓

Hetero chromatin.

Decreased expression.

Enzyme : Histone Deacetylase (HDAC).

Drugs inhibiting HDAC :

- Vorinostat .
- Valproic acid .

Functional consequences of histone modification :

1. Histone acetylation → Activation of gene expression.
2. Histone deacetylation → Decrease gene expression.
3. Histone phosphorylation → Increase/decrease gene expression.
4. H_1 phosphorylation → Chromatin condensation.
5. Histone methylation → Increase/decrease gene expression.
6. ADP Ribosylation → DNA repair.
7. Monoubiquitylation → Increase/decrease gene expression
8. Sumoylation (SUMO : Small ubiquitin-related modifier) → Chromatin condensation.

Physiological & pathological role of epigenetic modification

00:48:10

Physiological :

- Regulation of gene expression
- Genomic imprinting : Slight differences in identical alleles on paternal & maternal chromosomes.
- Aging.
- Embryogenesis.

Pathological :

- Fragile X Syndrome : FMR -1 gene is hypermethylated.
- Cancer : If oncogene is methylated → prevents cancer.
  If the tumor suppressor gene is methylated → causes cancer.
- Prader Willi syndrome & Angelman syndrome.

Molecular methods to detect epigenetic modification

00:55:21

1. Methylation Specific PCR
2. DNA Chromatin Immunoprecipitation (ChIP).

Antibody added to the chromatin with epigenetic modification.
The antigen-antibody reaction takes place.

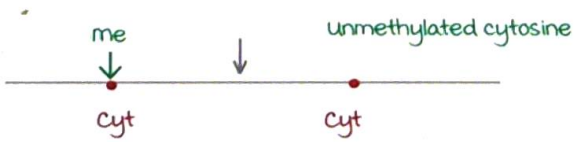
DNase added.
It lyses chromatin sparing the chromatin attached to the antibody-antigen complex.

DNA sequence taken out

Sanger's sequencing
ChIP Seq.

Microarray technique
(Chip technique)
ChIP chip.

3. Bisulfite sequencing :



Deamination by Sodium Bisulfite.

Sequencing.

Active space

HYBRIDIZATION TECHNIQUES

Hybridization techniques :

1) Blot techniques.
2) Microarray technique.
3) Fluorescent in situ hybridization.

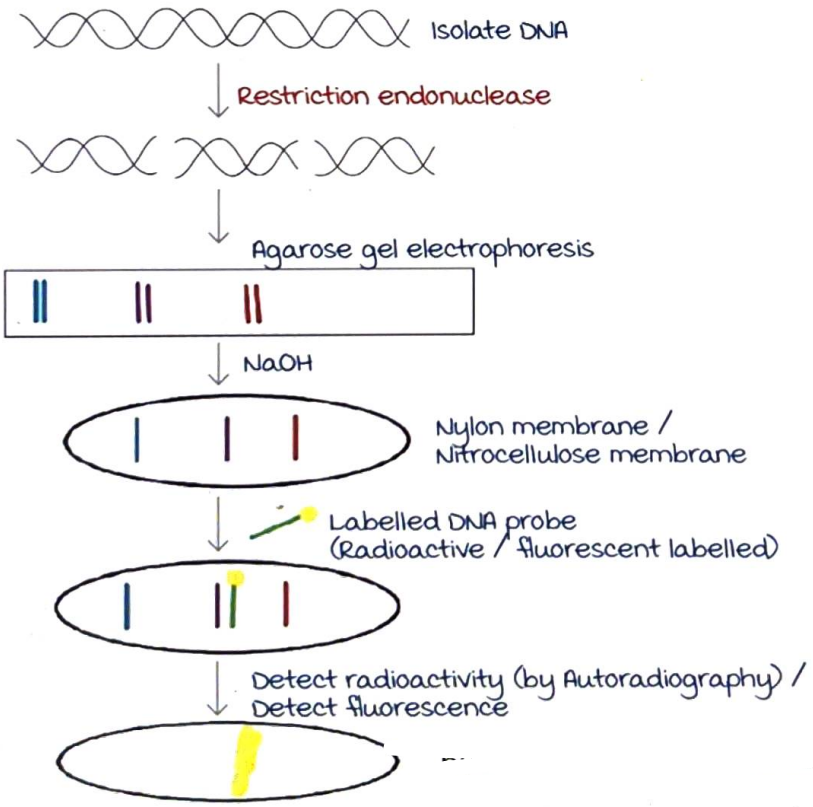
Blot techniques :

- Southern Blot.
- Northern Blot.
- Western Blot.

Southern Blot

00:01:49

- 1st blot technique invented.
- Named after E.M.Southern (invented in 1975).
- Detects DNA.
- Basic principle : DNA – DNA hybridization.



If the membrane has fluorescence at a particular location, then the sample is positive for particular bacteria.

Active space

Probe : A single stranded known oligonucleotide.
Labelled (radioactive/Fleuroscent).

Uses :

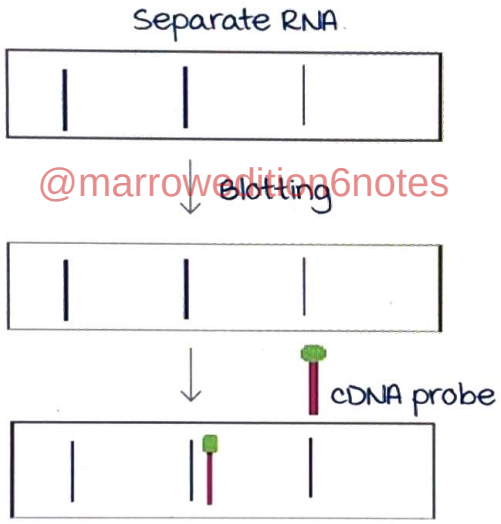
1) Microbiology (Viral/bacterial pathogens).
2) Screen inborn errors.
3) DNA mutation studies.
   - Large gene mutation.
   - Single gene mutation.
   - Point mutation.
4) Forensic medicine.

Northern blot

00:09:57

Detects RNA.

Principle : RNA- DNA (cDNA) hybridization.



If it detects fluorescence / radioactivity ,then it is positive for viral RNA.

cDNA probe is used.

It is complementary to viral RNA.

The viral RNA is converted to DNA using reverse transcriptase and cDNA probe.

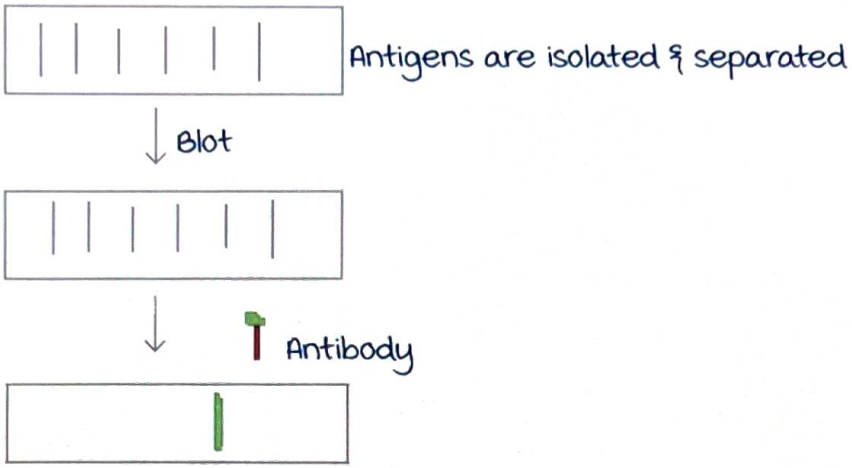
Uses :

1) To detect RNA.
2) To detect gene expression.

Western blot 00:14:12

A/k/a immunoblot

Detects protein.



If any fluorescence is detected, the sample is positive for the antigen

Principle : Ag-Ab interaction (immune reaction).

Labeled antibody is used which is complementary to antigen.

Other blot techniques 00:17:16

- Southwestern blot :
  Known as Overlay blot.
  Detecting DNA protein interaction.
- Dot blot/Slot blot :
  Blotting with nitrocellulose membrane is not done.
  It is blotted to several slots.
- Zoo blot :
  Used to study evolution.

Microarray technique 00:19:48

Thousands of known oligonucleotides is impregnated on a slide.

Uses :

- Genotyping.
- Gene sequencing.

Active space

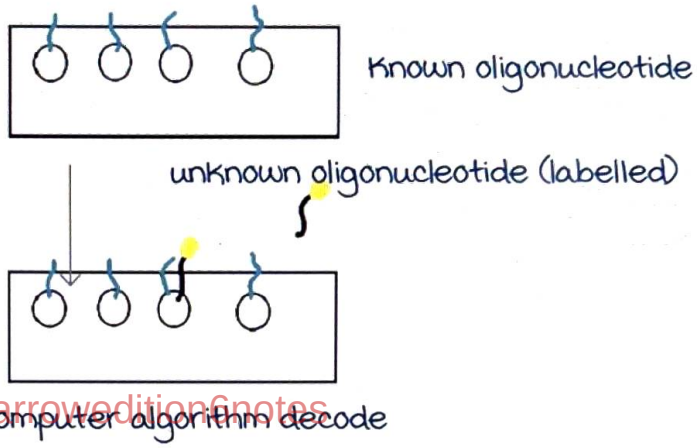
- Gene expression.
- Proteomics.

Classification :

1) DNA microarray.
2) cDNA microarray.
3) Protein microarray.
4) Array Comparative Genomic Hybridisation (CGH).

DNA microarray

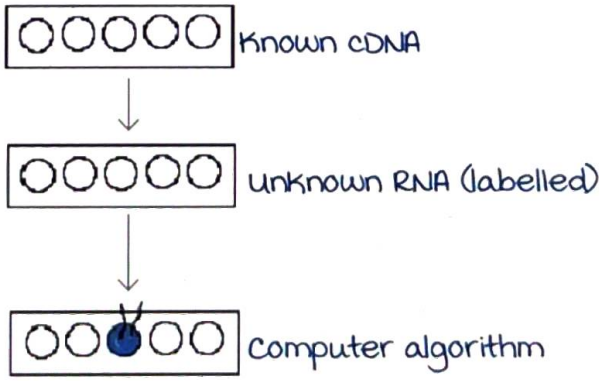
00:22:34



In DNA microarray there are several slides with different wells.
Each of these wells contain a known oligonuclotide.
In the next step, an unknown oligonucleotide is added which is tagged with a fluorescent label.
If it meets its complimentary pair it results in hybridisation.
If not, it gets washed off.
If fluorescence is detected.

cDNA microarray

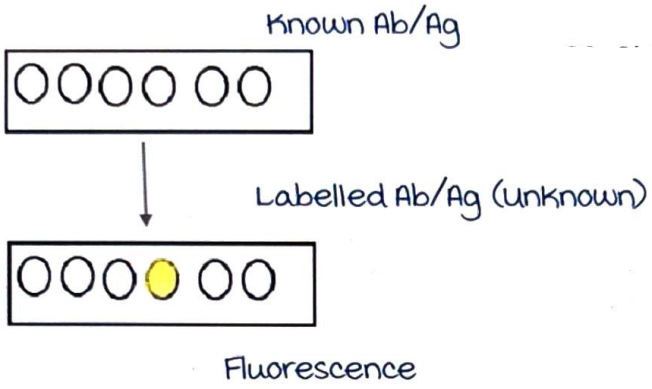
00:25:08



Active space

If a cDNA is combines with the unknown RNA that is added, then fluroscence in the third well can be detected.
Detect viral RNA, bacterial RNA.
Detect gene expression (especially oncogenes).

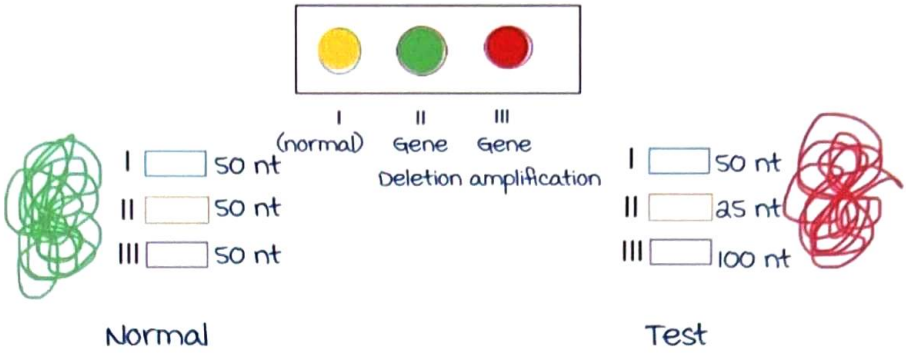
Protein microarray 00:28:30

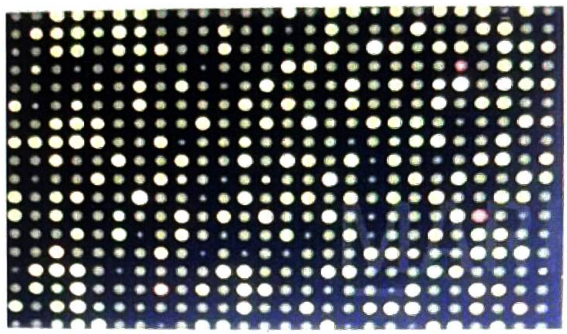


Antigen-Antibody reactions.
Uses : Proteomics.
The unknown protiens are added after tagging with protien microarray and the corresponding antigen-antibosy reaction will take place.
The Fluroscent will be detected.

Array comparative Genomic Hybridization 00:32:53

Based on microarray technique.
Genome chip : The entire genome of the organism is deposited in the slide.
2 genomes are compared.





Yellow : Normal.

Green : Gene deletion.

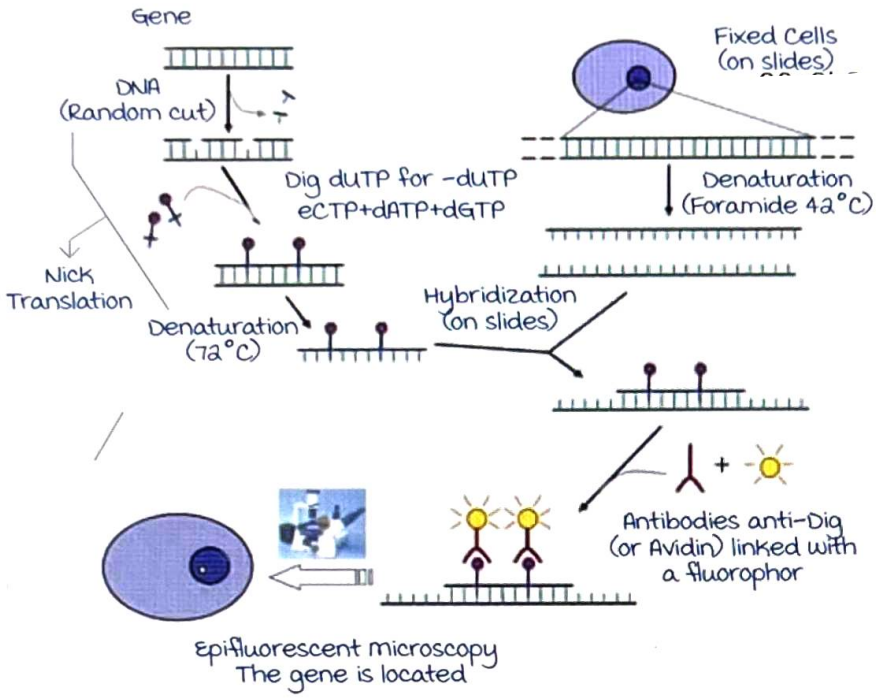
Red : Amplification of gene.

Uses :

- Detects gene deletion.
- Detects gene amplification.
- Detects genetic abnormalities in many diseases with unknown etiology (autism, dysmorphic features, cancers).
- It cannot detect balanced translocation.

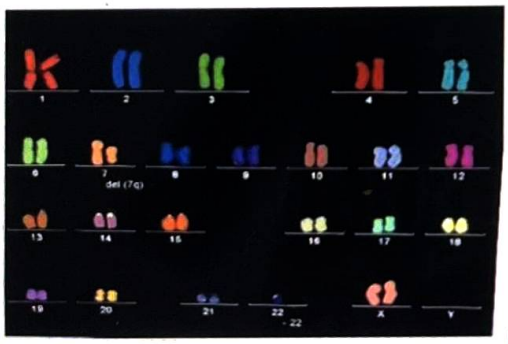
Active space

Fluorescent in situ hybridization (FISH) 00:48:53



A technique to detect a specific genetic element using fluorescent labeled probes in a morphologically intact tissue.

Multicolour FISH/metaphase FISH/Spectral Karyotyping :
Done at metaphase.



5 Fluorescent dyes
↓
mixed to form 23 distinct dye
↓
Each chromosome probe is labelled with a unique color.

Uses :

1) Detects gene deletion (subtle microdeletion).
2) Detects gene amplification (exaggerated colour).
3) Detects numerical abnormalities.

Active space

4) Detects structural abnormalities (translocation).

5) Locate newly identified gene to its correct position.

Nuclear FISH/Interphase FISH :

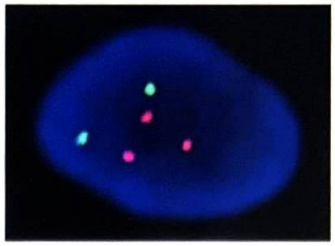
Done in interphase.

Rapid.

More sensitive than metaphase FISH.

For rapid result (prenatal sample, tumor cell).

Growing cell is not needed. (Growing cell is is needed in metaphase FISH.

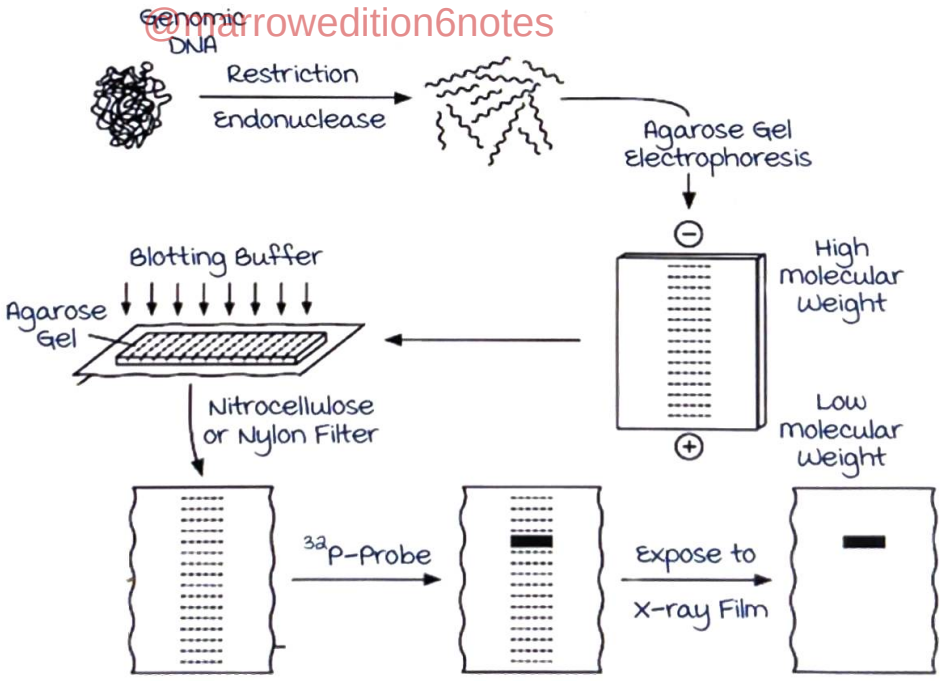


Chromosome painting

01:02:01

Labelling the chromosomes with different dyes (not unique).

Question. What is the technique shown?



Answer : Southern blot.

Active space

RECOMBINANT DNA

Definition 00:00:33

In vivo amplification of technique used to get a clone of the desired DNA fragment.

Restriction endonuclease 00:01:13

Discovered by Werner Arber.
Breaks covalent bond 3' → 5' Phosphodiester bond hence it is a hydrolase (Class 3).
It restricts the entry of phages into the bacterial cell.
It is present inside the bacteria.

Types :

- Type 1
  Cut the dsDNA at a random site.
- Type 2
  Cut dsDNA at the palindromic site.
  Used in molecular biology technique.
  Discovered by Hamilton Smith and Daniel Nathans.
  It is also known as Molecular scissors.

Action :

- ECORI : is specific to Palindromic site.

  G AATTC ECORI G AATTC
  CTTAA G E.coli CTTAA G

  Sticky end/ staggered end/ Cohesive end

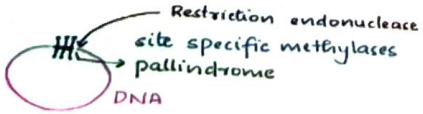
  Sticky end with over hanging sequence
- Hpa I in Palindromic site –

  GTT AAC Hpa I GTT AAC
  CAA TTG CAA TTG

  Blunt end

  Blunt end – No overhanging Sequences

Restriction endonuclease is specific for bacterial palindromic site.
Cannot cut its DNA due to site-specific methylases.



Active space

Vectors

00:10:00

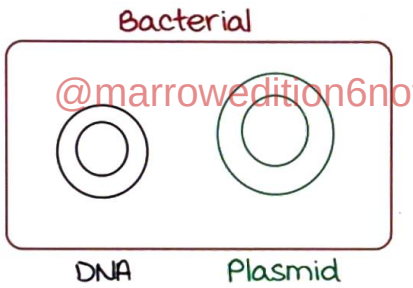
Vector is a molecule of DNA to which a fragment of DNA to be cloned can be attached/inserted.

Properties :

- Vectors should have autonomous replication.
- At least 1 restriction site should be present.
- At least 1 gene ~~can confer antibiotic resistance~~.

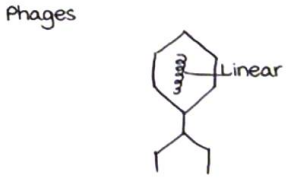
Plasmids :

These are circular dsDNA present outside the nucleus.
They are extrachromosomal DNA.
8 – 10 copies are present in one bacterial cell.
Function : To confer antibiotic resistance.
Plasmids can carry 0.01 – 10 Kbp (DNA insert size) of foreign DNA.
Plasmids have their origin of replication (ori).



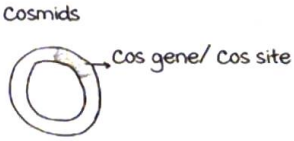
Phages :

Viruses that infect bacteria.
Linear DNA.
DNA insert size: 10 – 20 Kbp.



Cosmids :

Plasmids with cos site/cos gene.
Cos gene : It is required for packing phage DNA.
It has properties of plasmids + phages.
DNA insert size : 30 – 50 Kbp.



Artificial chromosomes :

Artificially created plasmids.

Active space

If similar to a bacterial chromosome called as BAC.

If similar to a phage chromosome called as PAC.

If similar to a yeast chromosome called as YAC.

BAC & PAC – DNA insert size: 50 – 250 Kbp.

YAC : 250 – 3000 Kbp.

HAC : Human artificial chromosome.

Steps of recombinant DNA technology 00:18:28

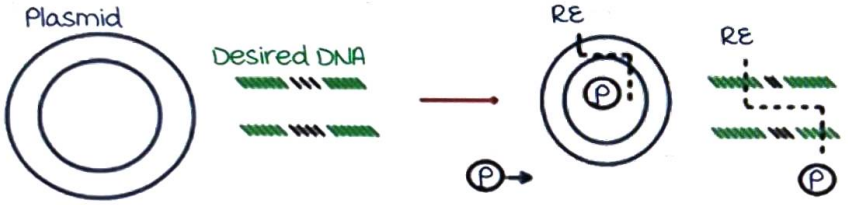
Isolation of desired DNA fragments.

Selection of vectors.

Formation of chimeric DNA or recombinant DNA.

Isolation of clones.

Synthesis of recombinant DNA :



Restriction endonuclease (RE)
(Same on both)

Complementary sticky end formed

Recombinant plasmid/chimeric DNA

↓

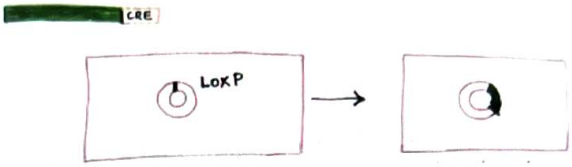
multiple as the bacteria replicates

Recombinases 00:23:58

Recombinases	Recognition site
CRE recombinases	Bacterial lox P site.
λ phage – INT protein	λ att site.
Yeast – Flp	FRT site.

Active space

They are adjunct to restriction endonucleases.
Mechanism of action : Helps in the site-specific insertion of the foreign DNA by a process called homologous recombination.
Foreign DNA inserted at LOx P site.



Site specific insertion of foreign DNA by Homologous recombination

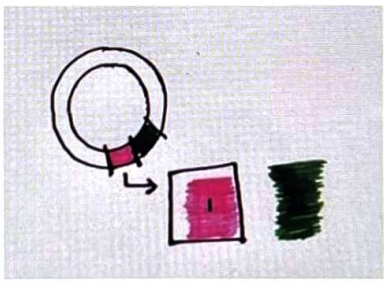
Gene library

00:26:21

A collection of recombinant clones from a specific sources.
Depending on the source :

- Genomic library :
  The clone of the gene is obtained & arranged
  The desired DNA fragment is taken from the genome and not from RNA.

  Disadvantages : difficult to replicate.



- cDNA library :
  The desired DNA fragment is taken from RNA

Collect mRNA —(Reverse transcriptase)→ cDNA

Advantages :

- Less in size and No introns.
- more informative and Easy to replicate.
- Can be used to study gene expression.

Active space

POLYMERASE CHAIN REACTION

Introduction 00:00:27

Example of uses :

- From a scene of crime blood sample obtained. To detect the culprit using the blood sample.
- To detect Covid 19 infection.

Amplification techniques :

In-vivo.

In-vitro.

In-vitro amplification technique :

- Thermal cycling : Temperature of the reaction mixture varies, e.g., PCR, ligase chain reaction (LCR).
- Isothermal cycling : Temperature of the reaction mixture doesn't vary, e.g., NASBA (nucleic acid sequence-based analysis), bDNA technique (branched DNA technology).

Amplification technique		
Target amplification: • PCR. • NASBA.	Probe/primer amplification : • LCR. • Qβ replicase.	Signal amplification : • bDNA technique.

Probe/primer amplification : Probe joined on target is amplified.

Target meets probe → Hybridization occurs → Attached with (fluroscent) dyes → Signal obtained → Amplified.

PCR 00:07:29

PCR is an in-vitro target amplification technique.

Uses 'thermocycler'.

Invented by Karry B Mullis, in 1989.

Ingenious technique.

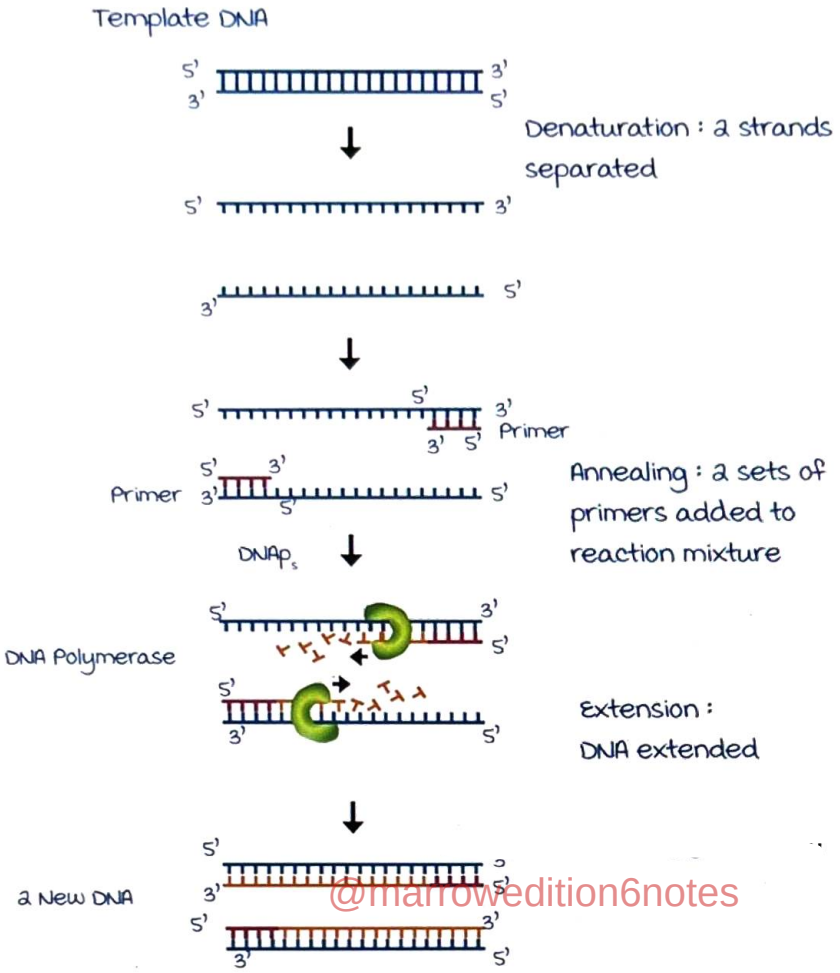
Prerequisites for PCR :

- Sample DNA (260/280 nm ratio).
  260nm : absorbed by DNA
  280 nm : absorbed by aromatic amino acids/proteins.
  Numerator > Denominator : Pure sample.
- Primers.
- dNTP : For extension of primers.
- Cations Mg^{a+} and K^{+}.
- Taq polymerase.
  DNA polymerase enzyme isolated from Thermus aquaticus.
  Thermus aquaticus : Bacteria living in hot geysers/hot springs.
  Acts at high temperature.

Steps of PCR :

- Denaturation.
- Annealing.
- Extension.
- Detection.

Initial denaturation :	Annealing :	Extension :
• Temperature required : 90 to 96°C • Time required : 3 minutes. Target DNA with flanking sequence. Target DNA is amplified.	• Temperature required : 54 To 60°C (like cooling) • Primers are required for annealing process. • Primers can be for ward or reverse type.	• Temperature required : 72°C • Requires dNTP, Mg^{++}, K^{+}, Taq polymerase.



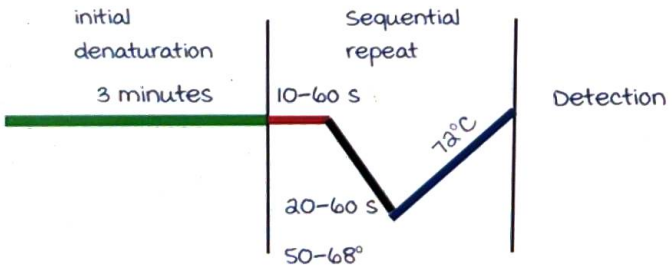
Once extension of target DNA done new cycle started denaturation (do not extend upto 3 minutes, only intial denaturation takes 3 minutes).

After 1st cycle of PCR : 2 samples of DNA are produced (i.e., 2^1 : 2 samples of DNA).

After 2nd cycle of PCR : 4 samples of DNA are produced (i.e., 2^2 : 4 samples of DNA).

Hence, after 2^n : (2xn) samples of DNA called as exponential amplification .

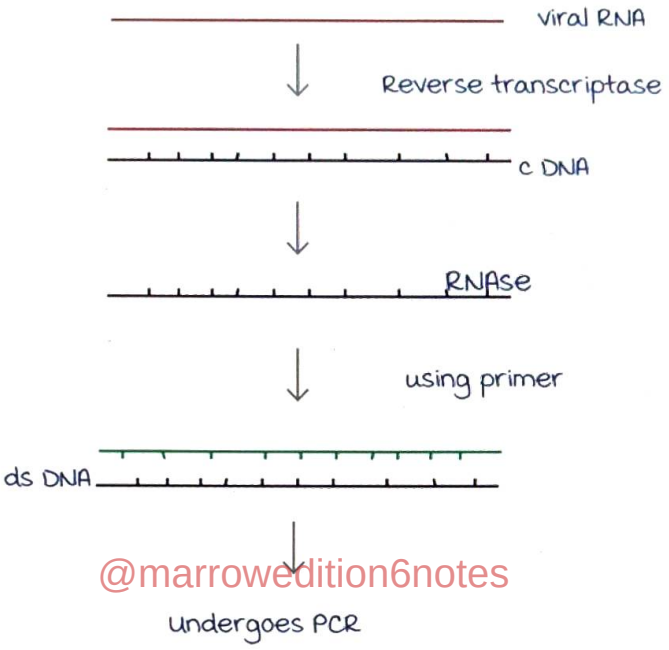
Approximate time required for 1 PCR cycle is 5 minutes.



Active space

Reverse transcriptase PCR (RT-PCR) 00:25:08

Variant of PCR.
It can amplify any RNA.
Enzyme used : T^{th} polymerase (has both reverse transcriptase + DNA polymerase activity).
T^{th} polymerase enzyme is extracted from Thermus thermophilus bacteria



Simplex PCR	multiplex PCR
One set of primer added at a time	Multiple sets of primers added at a time
One set of target DNA amplified at a time	more than one target DNA amplified at a time
Time consuming	Time saving (less time required)
more specific	Less specific

Real time PCR & rRT-PCR 00:30:27

Real time PCR :
Also known as quantitative PCR.
'Amplification and detection' occurs simultaneously.

Certain dyes (fluroscent), enzymes & probes added along reaction mixtures

Different techniques used :

- SyBR green
- Taqman probe
- FRET probe (fluorescence resonance energy transfer).
- molecular beacon.

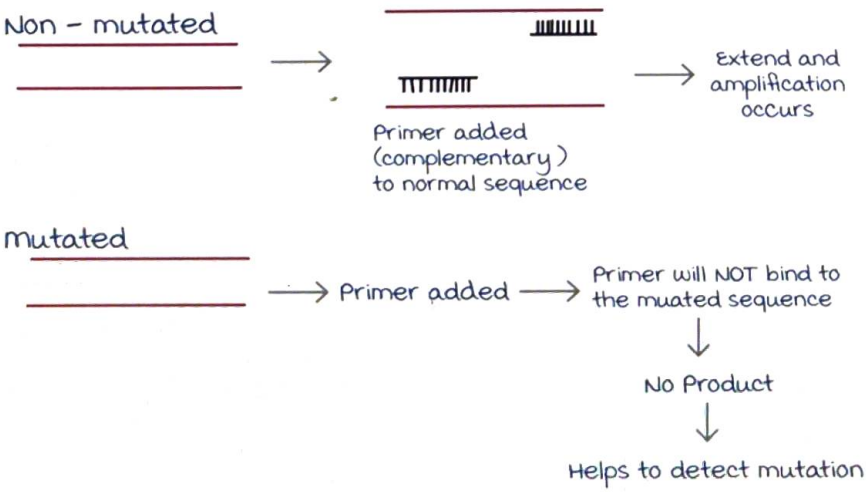
when target amplified fluroscenece emitted that will be detected.

Indicates amplification of target and can quantify DNA & RNA

rRT-PCR (real time reverse transcriptase PCR) : Used for COVID-19.

Applications of PCR 00:34:45

- Forensic medicine :
  example : DNA amplification of culprit from blood samples.
- Microbiology :
  Detection of RNA /DNA virus.
- Study of mutation.
- Repeat length polymorphism detection :
  Usually : 350nm
  Length will be increased hence more flurosence signals detected.
- Preliminary technique to other molecular biological tecniques.



Active space

DNA SEQUENCING

Various techniques 00:00:39

Maxam & Gilbert sequencing.
Sanger sequencing.
Pyrosequencing.
Next generation sequencing.

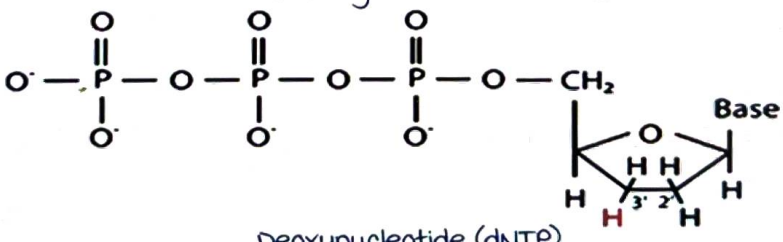
Maxam & Gilbert sequencing :

- It is also called as chemical cleavage method.
- It can only be used for small fragments of DNA.

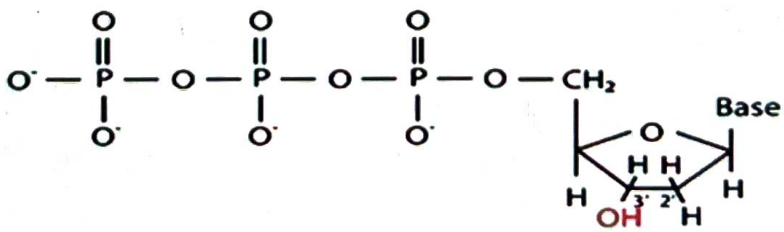
Sanger's sequencing :

- Invented by Frederick Sanger.
- It is also known as controlled chain termination method.
- Its an ingenious technique.
- most popular technique.
- Gold standard technique for detection of mutation.
- Disadvantage : Expensive technique.
- Requirements :
  - Sample DNA.
  - dNT (deoxynucleotides).
  - dideoxy NT.
  - Klenow polymerase (DNAP-I from which 5'→3' exonuclease activity is removed).

Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)



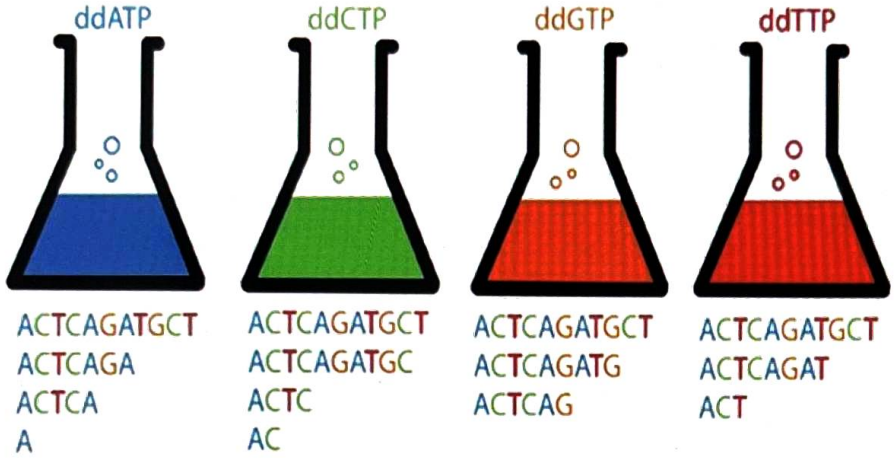
Active space

Basic principle of Sanger's sequencing :

- After DNA synthesis occurs, each reaction vial will have a unique set of single-stranded DNA molecules of varying lengths.

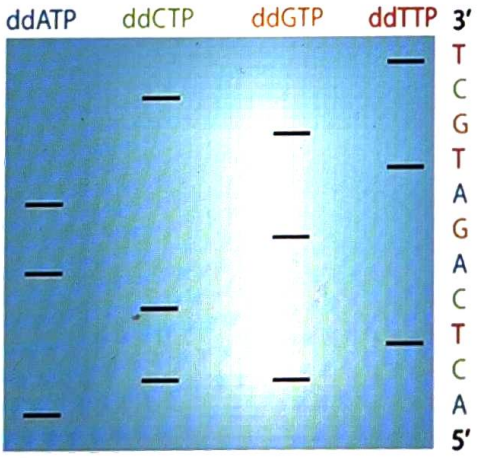
dATP + dCTP + dGTP + dTTP

DNA Polymerase
Template DNA
Primer



Since ddNTP is added, some of the strands cannot be elongated any further.

- The DNA strands are then ~~separated using~~ gel electrophoresis, then read from top to bottom (3'→5') to obtain the sequence.



Smaller strands migrate to the bottom, while larger strands stay up top. We can read each molecule in order to find the DNA sequence.

Active space

Pyro sequencing

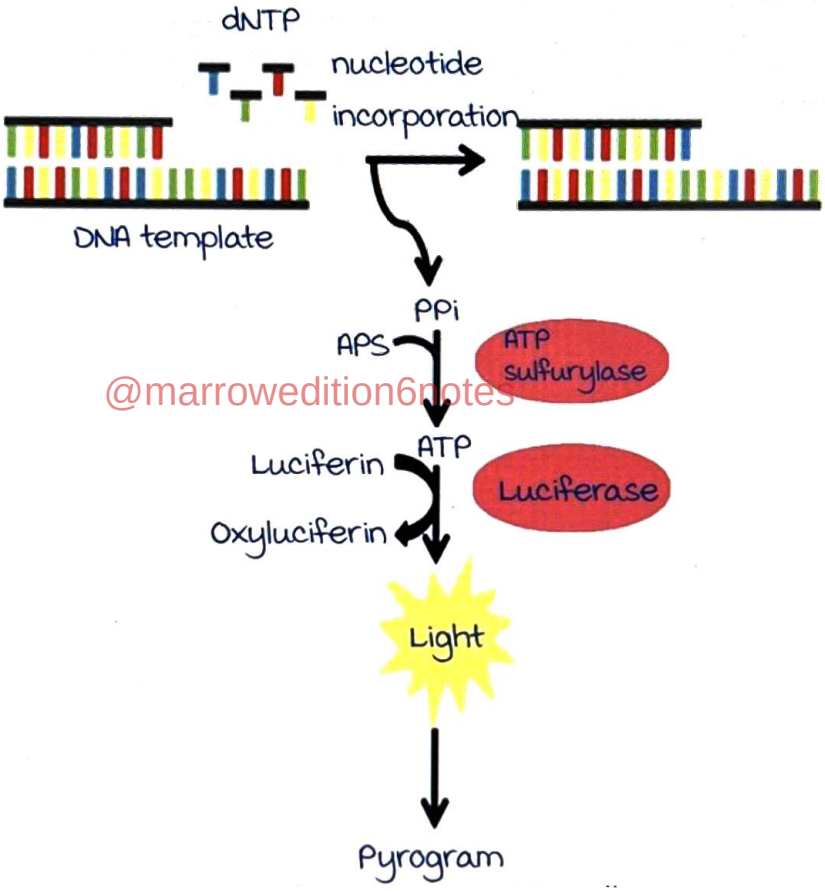
00:17:46

It is a synthesis type of approach of DNA sequencing.
more sensitive method than Sanger's technique.

The modified nucleotide is added with :

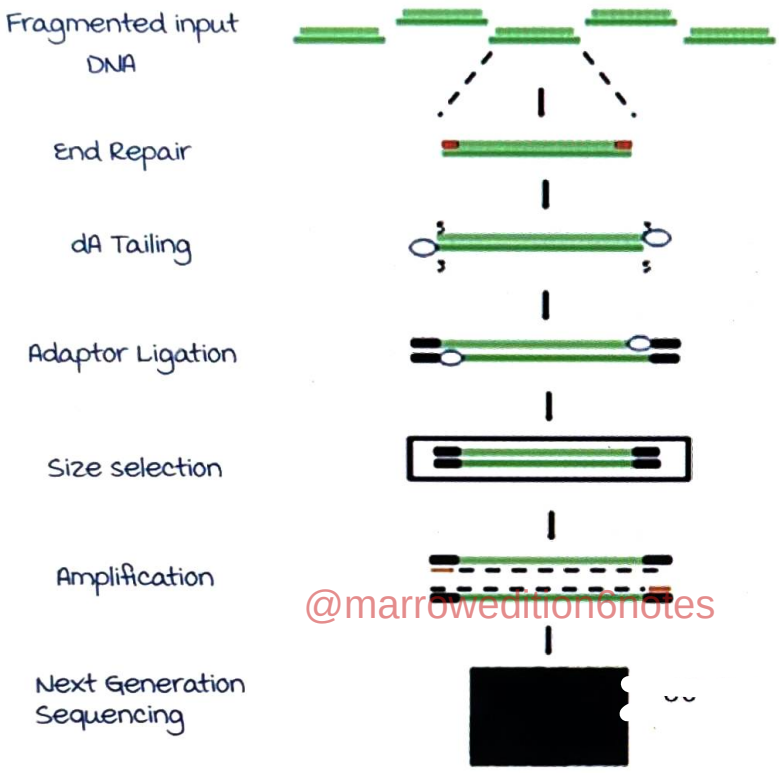
- Chemiluminescent signal.
- Bioluminescence.

Principle :



Next generation sequencing (NGS) 00:23:20

It's a high throughput sequencing method.
It is a massive parallel sequencing.
Principle :



Difference between Sanger's sequencing & NGS :

Sanger's sequencing	Next generation sequencing
One sequence is read, per sample	massive parallel sequencing
Approximately 1 million bases/day	Approximately a billion base pair/day
Time consuming	Time saving
Costly method	Less costly method

Active space

MUTATION

Definition and classification of mutations 00:00:55

Defined as any permanent change in the nucleotide sequence irrespective of functional consequences.
Occur in < 1% of population.

Mutation	Epigenetics	Polymorphism
Abnormal permanent change in nucleotide sequence	Reversible chemical modification. No change in nucleotide sequence.	Normal variation in nucleotide sequence.

Mutation in germ cell can be transmitted to next generation but not in somatic cell.
M/C mutation : Point mutation.

Class	Group	Type
Base substitution (M/C Point mutation).	Synonymous	Silent mutation
	Non synonymous	Missense mutation Non sense mutation

Silent mutation :
No change in amino acid sequence.
The mutation occurs due to degeneracy of codon.

UUU → UUC
↓ ↓
Phenylalanine(Phe) Phe

GAG → GUG
↓ ↓
Glutamic acid(Glu) Valine(Val)

Missense mutation :
Change in amino acid that is coded.

Classification of missense mutation 00:07:48

- Conservative :
  One amino acid is replaced by another with similar characteristics.
  Both Glu and Asp are polar and acidic.

GAA → GAC
↓ ↓
Glu Aspartic acid (ASP)

- Non conservative :

Active space

One amino acid is replaced by another amino acid with different characteristics.

Example : HbS mutation takes place in 6th position of β globin chain.

GAG → GUG
↓ ↓
Glu Val

Glu : Acidic, polar amino acid & Val : Non polar.

Classification of missense mutation :		
Acceptable	Partially acceptable	Unacceptable
No clinical symptoms. Example : Haemoglobin (Hb) Hikari : Alteration in electrophoretic mobility.	Clinical symptoms present. No change in function of protein. Example : HbS	Clinical symptoms present. Function is affected. Example : methaemoglobin (Hbm) : Iron is in ferric (Fe^{3+}) state. 6th valency (Oxygen) is lost.

Transition	Transversion
A ⟷ G C ⟷ T Purine ⟷ Purine Pyrimidine ⟷ Pyrimidine	A ⟷ G C ⟷ T Purine ⟷ Pyrimdine

Non sense mutation, insertion and deletion 00:15:37

Non sense mutation :

Coding codon is replaced by stop codon.

UGU → UGA
↓ ↓
Cysteine Stop codon

β° thalassemia : In the β globin
: CAG (coding codon) is replaced by UAG (stop codon)

Insertion :

- Insertion of a single nucleotide.
- Insertion of a single codon.
- Insertion of a large gene.

Deletion :

- Deletion of a single nucleotide.
- Deletion of a single codon.
- Deletion of a large gene.

Insertions and deletions are called indels : most deleterious mutations.

Frameshift mutation 00:18:17

Insertion or deletion that leads to garbled reading frame.

Example : TOM EAT PIE

1. TOM EAP IE
2. TOM EEA TPI E
3. TOM EAT LOT PIE — insertion of 3 nucleotide
4. TOM PIE — deletion of 3 nucleotide

If insertion or deletion not in multiple of 3 : Frameshift mutation.

If insertion or deletion in multiple of 3 : No frameshift mutation.

eg : In cystic fibrosis at 508 position codon for Phe is deleted

Truncated mutation, run on polypeptide and trinucleotide mutation 00:21:42

Truncated mutation :

1—2—3—4—5.....20 → Stop codon

↓

Changed to stop codon.

Resulting polypeptide has less amino acids than normal.

Run on polypeptide :

1—2—3—4—5—6—7—8—9—10.....20 → Stop codon.

↓

Stop codon changed to coding codon.

Polypeptide derived is longer than normal.

Example : Hb constant spring.

Trinucleotide repeat mutation :

Amplification of a sequence of 3 bases.

Example : FMR gene (CGG) in fragile X syndrome.

Dynamic mutation : Number of repeats changes during gametogenesis.

Mutation detection techniques 00:26:10

Numerical or structural abnormalities in chromosomes can be detected by cytogenetic analysis (FISH : Fluorescent in situ hybridization).

DNA sequencing :

Can detect point mutations, small deletions and insertions.

Gold standard method to screen for mutation, but expensive.

Sanger's technique : most widely used method for DNA sequencing.

Restriction fragment length polymorphism :

If a mutation creates or abolishes a restriction site.

Single strand conformational polymorphism (SSCP) :

Mutation results in conformational change and altered mobility in electrophoresis

Denaturing gradient gel electrophoresis (DGGE) :

Mutation results in conformational change and altered mobility in electrophoresis.

Oligonucleotide specific hybridization (OSH) :

Specific hybridization does not take place in case of mutation.

RNAse cleavage : used to detect small deletions and insertions.

Micro Array (DNA chip) : Genotyping of single nuclear polymorphism along with mutation.

Sequence alteration : PCR analysis.

Sanger's sequencing | Restriction fragment like polymorphism (RFLP) | Single base primer extension

Single base primer extension :

After amplification primer created 1 base prior to target DNA.

Detection of signals in amplification after hybridization.

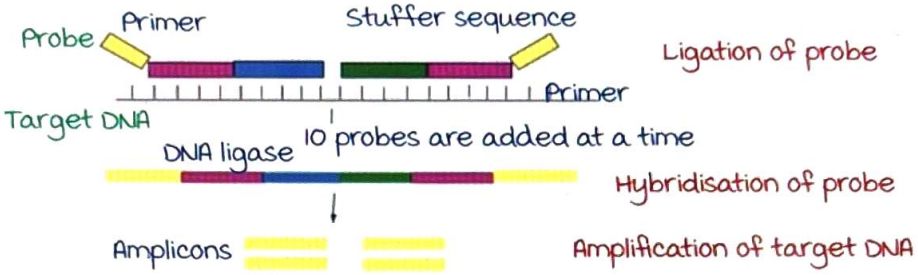
Active space

To detect mutation that affects length of DNA :

- Amplicon length analysis
- Real time PCR → more flurosence suggests increased length
- multiple ligation dependent probe amplification (MLPA).

Multiple ligation dependent probe amplification (MLPA)

00:32:46



Primer and stuffer sequence hybridised.
Gap between probes ligated by DNA ligase.

Analysis :

If exons deleted : No hybridization → No products for amplification.
If exons are duplicated : Large amplicons.
Copy number variations can be detected using MLPA.

Q. Describe mutation given below ?
normal transcript AUG UUU UGG GAG
met Phe Trp Glu
Mutant transcript AUG UUC UGG GAG
met Phe Trp Glu
A. Silent Mutation , Base Substitution, Transition

Active space

VITAMIN A

Vitamins

00:01:20

Definition : Vitamins are organic compounds present in small amounts in various food substances, needed for the growth and maintenance of the body.

Endogenously synthesised vitamins	
By the body : 1. Niacin (from tryptophan). 2. Vit.D (from 7-dehydro-cholestrol). skin → sunlight UV B rays (280-315nm)	In the body : by bacteria in body 1. Vit. K 2. Pantothenic acid. 3. Biotin. (all are synthesised from the intestinal flora).

Classification of vitamins :

Fat soluble : Vitamins A, D, E, K

Water soluble : B-complex vitamins & Vitamin C

B-complex vitamins		
Energy releasing : 1. B_1 : Thiamine. 2. B_2 : Riboflavin. 3. B_3 : Niacin. 4. B_5 : Pantothenic acid. Biotin.	Haematopoietic : Folic acid (B_9). Cobalamin (B_{12}).	Others : B_6 : Pyridoxine

Properties	Fat soluble vitamins	Water soluble vitamins
Absorption	Needs chylomicron	Doesn't need
Excretion	Not excreted in urine	Excreted in urine
Storage	In liver & adipose tissue	Not stored
Toxicity	Present	Absent (exception – Niacin & B_6).
Function	Various function (Vit K : Can act as coenzyme)	Acts as coenzyme (mostly)

Active space

Vitamin A

00:10:14

Types :

- Pro-vitamin A : Mainly from plant sources; known as carotenoids.
- Pre-formed vitamin A : Mainly from animal sources; collectively called as retinoids.

The ring structure present in Vit.A : β ionone ring + isoprenoid unit.

β carotene has a β ionone ring.

Metabolism of vitamin A :

Beta carotene is converted into retinol in the intestine, by the help of dioxygenase enzyme

↓

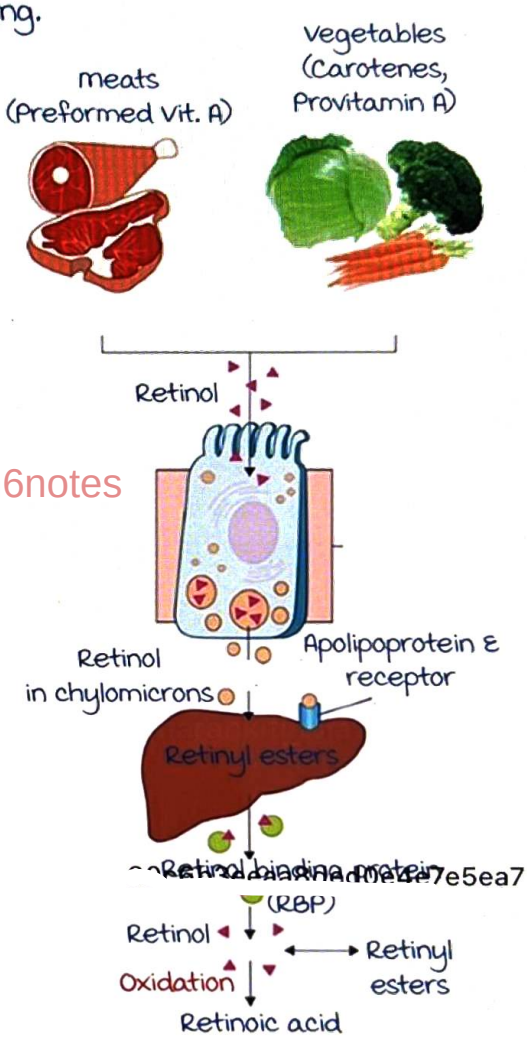
Carried to liver as retinyl ester in the chylomicrons

↓

Stored in the liver as retinyl ester (palmitate) in the perisinusoidal cells (Ito cells)

↓

Carried to target sites in the plasmas trimolecular complex bound to RBP and transthyretin



Retinoids :

All compounds related to retinol are called retinoids.

3 forms of retinoids :

- Retinal : All trans retinal and, 11-cis retinal (present in rhodopsin); required mainly for vision.
- Retinoic acid : All trans retinoic acid, 9-cis retinoic acid & 13-cis retinoic acid ; required for growth, morphogenesis and cellular differentiation.

Active space

- Retinol : Important for reproduction.

Carotenoids :

Carotenoids are provitamins.

Major source are the plants.

Most prevalent carotenoid with Pro-Vitamin A activity:

β carotene.

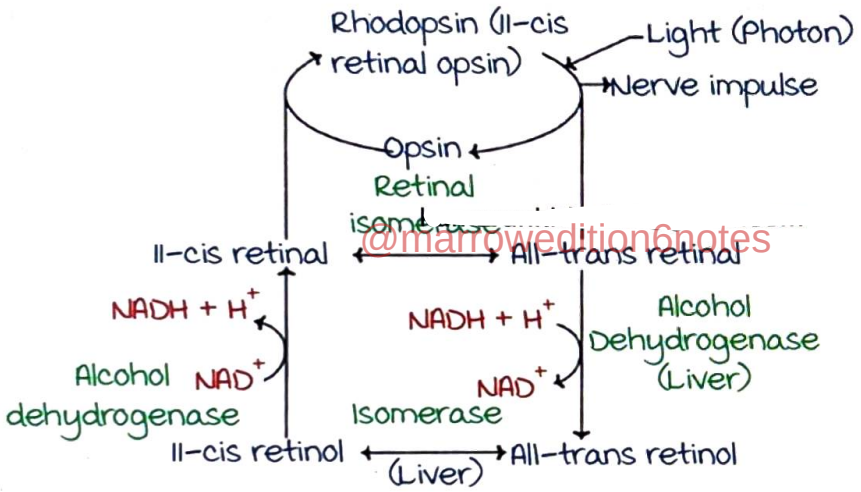
Non-provitamin A carotenoids :

- Lutein & Zeaxanthin : Used in Rx of macular degeneration.
- Lycopene : Used in Rx of prostate cancer.

Vitamin A in vision :

11-cis retinal + opsin forms important visual pigment, rhodopsin.

Wald's visual cycle



Series of isomerisation occurs in conversion to 11-cis retinal :

G protein coupled receptor : GPCR

Inactive GPCR $\xrightarrow{\text{metarhodopsin II}}$ active GPCR

Active G protein is called as Transducin

Inactive Phosphodiesterase $\xrightarrow{\text{Transducin}}$ active Phosphodiesterase.

cGMP $\xrightarrow{\text{active Phosphodiesterase.}}$ 5'GMP

↓

Closes sodium channel

↓

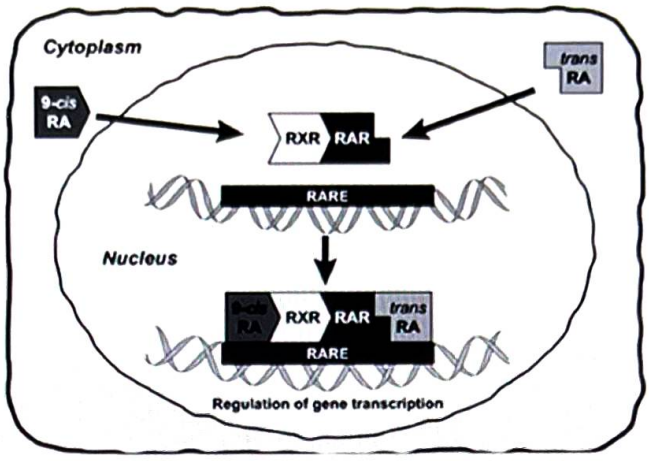
Hyperpolarisation & generation of nerve impulse

Active space

Regulation of gene expression by Vitamin A :

It acts via retinoic acid receptors (RAR).

ligand is retinoic acid.



- All trans-ra and 9 cis-ra are transported to the nucleus of the cell bound to cytoplasmic retinoic acid binding proteins.
- Within the nucleus, all-trans ra binds to retinoic acid receptors(rar) and 9-cis ra binds to retinoic acid x receptors(rxr).
- Rar and rxr form rar/rxr heterodimers, which bind to regulatory regions of the chromosome/gene called retinoic acid response elements(rare). Hence vit A & vit D are interrelated.

Vitamin A have steroid hormone like function.

Binding of all-trans ra and 9-cis ra to rar and rxr respectively allows the complex to regulate the rate of gene transcription.

Functions of Vit.A :

- Maintenance of normal mucosa &skin.
- Regulation of gene expression.
- Normal reproduction.
- It has antioxidant properties (especially β carotene).
- It also has photoprotective properties.

Active space

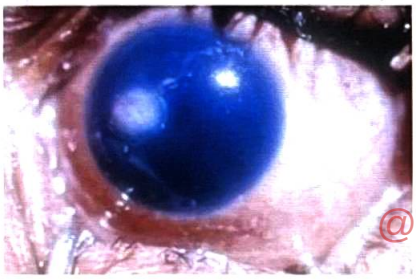
Deficiencies of vitamin A 00:29:24

Eyes : all manifestations of eye together called as Xerophthalmia.

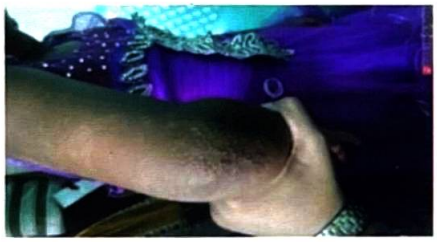
- Loss of sensitivity towards the green light (earliest manifestation).
- Night blindness / nyctalopia.
- Conjunctival and corneal xerosis(dryness).
- Bitot's spots (due to conjunctival keratinization).
- Corneal ulcer and keratomalacia (corneal degeneration).

Skin & mucosa :

Follicular hyperkeratosis/phrynoderma/toad skin.
Squamous metaplasia in the mucus secreting epithelium.

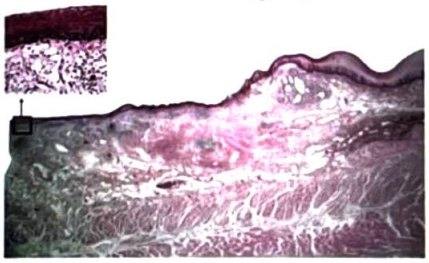


Adnexal gland plugging
↓
Hyperkeratosis and purple and dermatosis
↓
Toads skin



Dramaticimprovement after months of treatment with vitamin A can be expected.

Squamous metaplasia in the mucus secreting epithelium



Uses of vitamin A & vitamin A toxicity 00:35:12

most common cause of preventable blindness.

Uses of Vitamin A in treatment :

Beta carotene is used in cutaneous porphyria.
All trans retinoic acid are used in promyelocytic leukaemia as a differentiation therapy.
13-cis retinoic acid (isotretinoin) is used in cystic acne, psoriasis, and childhood neuroblastoma.

Active space

Vitamin A toxicity/Hypervitaminosis A :

Commonly seen in Arctic explorers (due to intake of polar bear liver).

Organelle damaged : Lysosomes.

Manifestations of acute toxicity :

- Pseudotumor cerebri.
- Exfoliative dermatitis.
- Hepatomegaly.
- Hyperlipidemia.

Manifestations of chronic toxicity :

- Nausea, restlessness, weight loss, anorexia.
- Bony exostoses.
- Hepatomegaly & cirrhosis.

In pregnancy : Isotretinoin is teratogenic.

Retinoic acid favours osteoclastic activity causing demineralization of the bone, hence increases the risk of fracture.

RDA, sources and assaying methods of Vitamin A

00:39:20

Recommended dietary allowance of Vit.A :

Children (1-6years) : 400mcg/day.
Men : 600mcg/day.
Women : 600mcg/day.
Pregnancy : 800mcg/day.
Lactation : 950mcg/day.

Sources of Vit.A :

- Animal sources : Egg, liver, cheese and other milk products.
- Plant sources : carrot, green leafy vegetables, mango, papaya, pumpkin.
- Halibut liver oil : Richest source.
- Cod liver oil : 2nd richest source.
- Carrots : Richest plant source.

Assay of Vit. A :

- Dark adaptation time.
- Serum vitamin A by Carr & Price reaction.

VITAMIN D, E, K

Vitamin D

00:00:43

Source : Sun.

Not a true vitamin since it is endogenously synthesized.

Group of sterols having a hormone like function.

Two sources :

Plant source : Commercially available vitamin.

Ergocalciferol (Vitamin D_2).

Fungus ergot.

Animal source : Cholecalciferol (Vitamin D_3).

Endogenous synthesis of vitamin D :

UV B (290 – 315 nm)

7 dehydrocholesterol → Cholecalciferol [D3]

Dietary Sources → Cholecalciferol

DBP

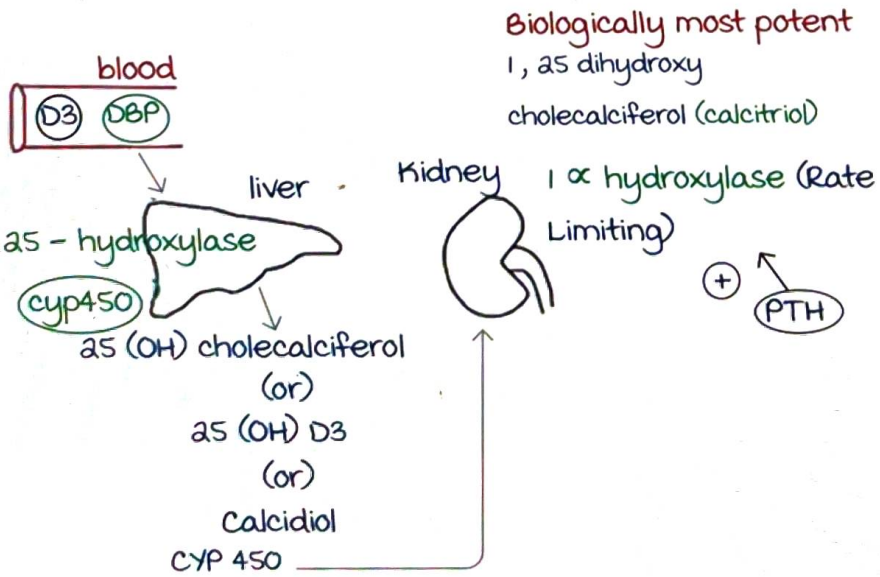
Cholecalciferol

1st step takes place in skin :

7-dehydrocholesterol → Cholecalciferol.

Cholecalciferol is transported in the blood by D-Binding protein (α_1 globulin).

2nd step takes place in the liver and 3rd step in the Kidney.



Active space

1α hydroxylase is activated by Parathyroid hormone and a decrease in phosphate level.

If the body does not need vitamin D,

1,25 dihydroxy Cholecalciferol (Calcitriol) is converted into 24,25 dihydroxy cholecalciferol (Calcitetrol).

Functions of Vitamin D :

- Regulation of calcium
- Immunomodulatory action

Regulation of Calcium

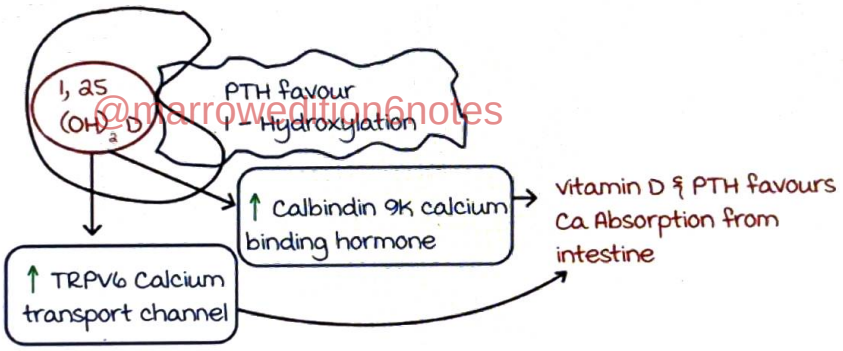
00:07:11

Primary function of vitamin D is to regulate Ca^{2+} and PO_4^{2-}.

Regulation happens at 4 different organs :

Intestine, Kidney, and bone.

- $1,25\ (OH)_2D$: Increases calcium and phosphorus.
- PTH : Increases calcium and decreases phosphorus.



Action on Kidneys : In distal tubules

↓

$1,25\ (OH)_2D$ → Increases level of Calbindin 28K and TRPV 5

↓

Favours re-absorption of calcium and phosphate

↓

Increased serum calcium and phosphate

Parathyroid hormone : Favour re-absorption of calcium and increases excretion/decreases reabsorption of phosphates (Phosphaturic).

Increases serum calcium and decreases serum phosphate.

Action on Bone : $1,25\ (OH)_2D$ and PTH favour RANK ligand

present on osteoblast.

Rank ligand + Rank ligand receptor on pro-osteoclast
leads to formation of mature osteoclast.

↓

Osteoclast → Demineralization of bone.

↓

Favour bone resorption (favour serum calcium level).

- Vitamin D also causes mineralization of bone :
  When serum calcium low → Bone resorption.
  When serum level is enough → Bone mineralization.
  Mineralization by stimulating the osteoblast

↓

Increase osteocalcin level (calcium binding protein)

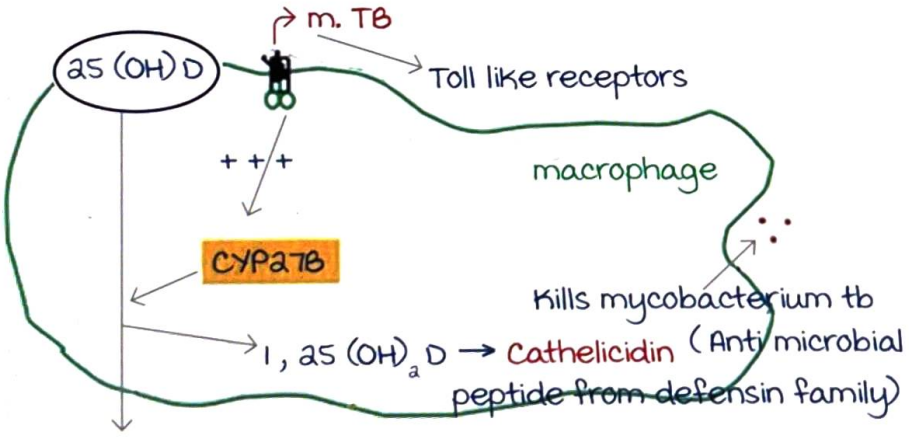
↓

Deposit calcium in the osteoid matrix and epiphyseal cartilage.

Immunomodulatory action of vitamin D

00:14:05

Exhibited in tuberculosis.



1, 25 (OH)$_2$ D
Cathelicidin destroys M. tuberculosis bacteria.

Vitamin D deficiency is a predisposing factor for Covid-19 and prescribed as preventive.

Vitamin D : The holy grail of cancer medicine.

- 25 OH D level <20 ng/ml is associated with an increase in the incidence of : Colon cancer and breast cancer.

Active space

- Vitamin D is protective of pre-diabetes and metabolic syndrome.

Assay of Vitamin D :

- Normal level of 25 cholecalciferol : 20 – 100 ng/ml.
- Serum osteocalcin is measured to assess the Vitamin D status of the body.

In rickets,

Rickety rosary, Wind-swept deformity

Biochemical changes :

Vitamin D deficiency

↓

Decreased serum calcium & decreased serum phosphate (serum Ca can be normal also).

↓

Secondary hyperparathyroidism

↓

Increase 1α hydroxylation

↓

1,25 dihydroxy cholecalciferol

↓

Increase serum calcium level but serum phosphorus level is decreased

molecular defects :

Vitamin dependant Rickets Type I(Pseudo-Vitamin D resistant rickets)	Gene encoding Renal α -hydroxylase
Vitamin dependant Rickets type 2(True vitamin D resistant rickets)	Vitamin D receptor

X-linked hypophosphatemic rickets [XLD] : MC	Mutation in PHEX gene → increased FGF - 23
Autosomal dominant hypophosphatemic rickets	Gene code for FGF - 23
Autosomal recessive hypophosphatemic	Gene encoding dentin matrix protein

Toxicity of vitamin D

00:28:00

Adults > 4000 IU and in infants > 2000 IU

Manifestations :

- Calcinosis : contraction of blood vessel (increased blood pressure)
- Metastatic calcification.

The richest dietary source of vitamin D : Halibut liver oil.

Only dietary source (animal) : Fish.

RDA :

- Children : 10 mcg/day : 400 IU (require more because bones are developing).
- Adults : 5 mcg/day : 200 IU.
- Pregnancy : 10 mcg/day : 400 IU.

Vitamin E

00:31:05

Also known as Alpha-tocopherol (Biologically most potent).

Stereoisomers of tocopherol.

Ring structure in Vitamin E : Chromane/Tocol ring.

most potent naturally occurring antioxidant.

Lipophilic chain breaking antioxidant : Prevent lipid peroxidation.

- Protects the LDL from oxidation
- Protects the PUFA in the membranes

Vitamin E deficiency :

- Axonal degeneration
- Peripheral neuropathy
- Spinocerebellar ataxia
- Haemolytic anaemia (RBC membrane integrity is lost)

- Eyes : Pigmented retinopathy.
  Ophthalmoplegia.
  Nystagmus.

Vitamin E used as treatment :

- Retrolental fibroplasia.
- Intermittent claudication.
- Bronchopulmonary dysplasia.
- Intraventricular haemorrhage of prematurity.
- Slow ageing.

RDA :

Males : 10mg/day.
Females : 8mg/day.
Pregnancy : 10mg/day.
Lactation : 12mg/day.

Vitamin E toxicity manifestation :

Interfere with platelet aggregation.
Interfere with vitamin K.

Selenium decreases the requirement of vitamin E.

Vitamin K

00:36:26

K stands for German word Koagulation.
Naphthoquinone derivative.
Three forms :

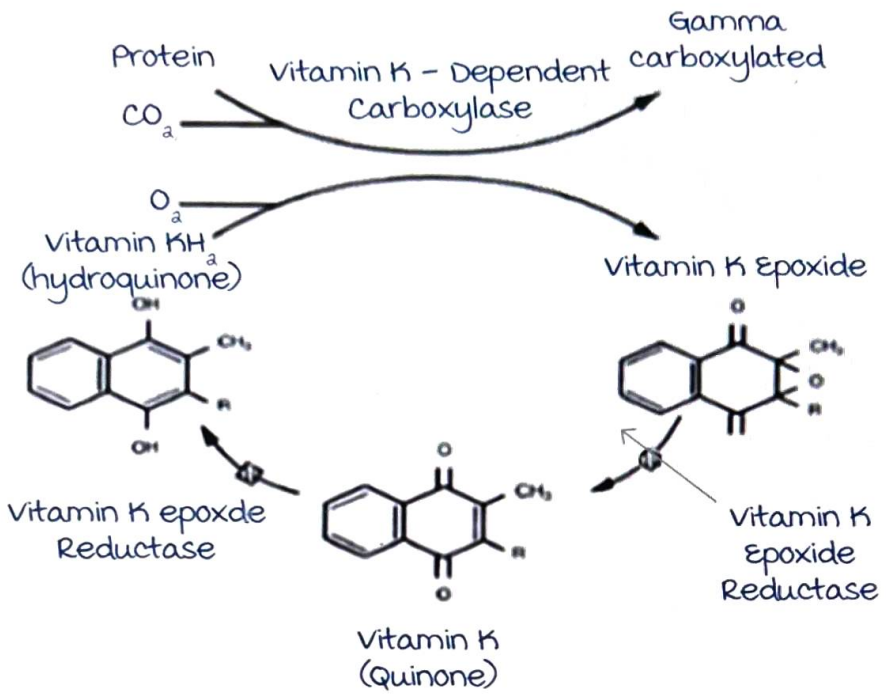
1. Vitamin K_1 : Phylloquinone (dietary sources).
2. Vitamin K_2 : Menaquinone (Bacterial flora).
3. Vitamin K_3 : Menadione (Synthetic water-soluble).

Functions :
Post-translational γ carboxylation of glutamic acid in :

- Factor 2.
- Factor 7, 9, 10.
- Protein C and Protein S.
- Nephrocalcin.
- Osteocalcin.
- Growth arrest-specific gene product (gas 6).

Vitamin K cycle :

Vitamin K epoxide reductase is competitively inhibited by Warfarin and Dicumarol (structural analogue).



Vitamin K deficiency :

Increased prothrombin time and bleeding time.

Bleeding manifestation.

New-borns are susceptible for deficiency of vitamin K because :

- Decreased fat stores.

(fat needed for transport of Vitamin K)

- Sterility of gut.
- Breast milk is a poor source.
- Less placental transport.
- Immaturity of lungs.

Vitamin K toxicity : Haemolysis

↓

Haemolytic jaundice

↓

Kernicterus and brain damage.

Active space

HEMATOPOIETIC VITAMINS

Hematopoietic vitamins : Vitamin B_9 and B_{12}.
Vitamin B_9 / Folic acid : mainly plant origin, rich in green leafy vegetables (folium : Leaves).
Vitamin B_{12} / Cobalamin : Animal origin.
Pure vegans develop Vitamin B_{12} deficiency.

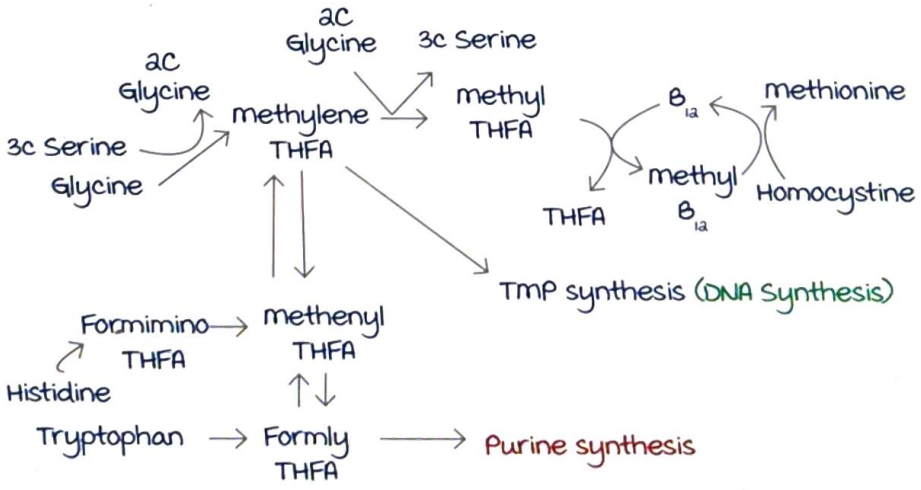
Folic acid

00:03:50

Active form : Tetra hydro folate (THFA).
Carrier of 1 carbon group.
One carbon group and their metabolism :
The 1 carbon groups are as follows :

- methyl (CH_3–).
- methylene (–CH_2–).
- methenyl (–CH=).
- Formyl (–CHO).
- Formimino (–CH=NH).

All these 1 Carbon groups are reversible except : methylene THFA to methyl THFA.



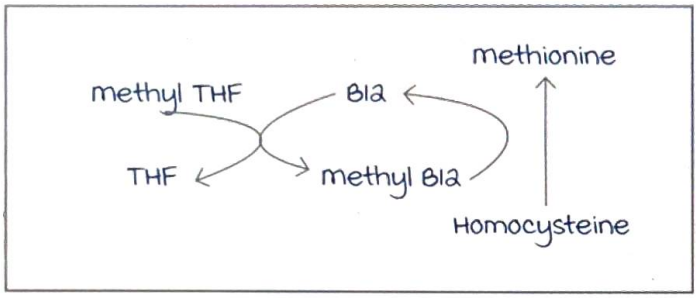
most important entry point to 1 carbon metabolism is serine to glycine conversion.
Enzyme involved in this conversion is serine hydroxy methyl transferase.

Active space

Folate trap may lead to decrease in THFA, thus carrier for 1 Carbon group decreases.
THFA deficiency leads to nuclear maturation defect.

Folic acid deficiency 00:14:30

Manifestations :
Decreased DNA synthesis (decrease in TMP/Purine synthesis).
Megaloblastic anemia.



Neural tube defects : Spina bifida, anencephaly.
Due to decreased DNA synthesis.
Vital to be supplemented during planning of conception itself, or atleast during pregnancy.
Homocysteinemia : Accumulation of homocysteine,
Homocystinuria may develop
methyl donor(SAM) decreases : Epigenetic modification affected and leads to cancer.

Normal diet is rich in folate yet deficiency possible as :
Folylpolyglutamate(Chemical form of folic acid) :
Sensitive to heat, on heating green leaves and vegetables for 5 to 10 minutes destroy 95% of folic acid.

Biochemical assessment :
- Serum folate and Red cell folate.
- Histidine load test :

Formimino THFA
THF
FIGLU (Formimino glutamic acid) ⟶ GLU
FIGLU is accumulated & excreted in urine.

Active space

- AICAR (Amino Imidazole Carboxamide Ribose 5 Phosphate) excreted in urine (Purine synthesis).
- Serum homocysteine.
- Peripheral smear :
  - Macrocytes.
  - Tear drop cell.
  - Hyper segmented neutrophils.
  - Anisopoikilocytosis.

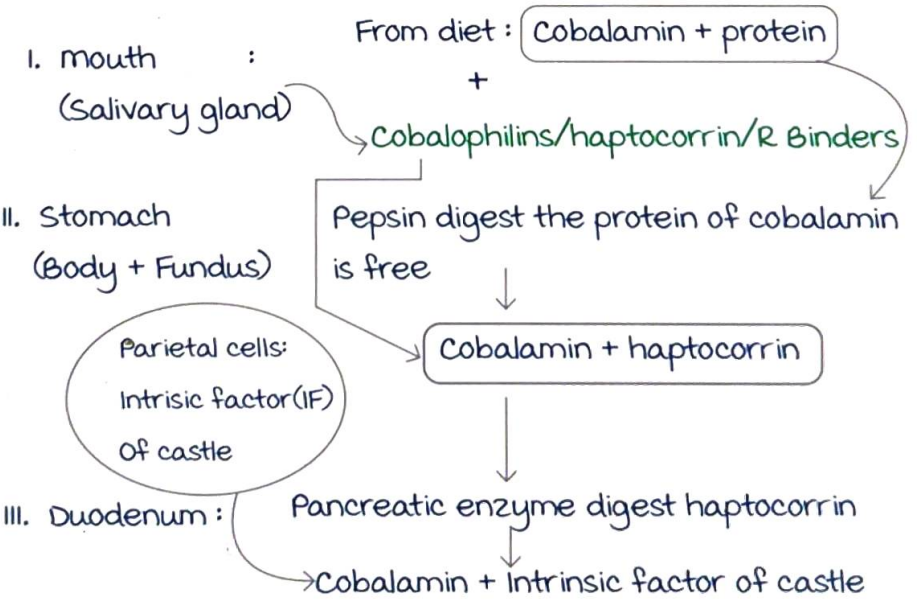
Methotrexate(Anti folate) : Causes folate deficiency.
Folinic acid/ 5 Formyl THFA : Treatment for folate deficiency caused by anti folate drugs.
Leucovorin : Racemic mixture of folinic acid can also be used. Called as leucovorin rescue.

Vitamin B_{12} / cobalamin

00:23:53

4 pyrrole rings with cobalt(4.35%) in the centre.
Intrinsic factor of castle.

Absorption : Mouth (passive form 1%) , Ileum (active form 99%).
Haptocorin : Cobalamin binding protein.



IV. Ileum : Has receptor for intrinsic factor → Cubilin.

Cubilin binds with IF and cobalamine taken up into ileal cells &

Active space

transported in blood using TC2 & TC1 t tagret organs..

Vitamin B_{12} / transport 00:31:00

Transcobalamin (Transporter protein) : Two types.

Transcobalamin 1 : For cobalamin analogs.

Transcobalamin 2 : main carrier protein.

Active forms of Vitamin B_{12} (Cobamide enzymes) :

- Adenosyl B_{12}.
- methyl B_{12}.

Adenosyl B_{12} plays coenzyme role in :

- methyl malonyl CoA mutase.
- Leucine aminomutase (Absent in humans).

Propionyl CoA enters gluconeogenesis pathway through this.

L-methyl malonyl CoA

Adenosyl B_{12} | methyl malonyl CoA mutase

↓

Succinyl CoA

methyl B_{12} plays coenzyme role in :

methionine synthase/Homocysteine methyl transferase.

Homocysteine $\xrightarrow[\text{methionine Synthase}]{\text{methyl } B_{12}}$ methionine

Deficiency of Vitamin B_{12} :

Nutritional deficiency (Strict vegans).

Malabsorption : Autoimmune gastritis (IF destruction)

Pernicious anaemia.

Gastric causes.

Intestinal causes : ileum site of cobalamine absorption.

- Ileal resection.
- Crohn's disease.
- Stagnant loop syndrome.

Fish tape worm (Diphyllobothrium latum)

Active space

Manifestations 00:37:16

megaloblastic anaemia (more common in THFA deficiency).
methyl THFA cannot be cnverted to THFA in vitamin B12 deficiency.
THFA deficiency causes defective nuclear maturation.
Homocysteinemia.
methyl malonic aciduria
Serum methyl malonate : Differentiates megaloblastic anemia due to folic acid deficiency or Vitamin B_{12} deficiency.

Neurological manifestations in Vitamin B_{12} deficiency :
methyl malonyl CoA mutase affected.

Accumulation of L methyl malonyl CoA and Propionyl CoA.
↓
Propionic acid level increases as a result.
↓
Abnormal fatty acid accumulation to the neuronal lipid.
↓
myelin breakdown occurs.
↓
Manifests as Sub acute combined degeneration.

Assessment of vitamin B_{12} deficiency 00:43:10

- Serum cobalamin.
- Serum homocysteine.
- Urine homocysteine.
- Serum methylmalonoic acid : unique for Vitamin B12 aetiology.
- Schilling test.
- Peripheral smear.
- Bone marrow cytology.

Q. Why megaloblastic anaemia in folic acid deficiency ?
A. Decrease in THFA ⟶ decrease in TMP ⟶ Affect DNA synthesis.

Q. Why strict vegans develop vitamin B12 deficiency ?
A. Because of pure plant source is deficient in vit B 12.

Q. Why neurological complications only in vitamin B 12 deficiency ?

Accumulation of L methyl malonyl CoA and Propionyl CoA.

↓

Propionic acid level increases as a result.

↓

Abnormal fatty acid accumulation to the neuronal lipid.

↓

myelin breakdown occurs.

↓

manifests as Sub acute combined degeneration.

Q. Why is it essential to exclude vitamin B 12 deficiency as the cause of megaloblastic anaemia?
A. In a case of megaloblastic anemia due to Vitamin B_{12} deficiency, if it is not diagnosed and folic acid is given in place of B12, It leads to aggravation of neurological symptoms.
As folic acid supplemented used for conversion on methyl THFA to THFA and B12 will be excessively used in conversion.
B12 store further depletes.

ENERGY RELEASING VITAMINS

Vitamin B1- Thiamine 00:02:03

Required for carbohydrate metabolism.

The sources of thiamine are :

- Aleurone layer (most important) : Between the white and brown layer of cereals.
- Unpolished rice, Whole wheat flour and Yeast.

The aleurone layer is lost when the rice is polished.

Parboiling is the preffered method of cooking rice to prevent this .

Coenzyme role :

Active form : Thiamine pyrophosphate (TPP) or Thiamine Diphosphate.

Thymine pyrophosphate acts as a coenxyme in the following enzymes :

1. Pyruvate dehydrogenase.
2. α Ketoglutarate dehydrogenase.
3. Branched chain Ketoacid dehydrogenase.
4. Transketolase (non oxidative phase of HMP).

Deficiency of Vitamin B1 00:06:18

Common in alcoholics.

Alcohol decreases absorption of thiamine.

Wet Beri Beri : Associated symptoms are ;

1. High output cardiac failure.
2. Dyspnoea.
3. Cardiomegaly.
4. Peripheral edema.
5. Pulmonary edema.

Case : A chronic alchoholic presented with pain and paresthesia of lower limbs , with cramps. Erythrocytes transeketolate activity is reduced. which is the vitamin deficient ?

Answer : Thiamine deficiency causing dry beri beri.

Active space

Dry Beri Beri :
Peripheral nervous system affected.
Symmetric motor and Sensory neuropathy.
Pain, paresthesia, loss of reflexes especially in lower limbs.
Muscle cramps, muscle atrophy(Severe cases).

Wernicke's Encephalopathy :
Case : A chronic alchoholic presented with ptosis of eyelid , confusion , nystagmus.
Diagnosis : Wernicke's Encephalopathy.
Central nervous sustem affected.
Horizontal nystagmus.
Ophthalmoplegia(Ptosis).
Truncal ataxia.
Confusion.

Wernicke's Korsakoff syndrome : Wernicke's Encephalopathy associated with memory loss (Dementia) and confabulatory psychosis.

Symptoms aggravated in Transketolase deficiency.
Thiamine phosphorylates chloride channels in nervous system, activates nerve conduction.

Biochemical assessment of thiamine deficiency :
Erythrocyte Transketolase.
Urinary thiamine excretion.
Required daily allowance (RDA) : 1-1.5 mg/day.
No reported toxicity.

Riboflavin/Vitamin B2

00:14:56

Questions :
During phototherapy which vitamin is supplemented ?
Answer : Riboflavin.

When we take B-complex vitamins , what is the reason for discoloration if urine ?
Answer : Riboflavin.

Active space

Which vitamin is a redox vitamin?
Answer : Riboflavin.

Cooking food will not destroy this vitamin?
Answer : Riboflavin.

Pigmented vitamin(Warburg yellow enzyme) : Gives urine yellow colour.
B2 (Riboflavin) and B3 (Niacin) are redox Vitamins.
Supplemented during phototherapy (Light sensitive vitamin).
Heat resistant vitamin.

Coenzyme role Riboflavin :
Active form : Flavin Adenine Dinucleotide(FAD), Flavin mono Nucleotide(FMN).
FMN coenzyme role :
Complex I in Electron transport chain(NADH dehydrogenase).
L-Amino acid oxidase.

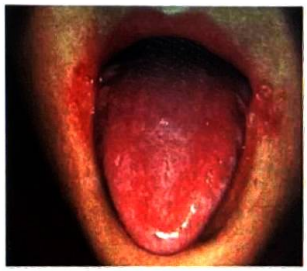
FAD coenzyme is associated with the following enzymes :

1. Acyl CoA dehydrogenase.
2. Succinate dehydrogenase (Complex II in ETC).
3. D Aminoacid oxidase.
4. Xanthine oxidase.
5. All multi enzyme complexes.

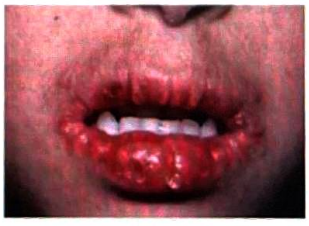
Riboflavin deficiency

00:19:56

Initially asymptomatic.



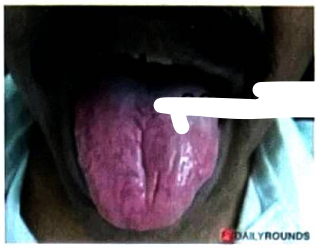
Cheilosis :
Pallor in angles of mouth.
Thinning and maceration of epithelium.
Fissuring extends radially to skin.



Glossitis :

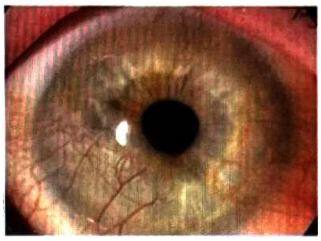
Magenta coloured tongue.

Tongue becomes smooth, loss of Papillae.



Eyes :

- Keratitis.
- Conjunctivitis.
- Photophobia.
- Lacrimation.
- Corneal vascularisation (Characterstic finding).



Other features :

Seborrheic dermatitis.

Normocytic normochromic anemia.

Biochemical assessment of Riboflavin :

Erythrocyte Glutathione reductase after giving FAD in vitro.

Urinary excretion of riboflavin.

RDA : 1.5 mg/day.

No reported toxicity.

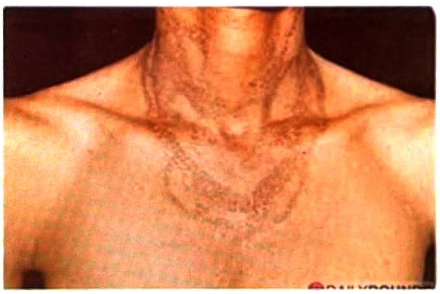
Niacin / Nicotinic Acid / Vitamin B3 00:24:00

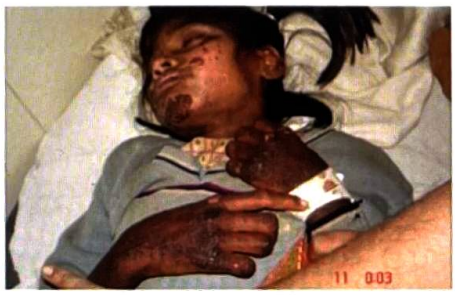
An endogenously synthesised vitamin from an amino acid,

Niacin active form are,

NAD^+(Nicotinamide adenine dinucleotide).

$NADP^+$(Nicotinamide adenine dinucleotide phosphate).





symmetrical erythmatous sclay lesions on the sun exposed areas.

Active space

"Casal Necklace" due to photosensitive dermatitis.

The lichenified lesions on both the hand is known as Gauntlet of pellagra.

NAD^+ Coenzyme role :

Every dehydrogenase requires NAD+, Except enzymes that reauire FAD Or NADP+.

$NADP^+$ and NADPH coenzyme role :

$NADP^+$ generating reactions :

1st two enzymes of oxidative phase of Hexose monophosphate Pathway :

- Glucose -6- Phosphate dehydrogenase.
- 6- Phospho gluconate dehydrogenase.
- Cytoplasmic isocitrate dehydrogenase.
- Malic enzymes.

NADPH requiring enzymes :

All reductase require NADPH.

- Enoyl reductase : For fatty acid synthesis.
- Ketoacyl reductase : For fatty acid synthesis.
- HMG COA reductase : For cholesterol synthesis.
- Glutathione reductase : Free radical scavenging.
- Ribonucleotide reductase : Conversion of Ribonucleotide to Deoxy ribonucleotide.
- Folate reductase.

Niacin Deficiencies :

Initially present with vague symptoms.

Progresses to Pellagra.

Cutaneous manifestations :

- Photosensitive dermatitis (Casal's necklace).
- Diarrhoea.
- Dementia.
- Depressive psychosis.
- Death (Advanced cases).
- Casal's necklace develops in sun exposed areas symmetricaly.

Active space

Pellagra like symptoms :
Hartnup's disease : Due to decreased tryptophan.
Carcinoid syndrome : Tryptophan used for serotonin production.
B_6 deficiency : Inhibits Kynureninase.
Maize/corn diet : Niacin in bound form.
Sorghum(Jowar) : High leucine (Inhibits Q PRTase).

Niacin toxicity :
Prostaglandin mediated Cutaneous flushing.
Most fatal : Fulminant hepatitis.
Glucose intolerance.
Hyper uricemia.
Gastric irritation.
Macular edema.
Liver enzymes are increased in fulminant hepatitis.

Treatment :

Premedication with Aspirin.
Laropiprant(Prostaglandin D_a inhibitor).
Niacin : Lipid modifier drug.
Decreases Triglycerides,
Increases HDL, Decreases LDL.

Pantothenic acid/Vitamin B5 00:35:02

Beta alanine is present in Pantothenic acid which is inturn present in CoA.
Endogenously synthesised in intestinal flora.
Coenzyme role :
Present in Coenzyme A.
Required for:

- Succinyl CoA and Acetyl CoA(TCA cycle)
- HMG CoA(Cholesterol and Ketone body synthesis, Leucine catabolism).
- Acyl carrier protein(Fatty acid synthase complex)

Active space

Deficiency manifestation :
Gopalan's burning foot syndrome/ Nutritional melalgia.

Biotin / Vitamin H/ Vitamin B7 00:39:16

Case : A young adult who is fond of eating raw eggs daily developed erythematous rash around nose, eyes and mouth. Which enzyme activity is affected in this person ?

Ans : Raw egg contains avdin which inhibits biotin.

Active form : Carboxy biocytin.
Coenzyme role : Biotin dependent carboxylation reaction.

- Propionyl CoA carboxylase.
- Pyruvate carboxylase.
- Acetyl coenzyme A carboxylase.

Biotin Independent carboxylation :

- Gamma carboxylation(Vitamin K).
- Malic enzyme.
- AIR carboxylase for purine synthesis.
- Carbomylphosphase synthetase I and 2 .

Biotin deficiency :
Mental changes(Depression, Hallucination).
Scaling, seborrheic and erythematous rash.
~~Biotinase~~(Releases active form of biotin) deficiency :
Leiner's disease.

Other uses :
Biotin affinity toward avidin is very strong.
Streptavidin : Capable of binding to 4 biotin, used in laboratory investigations.

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 Alanine amino transferase

Glycine

require PLP

Alanine Pyruvate

In case of PLP deficiency

Glyoxylate → Oxalate

results in "Oxaluria"

2. Amino acid decarboxylation :

Histidine $\xrightarrow{DLP}$ 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-Hydroxy Kynurenine
PLP ↓ Kynureninase
3-hydroxyanthranilate
↓ ↓
Ketogenic fate NADPH metabolism

In case of PLP deficiency
↓
3-Hydroxykynurenine → Xanthurenic acid
It leads 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)

Manifestations of Vitamin B6 deficiency 00:12:00

Neurological :
- Peripheral neuropathy.
- Personality changes (depression & confusion).
- Convulsion.

microcytic hypochromic anemia.
Pellagra.

Urinary analytes excreted in PLP deficiency :
- Homocysteine.
- Xanthurenic acid
- Oxalate (causing oxaluria)

Active space

PLP and hormone dependent cancer :

Vitamin B_6 : Inhibit binding of Hormone receptor complex to hormone receptor elements.

Deficiency of vitamin B_6 leads to enhanced binding

↓

↑ action of hormone.

Toxicity : Sensory neuropathy

Biochemistry assay :

Erythrocyte transaminase.

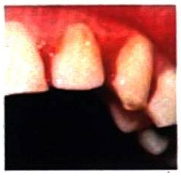
Tryptophan load test.

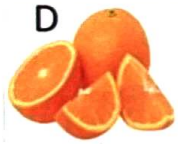
measurement of PLP in blood.

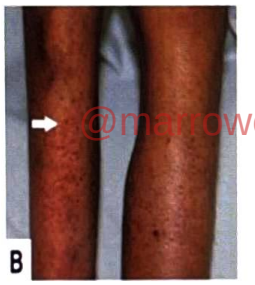
RDA : 1 – 2 mg/day

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. Co-enzyme for copper containing hydroxylases.
   - Dopamine β hydroxylase
   - Peptidyl glycine hydroxylase

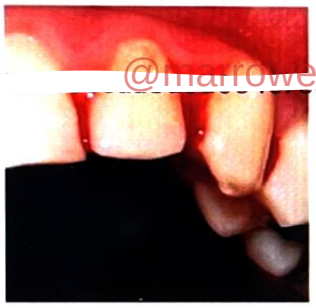
Active space

2. Coenzyme for α ketoglutarate linked iron containing enzyme : Proline and lysyl hydroxylase.
Decreased hydroxylation of proline & lysine
↓
defective collagen → bleeding manifestation
↓
Anemia

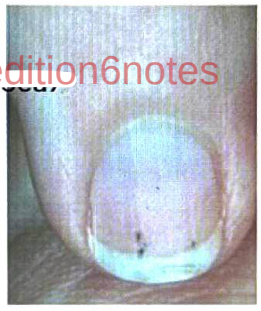
Ferrireductase (need vitamin C)
↓
$Fe^{3+} \longrightarrow Fe^{2+}$ (ferrous form)
(absorbed from intestine)

Hence, vitamin C deficiency causes anemia.

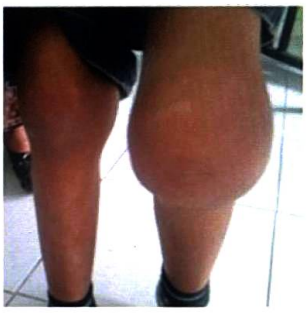
Clinical manifestations of vitamin C deficiency 00:23:14



Bleeding gums



Splinter hemorrhages

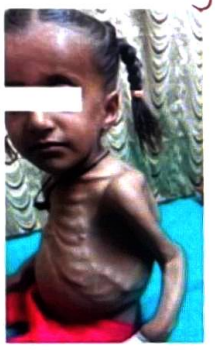


Hemarthrosis

Scorbutic rosary

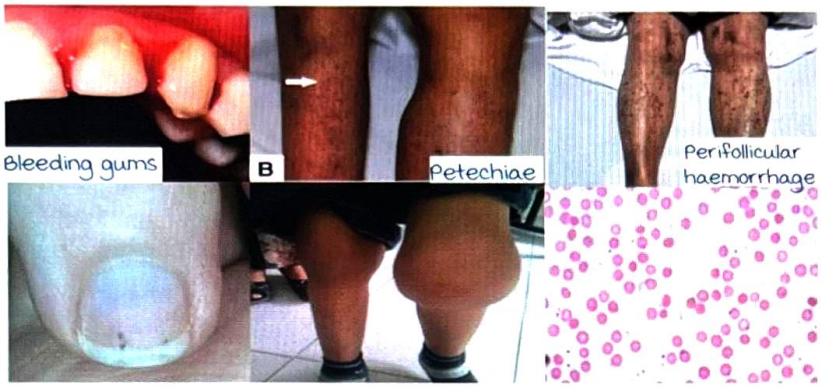
Sharp angulation with or without beading due to backward displacement or pushing in of sternum.

Rachitic rosary



Bead like enlargement of costochondral junction

1. Bleeding gums, petechiae, ecchymosis
2. Splinter hemorrhage, perifollicular haemorrhage
3. Hemarthrosis
4. Pseudoparalysis : 'Pithed frog leg' appearance
5. Scorbutic rosary.



Splinter hemorrhage Hemearthrosis Anemia

Infantile scurvy :

a/k/a Barlow's disease.

Infants between 6 - 12 months are affected.

When weaning starts, vitamin C deficiency occurs.

So they should be supplemented with vitamin C.

Active space